



Investigation of Electrochemical Properties of Uric Acid at Carbon Paste Electrode with Aluminium Oxide Nanoparticles and Development of Voltammetric Method for its Determination in Serum

Ürik Asitin Alüminyum Oksit Nanopartiküllü Karbon Pasta Elektrotta Elektrokimyasal Özelliklerinin İncelenmesi ve Serumda Tayini için Voltammetrik Yöntem Geliştirilmesi

Rüveyda Ünvert[✉] and Funda Öztürk*[✉]

Tekirdağ Namık Kemal University, Faculty of Science and Art, Department of Chemistry, Tekirdağ, Turkey.

ABSTRACT

The electrochemical effects of uric acid (UA) were determined by cyclic voltammetry (CV), and square wave voltammetry (SWV) at a carbon paste electrode modified with aluminum oxide nanoparticles (Al_2O_3 NP/CPE) in 0.05 M phosphate buffer (pH 5.0). The adsorption properties of the molecule on Al_2O_3 NP/CPE were studied, and the electrons assigned in the electrode reaction were calculated. A new procedure for the quantification of UA in human serum has also been proposed. The linear operating range, and limit of detection (LOD) of the method were calculated as 0.1 μ M-230 μ M, and 0.1 μ M, respectively. The developed method for UA in serum showed high reliability, repeatability, accuracy, and precision.

Key Words

Uric acid, voltammetric method, sensor, aluminum oxide nanoparticle.

Öz

Ürik asidin (ÜA) elektrokimyasal özellikleri, 0.05 M fosfat tampon çözeltisinde pH 5,0'de alüminyum oksit nanopartiküllü karbon pasta elektrotta (Al_2O_3 NP/KPE) döngüsel voltametri (DV) ve kare dalga voltametri (KDV) ile incelenmiştir. Molekülün Al_2O_3 NP/KPE'de adsorpsiyon özellikleri araştırılmış ve elektrot reaksiyonunda aktarılan elektron sayısı hesaplanmıştır. Ayrıca insan serumunda ÜA tayini için yeni bir voltammetrik yöntem önerilmiştir. Yöntemin doğrusal çalışma aralığı ve gözlenebilirlik sınırı sırasıyla 0,1 μ M-230 μ M ve 0,1 μ M olarak belirlenmiştir. Serumda ÜA tayini için geliştirilen yöntem yüksek güvenilirlik, tekrarlanabilirlik, doğruluk ve kesinlik göstermiştir.

Anahtar Kelimeler

Ürik asit, voltammetrik yöntem, sensör, alüminyum oksit nanopartikül.

Article History: Received: Dec 28, 2022; Revised: Jan 12, 2023; Accepted: Jan 12, 2023; Available Online: Jan 20, 2023.

DOI: <https://doi.org/10.15671/hjbc.1225829>

Correspondence to: F. Öztürk, Tekirdağ Namık Kemal University, Faculty of Science and Art, Department of Chemistry, Tekirdağ, Turkey.

E-Mail: fozturk@nku.edu.tr

INTRODUCTION

UA is the final product in purine metabolism. High levels of UA in biological fluids can cause gout/arthritis, renal diseases, hyperuricemia, lesch-nyhan syndrome, leukemia, pneumonia, and renal failure [1]. Studies have shown that the likelihood of cardiovascular diseases increases with increasing levels of UA in serum. In contrast, low UA levels are also correlated with neurological diseases such as multiple sclerosis, Parkinson's, and Alzheimer's [2]. Determination of UA in biological fluids is therefore very important. In the literature, there are different methods for UA determination such as chemiluminescence [3], chromatography [4], spectrofluorimetric [5], enzymatic system [6]. However, these methods are laborious, expensive, and time consuming. In addition, the application of these methods is complicated. [1]. On the other hand, electrochemical methods provide fast response, high sensitivity, and low cost [7]. However, the lack of good selectivity of unmodified electrodes is a disadvantage of electrochemical methods.

Carbon is widely used as a working electrode in electrochemical practices. The carbon paste electrode (CPE) is of special importance among carbon-based electrodes. CPEs have advantages over other carbon electrodes due to the ease of preparation, and obtaining new reproducible surfaces, low residual current, porous surface, and low cost. Therefore, CPE can be a suitable material for the fabrication of modified electrodes [8]. However, these electrodes have several drawbacks such as low sensitivity, slow electron transfer, the need for higher overpotential for the electrocatalytic process. Incorporation of metal oxide nanoparticles into the carbon paste mixture is a promising approach to overcome these limitations [9-12].

Metal oxide nanoparticles are widely used in electrochemistry applications such as electrochemical sensors [13], lithium-ion batteries [14], and electrochemical capacitors [15]. Metal oxide nanoparticles used in the modification of many electrochemical sensing platforms are very popular due to their high surface area, simple fabrication methods, small size [16], chemical stability, low toxicity, good electrical conductivity, catalytic activity, and good biocompatibility [15].

UA, and ascorbic acid (AA) are two antioxidants often found together in food, and biological samples [2]. The main problem in the determination of the two molecules at unmodified electrodes is that both species are oxidized at the same potentials. For this reason, the separation of UA, and AA oxidation potential values has been an important goal for researchers working in the field of electroanalytical chemistry. In literature, studies on the quantification of UA in the existence of AA by electrochemical methods have been reported [2, 17-19]. No voltammetric technique for the determination of UA in biological fluids using $\text{Al}_2\text{O}_3\text{NP}/\text{CPEs}$ has been reported in the literature. In this study, the electrochemical properties of UA were studied on $\text{Al}_2\text{O}_3\text{NP}/\text{CPE}$ by CV, and SWV methods, and a new method for the quantitation of UA in serum was proposed.

