

Determination of Uric Acid in the Presence of Dopamine on the Activated Pencil Lead Electrode

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The electrochemical properties of uric acid and dopamine on the activated pencil lead electrode (APLE) have been investigated. Cyclic voltammetry was applied for activation of pencil lead electrode and differential pulse voltammetry (DPV) was considered as suitable procedure for determination of dopamine in the presence of uric acid. The effect of different parameters such as pH and composition of support electrolyte, range of potential, number of cycles has been investigated. On the APLE in optimum condition, anodic peaks of dopamine and uric acid shift to negative potentials. The peak current is linearly proportional to the bulk concentration in the range of 6.8×10^{-6} – 1.8×10^{-5} and 5.0×10^{-6} – 1.1×10^{-4} M for uric acid and dopamine, respectively. Correlation coefficient was calculated from linear regression 0.9992 for uric acid and 0.9958 for dopamine. The detection limit was 3.2×10^{-6} M for uric acid and 8.6×10^{-7} M for dopamine. The APLE was used for determination of UA in biological samples such as serum. The results obtained were compared with those of standard spectrophotometric method. No significant difference is observed at 95% confidence level between the results of two procedures. Dopamine in pharmaceutical samples was determined using standard addition procedure with APLE.

Key Words: Uric acid, Dopamine, Pencil lead electrode, Activated electrode, Differential pulse voltammetry, Electrochemical determination.

INTRODUCTION

Uric acid (UA) is a primary end product of purine metabolism¹ and abnormal levels of uric acid are symptoms of several diseases like gout, hyperuricemia and Lesch-Nyhan syndrome². Gout occurs when sodium urate crystals are deposited in the joints. Lesch-Nyhan syndrome is an X-linked chromosome disorder that results in the absence of the enzyme hypoxanthineguanosine phosphoribosyltransferase (HGPRT)². Hyperuricemia (evaluated concentrations of UA) may indicate other medical conditions such as kidney injury³.

Catechol amines are compounds that consist of amines attached to a benzene ring bearing two hydroxyl groups. As one of the catechol amines, dopamine

(3,4-dihydroxyphenethylamine) is a central neurotransmitter particularly important in the regulation of movement and possesses important intrinsic pharmacological properties. It is used for the correction of hemodynamic disorders associated with shock episode and changes in its concentration may lead to serious diseases such as Parkinson's⁴. Various methods including chromatographic, enzymatic and electrochemical techniques have been used for detection and determination of dopamine in the presence of uric acid or simultaneously⁵⁻¹⁰. Uric acid (UA) and dopamine (DA) are some of the biological compounds, which are electrochemically active. The ability to determine UA and DA has been a major goal of electroanalytical research¹¹. The electrochemical techniques received much attention, as they are more selective and less time consuming than those based on other colorimetric or spectrophotometric methods¹⁰. Hence, much research work has been done on the determination of UA or DA in the presence of some interferences such as AA^{12,13}. One of the major problems frequently encountered in the electrochemical determination of these compounds is the serious interference caused by coexisting of DA and UA. Various methods, such as an adsorption/medium exchange approach¹⁴, enzyme-based techniques¹⁵, the use of polymers modified electrodes with¹⁶ and without¹⁷ catalyst, and use of carbon paste electrode¹⁸, were developed to solve these problems. Among the electrochemical techniques, electrochemical pre-anodization of glassy carbon electrode was named electrochemical activation and electrochemical modification. The electrochemical activation usually consists of anodization about 1.8 or 1.6 V (vs. Ag/AgCl) or cycling between fixed potential values¹⁹. These electrochemical treatments are sensitive not only to the potentiostatic conditions but also to the composition of solution, nature and concentration of the electrolyte.