MATERIAL and METHODS

Chemicals and Samples

AA, graphite powder (powder > 20 μm), $\text{Al}_2\text{O}_3\text{NP}$ (particle size <50 nm), glucose, paraffin oil, UA, lithium carbonate (Li_2CO_3), human serum, xanthine, ethanol, acetic acid, sodium phosphate, sodium dihydrogen phosphate, hydrochloric acid, and sodium hydroxide were from Sigma-Aldrich. Phosphate buffer solution (PBS) was made by mixing appropriate amounts of sodium phosphate, and sodium dihydrogen phosphate and diluting with distilled water. The stock solution of UA (10.0 mM) was prepared by dissolving a mixture of UA, and Li_2CO_3 in distilled water. Stock AA solution (10.0 mM) was prepared by dissolving appropriate amounts of AA in distilled water. For CV and SWV experiments, solutions diluted with PBS were used. Methanol was used at a 1:1 ratio to precipitate proteins in serum. The precipitated proteins were centrifuged at 2000 rpm for 5 minutes. 1.0 mL of the protein precipitated serum was taken and diluted to 10.0 mL with pH 5.0 PBS.

Apparatus

The studies were performed on BAS C3 cell stand with Dropsens μstat 400 (Spain) electrochemical system. A three-electrode system consisting of a CPE working electrode with an electrode body of 15.0 mg internal volume, Pt wire (BAS MW-1034), and Ag/AgCl reference electrode (BAS MF-2052) was used for voltammetric measurements. The reference electrode was kept in 3.0 M KCl after the measurements. pH studies were performed with a HANNA Instruments HI 2211 pH meter. Solution preparation, and stirring were performed

on a WISD Laboratory Instrument MHS-20 D Wise Stir magnetic stirrer. High purity (99.99%) nitrogen gas of HABAS brand was used for electrochemical measurements. Purified water for the preparation of solutions was obtained from PURELAB Option Q DV 25. OHAUS Pioneer brand precision balance was used for weighing. AXYGEN micropipettes were used in the experiments. All experiments were recorded at room temperature. Before voltammetric measurements, the UA solution prepared at pH 5.0 PBS was treated with nitrogen gas for one minute to provide an oxygen-free environment. Cyclic voltammograms were recorded by varying the potential range and the appropriate operating potential range (0.0 V-1.0 V) was determined. The optimum device parameters (amplitude 10 mVs⁻¹, step potential 0.01 V) were selected based on the current value and peak shape of the recorded peaks. Calibration curves were constructed with peak current values obtained from square wave voltammograms of UA at different concentrations.

Fabrication of CPE and Al₂O₃NP/CPE

CPEs were formed by crushing graphite powder (15.0 mg) with paraffin oil (10 µL) until a homogeneous mixture was obtained. The resulting paste-like mixture was filled into the electrode body with an internal volume of 15 mg. The electrode surface was then smoothed on a plain paper.

Al₂O₃NP/CPEs were obtained by homogeneously mixing Al₂O₃ nanoparticles (3.0 mg), graphite powder (12.0 mg) and paraffin oil (10 µL). The homogeneous paste mixture was filled into the electrode body and the electrode surface was smoothed.

RESULTS and DISCUSSION

In the first part of this study, the electrochemical behavior of UA (Figure 1) on Al₂O₃NP/CPE surface in 0.05 M PBS (pH=5.0) was evaluated. By evaluating the findings

obtained from voltammetric experiments, the adsorption properties of UA were determined, and a possible oxidation reaction was proposed. A possible oxidation reaction was proposed by considering the groups present in the structural form of UA. In the second part of the study, a new and easy voltammetric method for the quantification of UA in serum was presented. The results obtained are given below.

Electrochemical properties of UA

Cyclic voltammetry (CV) studies

The electrochemical effects of UA at Al₂O₃NP/CPE were examined by CV, and SWV methods in 0.05 M PBS (pH 5.0) containing 1.0 mM UA. In the cyclic voltammograms recorded in the determined potential range, a sharp oxidation peak was obtained at +0.57 V and +0.58 V in simple CPE and Al₂O₃NP/CPE, respectively (Figure 2). When the voltammograms obtained were compared, it was observed that the peak intensity obtained in Al₂O₃NP/CPE was significantly higher than the peak intensity obtained in unmodified CPE. This significant increase in the peak current of UA in Al₂O₃NP/CPE can be explained by the larger effective surface area and higher electron transfer rate of the modified electrode.

The reversibility of the electrochemical oxidation reaction of UA on Al₂O₃NP/CPE was investigated by CV. Figure 2 shows that no cathodic peak was detected in the recorded reverse scan, indicating that the electrode reaction may be irreversible. Cyclic voltammograms of UA recorded at different scan rates were used to support this idea. Cyclic voltammograms of 1.0 mM UA solution in 0.05 M pH 5.0 PBS at Al₂O₃NP/CPE were recorded at scan rates (*v*) ranging from 0.01 to 1.0 Vs⁻¹ (Figure 3A). When the voltammograms were analyzed, it was shown that as the scan rate increased, the peak current intensity increases, and the peak potentials shift to positive values. The change in peak current and peak potential with increasing scan rate indicated that the oxidation reaction of UA on Al₂O₃NP/CPE is irreversible. [20]. Furt-

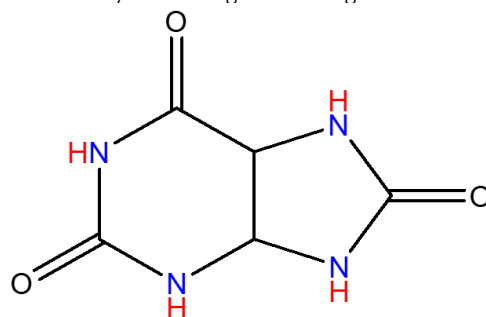


Figure 1. Molecular structure of UA

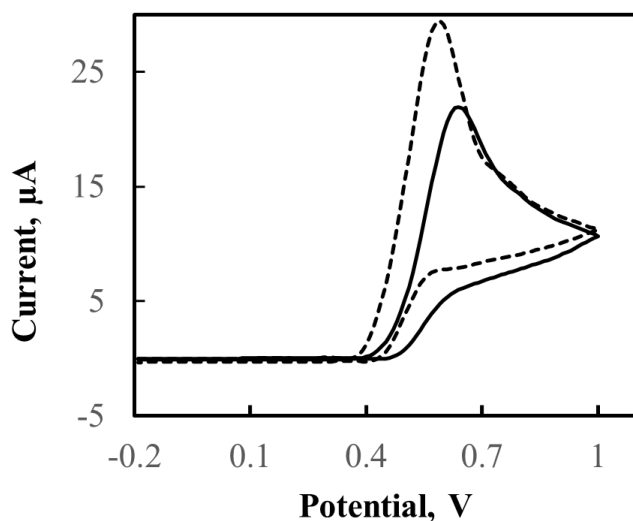


Figure 2. Cyclic voltammograms of 1.0 mM UA at (A) simple CPE and (B) $\text{Al}_2\text{O}_3\text{NP/CPE}$ (0.05 M pH=5.0 PBS).

hermore, the reversibility of the oxidation reaction of UA on $\text{Al}_2\text{O}_3\text{NP/CPE}$ was investigated by utilizing the variation plot of the current function ($ip_a/v^{1/2}$) with scan rate (v) (Figure 3B). The change of $ip_a/v^{1/2}$ with v is another indication that the electrode reaction is not reversible [21, 22]. The findings showed that the oxidation of UA on $\text{Al}_2\text{O}_3\text{NP/CPE}$ is irreversible.

Adsorption properties of UA

Determination of the adsorption properties of molecules is important for elucidating electrode reactions and calculating some electrochemical quantities. CV is the most important voltammetric technique used for this. CV experiments at different scan rates were utilized to determine the adsorption properties of UA on $\text{Al}_2\text{O}_3\text{NP/CPE}$. For this purpose, the logarithm of peak current ($\log ip_a$) versus logarithm of scan rate ($\log v$) graph was plotted (Figure 3C). According to the Randless-Sevcik equation, a line equation with slope 0.5 is obtained from the $\log ip_a$ - $\log v$ plot [22]. The slope of the $\log ip_a$ - $\log v$ plot is approximately 0.5, indicating that the oxidation of UA on $\text{Al}_2\text{O}_3\text{NP/CPE}$ is diffusion controlled.

Effect of pH on the electrode reaction

pH is the most critical parameter affecting the position (E_p) and intensity (ip) of the electrochemical signal. For this purpose, SW voltammograms of 1.0 mM UA solution in 0.05 M PBS were recorded between pH 3.0-8.0 (Figure 4A). In Figure 4, it was observed that the peak potentials shifted to more negative values and the peak

intensity decreased with increasing pH values. After pH 6.0, the peak shapes were observed to be distorted. The change in peak potential with pH indicates that electron transfer is accompanied by proton transfer [23-25].

$$E_p = E^0 + \frac{RT}{nF} \ln \frac{[O]}{[R]} - \frac{\partial RT}{nF} \ln [H^+] \quad (1)$$

In Equation 1, ∂ is the protons number, and n are the electrons number. Using the values of the known quantities in the equation, and the slope of the plot of pH versus peak potential, the ratio ∂/n was determined to be 0.81 (Figure 4B). This result was close to one, indicating that each electron in the oxidation reaction of UA was accompanied by an equal number of protons. The number of electrons in the electrode reaction of UA was calculated from Equation 2 [26].

$$E_p = k + \frac{RT}{(n\beta)F} \ln v \quad (2)$$

In Equation 2, n : the number of electrons, mol electrons/mol molecule; β : the anodic charge transfer coefficient. The variation of the peak potential of UA in $\text{Al}_2\text{O}_3\text{NP/CPE}$ with the logarithm of the scan rate was investigated (Figure 3D). When the slope of the obtained curve was substituted in Equation 2, the value of $n \times \beta$ was determined as 1.06. The fact that β can take values ranging from 0 to 1 [27], and this value is 0.50 for most electrodes indicates that the oxidation of UA on the $\text{Al}_2\text{O}_3\text{NP/CPE}$ surface occurs with two electron transfer. According to these results, the possible electrode reac-