It has been shown that this type of treatments leads to a disruption of normal surface in which the oxygen proportions increase forming a graphitic oxide film. It is usually assumed that the surface functional groups generated during the activation process are phenolic, quinonic, carboxylic, carbonylic and epoxidic-like, all species being immobilized and covalently bonded on to the surface modified GC electrodes²⁰. Durall *et al.*²⁰ showed that electron transfer kinetics of several reactions at glassy carbon electrodes was affected by electrochemical activation. Liu *et al.*²¹ applied electrochemical pretreatment GC for detection of catechol derivative.

In this work, electrochemical activation of pencil lead electrode was used for differentiation of oxidation signals of DA and UA. The method was applied for determination of UA in biological samples such as serum and DA in pharmaceutical sample.

EXPERIMENTAL

Deionized water was used to prepare solutions. Uric acid and dopamine were purchased from Sigma and other analytical grade reagents were obtained from Merck and used without further purification. Solutions of uric acid and dopamine were prepared with double distilled water immediately prior to use. Dopamine

hydrochloride solutions were prepared and were stored in a refrigerator. Serum samples were obtained from a clinical laboratory. To fit into the linear range all the samples used for determination were diluted 100 times.

The electrochemical experiments were carried out using on Autolab electrochemical analyzer (PGSTAT20, Ecochemie, Netherlands) connected to a PC for control and data storage. A pencil lead electrode (Rotring HB, 2 mm in diameter) was used as working electrode. The counter electrode was a platinum bare electrode. All potentials reported in this paper are referenced to a calomel electrode (SCE). All experiments were performed at room temperature, without removing the dissolved oxygen.

For preparation of activated pencil lead electrode, a pencil lead was placed in phosphate buffer solution and activated by cyclic voltammetry in the potential range of 0.5–2 V with 30 cycles and scan rate of 100 mV/s. All measurements were carried out in phetalat pH = 5 buffer using differential pulse voltammetry (DPV).

RESULTS AND DISCUSSION

Fig. 1a shows differential pulse voltammograms obtained for the mixture of UA and DA at the inactive pencil lead electrode. The inactive pencil lead electrode shows a weak voltammetric signal for DA and UA. Fig. 1b shows DPV obtained for same solution as Fig. 1a at the activated PLE. As can be readily seen from Fig. 1b the activated pencil lead electrode successfully separates the voltammetric signals of both analytes. The voltammetric peaks of DA and UA are well separated with a potential difference of 160 mV, which is larger than the values reported in literature⁵. As shown in Fig. 1b the peak potentials shift to the negative and peaks current increases. The above results suggest that the rate reach of DA and UA to surface of electrode increases and this refers to the interaction of DA and UA with functional groups of the surface of electrode. As the oxidation peaks are well separated, the determination of UA in the presence of DA or the simultaneous determination of UA and DA would be possible with the activated pencil lead electrode.

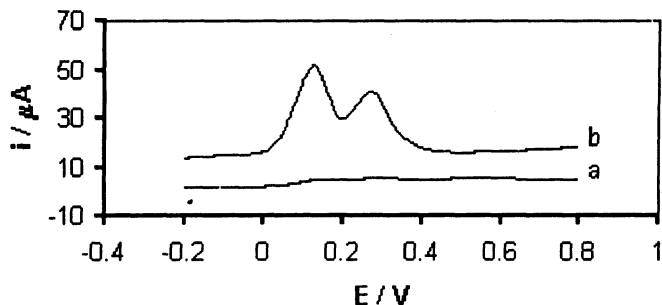


Fig. 1. Differential pulse voltammograms (DPVs) of 1×10^{-3} M UA and 5×10^{-4} M DA obtained at (a) inactive and (b) activated pencil lead electrode (APLE); activation condition: 30 cycles in the range of 1.5–2 V in phosphate buffer pH = 7.

Optimization of activation parameters

The optimum peak separation and peak currents were obtained when cyclic voltammetry method was used for activation. In cyclic voltammetric method for activation, parameters such as potential range, scan rate and number of cycles must be optimized. Optimum potential range values were obtained at the potential 0.5–2V.