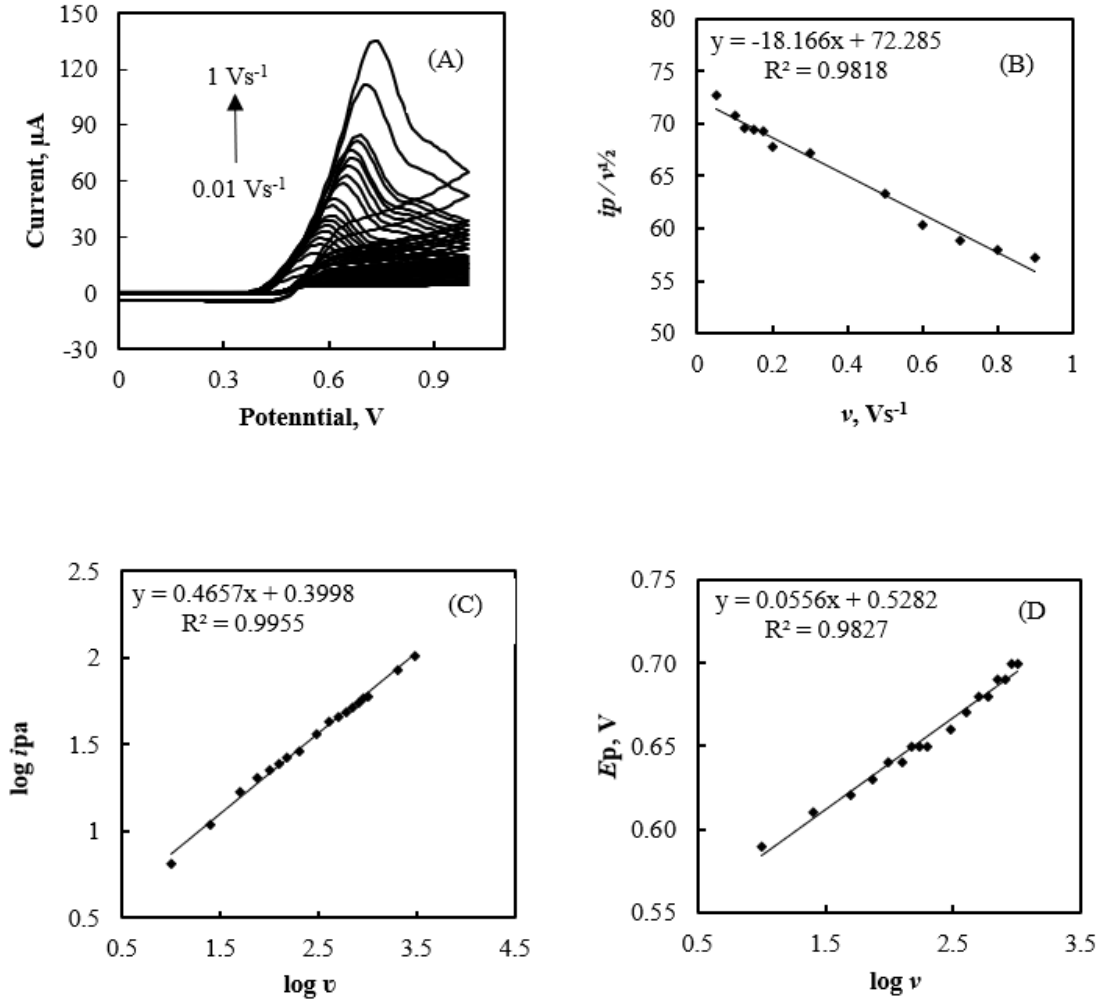


Figure 3. Cyclic voltammograms of 1.0 mM UA in $\text{Al}_2\text{O}_3\text{NP/CPE}$ in pH 5 PBS (A) at different scan rates (0.01; 0.025; 0.05; 0.075; 0.1; 0.125; 0.150; 0.176; 0.2; 0.25; 0.3; 0.4; 0.5; 0.5; 0.6; 0.7; 0.7; 0.8; 0.8; 0.9; 1.0 Vs^{-1}) (B) plot of the current function $i_p, a/v^{1/2}$ versus scan rate (v) (C) $\log i_p, a$ - $\log v$ graph (D) $\log v - E_p, a$ graph.

tion mechanism of UA is given in Figure 5 [28, 29].

Analytical parameters of the SWV method

In the second part of the study, a new voltammetric approach was designed on $\text{Al}_2\text{O}_3\text{NP/CPE}$ for the quantification of UA in the presence of AA, the main interferant in serum. For this purpose, cyclic voltammograms of 1.0 mM AA and 1.0 mM UA solutions at pH 5.0 PBS were recorded on simple CPE, and $\text{Al}_2\text{O}_3\text{NP/CPE}$ (Figure 6). A difference of 110 mV and 140 mV was observed between the oxidation potentials of AA, and UA on simple CPE, and $\text{Al}_2\text{O}_3\text{NP/CPE}$, respectively. Moreover, the oxidation peak intensity of UA was significantly increased on $\text{Al}_2\text{O}_3\text{NP/CPE}$.

Determining the Analytical Parameters of the SWV Method

Voltammetric measurements of the solutions of UA prepared at different concentrations in $\text{Al}_2\text{O}_3\text{NP/CPE}$ at 0.05 M pH 5.0 PBS were recorded by SWV method at optimum device, and method parameters (Figure 7A). The peak current values obtained from the voltammograms were plotted against changing concentration values, and a calibration curve was derived (Figure 7B). The linear range for UA determination by SWV on $\text{Al}_2\text{O}_3\text{NP/CPE}$ was determined as 0.1 μM - 230 μM . LOD,

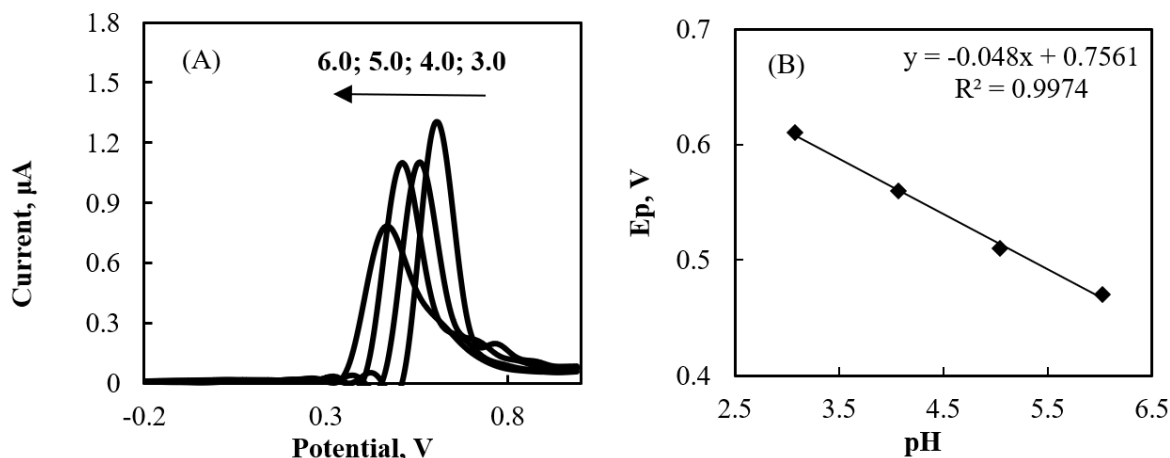


Figure 4. SW voltammograms of 0.1 mM UA on Al₂O₃NP/CPE (A) recorded at different pH values (B) variation of peak potential with pH. The ratio of the number of protons to the number of electrons in the oxidation reaction of UA in Al₂O₃NP/CPE was calculated from Equation 1 using the slope of the graph given in Figure 4B [26].

and the limit of quantification (LOQ) for the suggested SWV method were calculated with the relations $3s/m$, and $10s/m$, respectively [30]. In these equations, s is the standard deviation of the starting point, and m is the slope of the calibration plot. According to the equations, the LOD, and the LOQ of the SWV method were calculated as $0.1\mu\text{M}$, and $0.4\mu\text{M}$, respectively.

Interference studies

In electrochemical studies, it is important to determine the contribution of species present in real samples, and electroactive at potentials close to the reduction / oxidation signal of the species to the measurement results. Therefore, the response of the modified CPE to 2.5×10^{-4} M UA solution was investigated in the existence of interferences such as AA, glucose, glutamic acid, xanthine, urea, oxalic acid. For this purpose, first uric acid, and then other interferences were added successively to 0.05 M PBS (pH=5.0). From the voltammograms obtained, the contribution of the current readings to the UA

current was calculated (Tables 1, 2). When Table 1 was analyzed, it was seen that the contribution of AA, the largest interferences of UA in serum, to the voltammetric response ranged between 6.8%-11.9% in simple CPE, and between 0.9%-2.6% in Al₂O₃NP/CPE. Table 2 shows the effect of other interferences on the UA reply. The contribution of interfering species to the UA response was less than 5%. Since AA is the main interferent for the determination of UA in serum, interference studies were performed separately at both electrodes. According to the results given in Table 1, the use of Al₂O₃NP/CPE instead of simple CPE significantly reduced the interference effect in the quantification of UA in serum by SWV.

Sample analysis

The recovery studies of the proposed SWV method were performed by direct calibration. Voltammetric measurements were recorded by adding varying volumes of the stock solution of UA to the serum samples

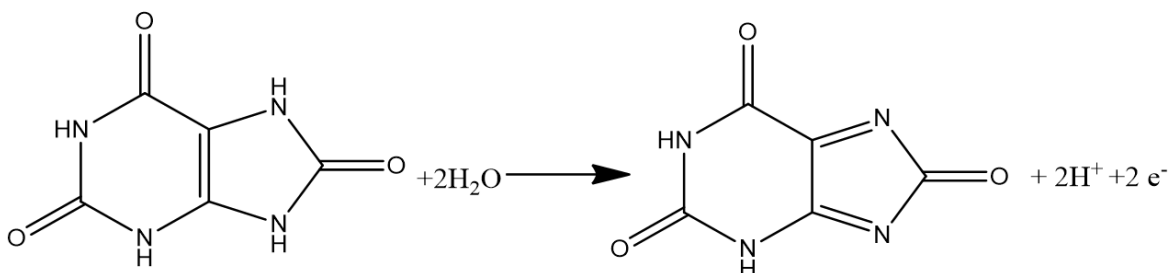


Figure 5. Oxidation mechanism of UA

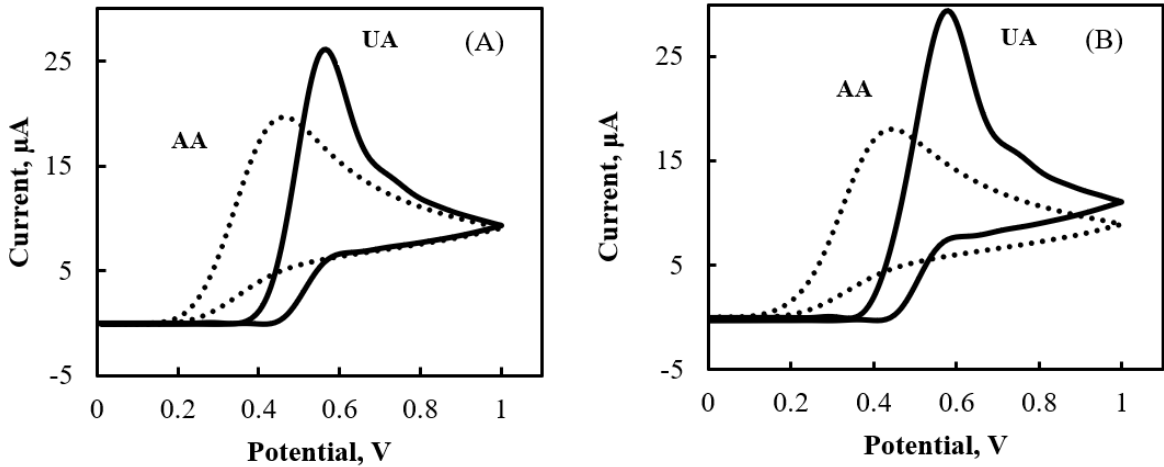


Figure 6. Cyclic voltammograms of 1.0 mM UA, and 1.0 mM AA solutions (A) in simple CPE (B) in $\text{Al}_2\text{O}_3\text{NP/CPE}$ (ν : 0.1 V s⁻¹, 0.05 M pH 5.0 PBS).

(Table 3). Table 3 shows that the recovery values obtained range from 101% to 104%. The SWV method developed for the quantification of UA in serum was found to be comparable to other methods in the literature.

DISCUSSION

In this study, the electrochemical response of UA was explored for the first time in $\text{Al}_2\text{O}_3\text{NP/CPE}$. The irreversible, and diffusion-controlled oxidation of UA by $2e^-/2H^+$ on $\text{Al}_2\text{O}_3\text{NP/CPE}$ was determined. A new method for the quantification of UA in the existence of AA was

developed, and applied to serum samples. The working range, and LOD of the suggested method were determined, and recovery values in serum were calculated. The significant advantage of the modified CPE with Al_2O_3 nanoparticles added to the electrode composition in this study is that it does not respond to species present in serum, which are thought to make significant contributions to the electrode response in UA determination. It was concluded that the proposed modified CPE for the quantification of UA showed superior properties that can compete with other reported voltammetric electrodes, and the results obtained can make important contributions to the literature.

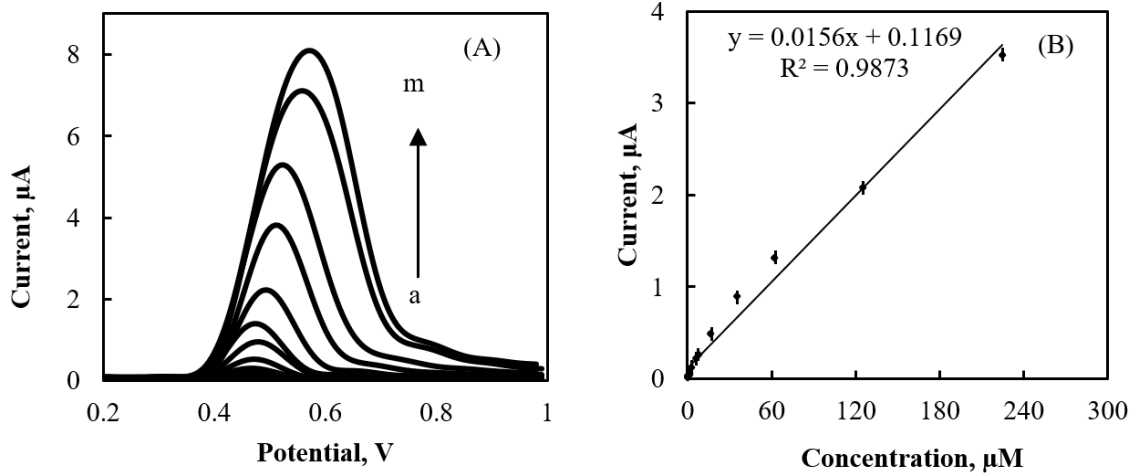


Figure 7. (A) SW voltammograms of different concentrations UA at $\text{Al}_2\text{O}_3\text{NP/CPE}$ (B) Current-concentration graph (in pH:5.0 PBS) (a) 0.1 μM , (b) 0.3 μM (c) 0.6 μM (d) 0.8 μM (e) 1.0 μM (f) 2.9 μM (g) 5.7 μM (h) 7.4 μM , (i) 17 μM (j) 35 μM (k) 63 μM (l) 125 μM , (m) 230 μM).

In this study, the analytical parameters of the proposed method for UA detection were compared with previous studies in the literature. Table 4 shows that the proposed method exhibits lower LOD values, and wider linear range than most of the previously reported studies.

ACKNOWLEDGEMENT

We are grateful for the financial support of the research project numbered NKUBAP.00.10.AR.12.12.

Table 1. Effect of AA on the response of Al₂O₃NP/CPE and simple CPE.

AA concentration	Interference, %	
	CPE	Al ₂ O ₃ NP/CPE
3.5 × 10 ⁻⁵	6.8	0.9
5.0 × 10 ⁻⁵	9.7	0.9
7.0 × 10 ⁻⁵	11.0	1.7
8.0 × 10 ⁻⁵	11.9	2.6

Table 2. Effect of Interference species on the response of Al₂O₃NP/CPE electrode.

Interfering concentration, M	Interference, %				
	Xanthine	Urea	Glucose	Glutamic acid	Oxalic acid
1.0 × 10 ⁻⁵	2.1	0.6	3.3	0.1	1.1
2.5 × 10 ⁻⁵	3.8	3.2	4.2	0.5	2.0
5.0 × 10 ⁻⁵	4.1	3.4	4.4	2.4	3.4

Table 3. Results for quantification of UA in serum samples.

spiked amount, mg/L	Found amount, mg/L	Recovery value*, %	RSD, %	t exp
41.6	40.6; 43.4; 40.9	101±3.77	3.72	0.56
16.8	17.1; 17.5; 17.8	104±1.92	1.85	3.54

*Results of recovery values are given as mean ± ts/VN (at 95% confidence level).

Table 4. Characteristics of various voltametric uric acid sensors.

Method	Working electrode	Linear range, μM	LOD, μM	Recovery serum %	Recovery urine %	Ref
SWV	ZnO-graphene/ITO	5.0 – 80	5.0	-	99.3 – 100.8	[17]
DPV	ERGO-ITO	0.3 – 100	0.3	-	101.3 – 101.5	[18]
DPASV	ZIF11 modifie GCE	20 – 540	0.5	-	94.5 – 104.5	[31]
SWV	GCE	0.04 – 2.0	0.009	-	95.0 – 106.5	[32]
SWV	MBI-Au	1.0 – 300	1.0	92.6	-	[33]
DPV	P-PPD/GCE	50 – 1600	2.5	104.5 – 105.5	-	[34]
DPV	HNP-PtTi	100 – 1000	5.7	≥105	-	[35]
SWV	Al ₂ O ₃ NP/CPE	0.1 – 230	0.1	101.0 – 104.0	-	This study

DPASV: Differential pulse anodic stripping voltammetry, ZIF: Zeolite Imidazolate Framework, CV: Cyclic voltammetry, CA: Chronoamperometry, OMC: Ordered mesoporous carbon, GCE: Glassy carbon electrode, DPV: differential pulse voltammetry, ERGO: electrochemically reduced graphene oxide, ITO: Indium tin oxide, NGr: Nitrogen doped graphen, CPE: carbon paste electrode, SWV: Square wave voltammetry, Al₂O₃NP/CPE: Aluminium oxide nanoparticles carbon paste electrode, P-PPD: para-phenylenediamine, MBI-Au: Mercaptobenzimidazole-gold, HNP: hierarchical nanoporous

References

1. T. Fukuda, H. Muguruma, H. Iwasa, T. Tanaka, A. Hiratsuka, T. Shimizu, K. Tsuji, T. Kishimoto, Electrochemical determination of uric acid in urine and serum with uricase/ carbon nanotube /carboxymethylcellulose electrode, *Anal Biochem.*, 590 (2020) 113533.
2. F. Mazzara, B. Patella, G. Aiello, A. O’Riordan, C. Torino, A. Vilasi, R. Inguanta, Electrochemical detection of uric acid and ascorbic acid using r-GO/NPs based sensors, *Electrochim. Acta.*, 388 (2021) 138652.
3. F. Wu, H. Yuming, L. Qian, Animal tissue-based chemiluminescence sensing of uric acid, *Anal Chim Acta.*, 536 (2) (2005) 107–113.
4. J. Perello, P. Sanchis, F. Grases, Determination of uric acid in urine, saliva and calcium oxalate renal calculi by high-performance liquid chromatography/mass spectrometry, *J Chromatogr B.*, 824 (2005) 175–180.
5. PD. Martinez, M.L Ferrer, M.C. Reyes, A reagent less fluorescent sol-gel biosensor for uric acid detection in biological fluid, *Anal Biochem.*, (2003) 322 238–242.
6. E. Akyilmaz, M. K. Sezgintürk, E. Dincaya, A biosensor based on urate oxidase–peroxidase coupled enzyme system for uric acid determination in urine. *Talanta.*, 61 (2003) 73–79.
7. J. Premkumar, S. B. Khoo, Electrocatalytic oxidations of biological molecules (ascorbic acid and uric acids) at highly oxidized electrodes, *J Electroanal Chem.*, 576 (2005) 105–112.
8. HR. Zare, N. Nasirizadeh, M. M. Ardakani, Electrochemical properties of a tetrabromo-p-benzoquinone modified carbon paste electrode. Application to the simultaneous determination of ascorbic acid, dopamine and uric acid, *J Electroanal Chem.*, 577 (2005) 25–33.
9. S. Tajik, H. Beitollahi, F. G. Nejad, M. Safaei, K. Zhang, Developments and applications of nanomaterial-based carbon paste electrodes, *Roy Soc Ch.*, 10(36) (2020) 21561-21581.
10. PE. Erden, B. Zeybek, Ş. Pekyardımcı, E. Kılıç, Amperometric carbon paste enzyme electrodes with Fe₃O₄ nanoparticles and 1, 4-Benzoquinone for glucose determination, *Artif Cell Nanomed B.*, 41(3) (2013) 165-171.
11. B. Çölkesen, F. Öztürk, P. E. Erden, Electroanalytical characterization of montelukast sodium and its voltammetric determination in pharmaceutical dosage form and biological fluids, *J Brazil Chem Soc.*, 27 (2016) 849-856.
12. AN. Amro, K. Emran, H. Alanazi, Voltammetric determination of itopride using carbon paste electrode modified with Gd doped TiO₂ nanotubes, *Turk J Chem.*, 44(4) (2020) 1122-1133.
13. JM. George, A. Antony, B. Mathew, Metal oxide nanoparticles in electrochemical sensing and biosensing: a review. *Microchim Acta.*, 185(7) 2018 1-26.
14. J. Jiang, Y. Li, J. Liu, X. Huang, C. Yuan, X.W.D. Lou, Recent advances in metal oxide-based electrode architecture design for electrochemical energy storage, *Adv Mat Res.*, 24(38) (2012) 5166-5180.
15. MY. Ho, P. S, Khiew, D. Isa, T. K Tan, W. S Chiu, C. H. Chia, A review of metal oxide composite electrode materials for electrochemical capacitors, *Nano.*, 9(06) (2014) 1430002.
16. R. Shashanka, G. K. Jayaprakash, B. G. Prakashaiah, M. Kumar, B. E. Kumara Swamy, Electrocatalytic determination of ascorbic acid using a green synthesised magnetite nanoflake modified carbon paste electrode by cyclic voltammetric method, *Mater Res Innovations.*, 26(4) (2022) 229-239.
17. Q. Wang, H. Yue, J. Zhang, X. Gao, H. Zhang, X. Lin, B. Wang, D. Bychano, Electrochemical determination of uric acid in the presence of ascorbic acid by hybrid of ZnO nanorods and graphene nanosheets, *Ionics.*, 24 (2018) 2499–2507.
18. MM. I. Khan, Al-M. J. Haque, K. Kim, Electrochemical determination of uric acid in the presence of ascorbic acid on electrochemically reduced graphene oxide modified electrode, *J Electroanal Chem.*, 700 (2013) 54–59.
19. RM. Berriozabal, L. S. Galicia, Gutierrez-Granados, J. Sandoval Cortes, P. Herrasti, Selective Electrochemical Determination of Uric Acid in the Presence of Ascorbic Acid Using a Carbon Paste Electrode Modified with b-Cyclodextrin, *Electroanalysis.*, 20(15) (2008) 1678-1683.
20. RS. Nicholson, I. Shain, Theory of stationary electrode polarography. Single scan and cyclic methods applied to reversible, irreversible, and kinetic systems. *Anal Chem.*, 36(4) (1964) 706-723.
21. R. Greef, R. Peat, L. M. Peter, D. Pletcher, J. Robinson, *Instrumental methods in electrochemistry.* Ellis Horwood Ltd., (1990). New York.
22. AJ. Bard, L. R. Faulkner, *Electrochemical Methods: Fundamentals and Applications.* John Wiley and Sons. Inc. (2001). New York.
23. WU. Malik, R. Jain, S. Agarwal, Redox Behavior of Some 2 benzothiazolylylhydrazones of Ethyl 2-cyanoethanoate, *P Indian AS-Phy Sci.*, 48 (1982) 85-91.
24. V. Kameswara, C. S. Venkatachalam, C. Kalidas, Electrochemical Reduction of Hydrazono Compounds Derived from Meldrum’s acid in Methanolwater Mixtures. *Indian J Chem.*, 26 (1988) 202–204.
25. IS. El-Hallag, G. B. El-Hefnawy, Y. I. Moharram, E. M. Ghoneim, Electrochemical Studies of Schiff Base Compounds Derived from Antipyrinenucleus in Ethanolic Buffer Solutions. *Can J Chem.*, 78 (2000) 1170-1177.
26. J. Wang, *Analytical electrochemistry,* (2006) New York, USA: Wiley.
27. CM. A. Brett, A. M. O. Brett, *Electrochemistry, Principles, Methods and Applications,* Oxford University Press, (1994). New York, USA.
28. A. Kedija, H. Tadele, T. Mekonen, P. Rishi, R. C. Saini, Electrochemical determination of uric acid in human urine using nickel hexa-cyano ferrate modified carbon paste electrode, *Int J Pure App Res.*, 1 (2015) 43 60.
29. M. Arvand, M. Hassannezhad, Magnetic core-shell Fe₃O₄@ SiO₂/MWCNT nanocomposite modified carbon paste electrode for amplified electrochemical sensing of uric acid, *Mater Sci Eng.*, 36, (2014) 160-167.
30. M. Hosseinian, G. Najafpour, A. Rajimpour, Amperometric urea biosensor based on immobilized urease on polypyrrole and macroporous polypyrrole modified Pt electrode, *Turk J Chem.*, 43 (2019) 1063-1074.
31. S. Tran Thanh, P. T. Qui, N. T. Thanh Tu, T. T. Tam Toan, T. T. Bich Hoa, L. V. Thanh Son, D. M. Nguyen, T. N. Tuyen, D. Q. Khieu, Electrochemical Determination of Uric Acid in Urine by Using Zeolite Imidazolate Framework-11 Modified Electrode, *J Nanomater.*, (2021). 9914062-9914075.

32. K. Shi, K. K. Shiu, Determination of Uric Acid at Electrochemically Activated Glassy Carbon Electrode, *Electroanalysis*, 13(16) (2001) 1319-1325.
33. C. Retna Raj, T. Ohsaka, Voltammetric detection of uric acid in the presence of ascorbic acid at a gold electrode modified with a self-assembled monolayer of heteroaromatic thiol, *J Electroanal Chem.*, 540 (2003) 69-77.
34. M. Mazloun-Ardakani, M. A. Sheikh-Mohseni, A. Benvidi, Electropolymerization of Thin Film Conducting Polymer and Its Application for Simultaneous Determination of Ascorbic Acid, Dopamine and Uric Acid, *Electroanalysis*, 23 (12) (2011) 2822-2831.
35. D. Zhao, G. Yu, K. Tian, C. Xu, A highly sensitive and stable electrochemical sensor for simultaneous detection towards ascorbic acid, dopamine, and uric acid based on the hierarchical nanoporous PtTi alloy, *Biosens. Bioelectron.*, 82 (2016) 119-126.