Fig. 2 shows differential pulse voltammetry of 5×10^{-6} M DA and 2.5×10^{-5} M UA at the activated lead electrode for potential range 0.5–2 V with

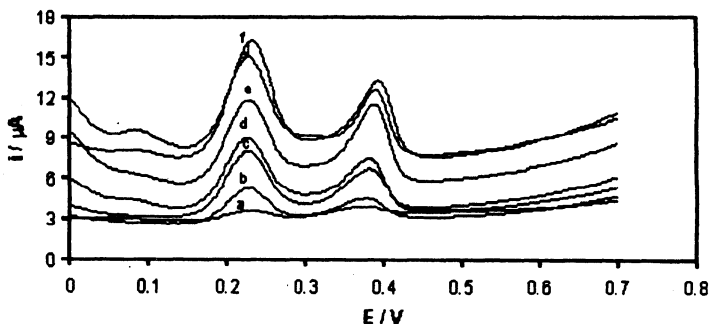


Fig. 2. Peak separation ($\Delta E_p = E_{p_{UA}} - E_{p_{DA}}$) of 2.5×10^{-5} M UA and 5×10^{-6} M DA vs. pH of supporting electrolytes at activated PLE with 30 cycles in the range of 0.5–2 V

scan numbers of 5, 10, 15, 20, 25, 30, 50. The potential separate (ΔE_p) in scan numbers of 25, 30, 50 are equal and among them scan number of 30 that has highest peak currents refers to DA and UA. So it has been chosen as the optimum scans number.

The highest peak currents have been observed at scan rate of 100 mV/s for DA and UA, therefore, this rate has been chosen as the best scan rate. Also, the effects of pH and supporting electrolyte compositions during activation were investigated. It was found that the phosphate buffer solution, pH = 7 has highest ΔE_p (Fig. 3); so it has been chosen as optimum pH for activation of electrode.

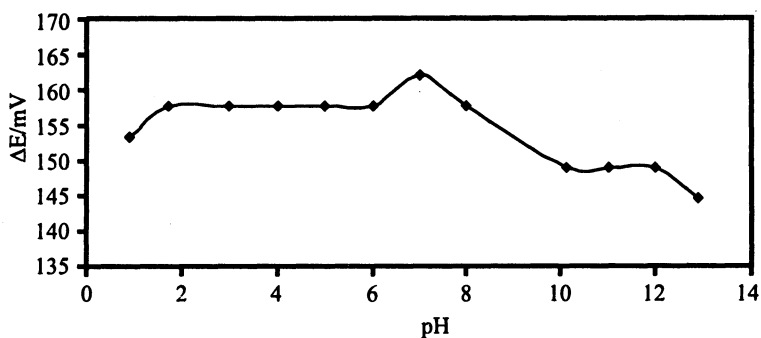


Fig. 3. Peak separation ($\Delta E_p = E_{p_{UA}} - E_{p_{DA}}$) of 2.5×10^{-5} M UA and 5×10^{-6} M DA vs. pH of supporting electrolytes at activated PLE with 30 cycles in the range of 0.5–2 V

Effect of pH on the oxidation of DA and UA

The effect of pH on the peak separation potential ($\Delta E_p = E_{pUA} - E_{pDA}$) and peak currents for the oxidation of DA and UA were investigated at an activated PLE, under optimum conditions (Fig. 4). Results are shown in Figs. 4A and 4B. It has been clear, while increasing pH, that the peak potentials slowly decrease (Fig. 4B) and the current responses of DA and UA at pH of 5, 6 reach a maximum respectively (Fig. 4A). For selection of optimum pH, DA has been given importance, because the concentration of DA in real samples is very little and must be worked on condition of the most sensitivity to DA. On the other hand, since peak separation potentials decrease slowly with increasing pH, so pH = 5 phetalat buffer is used as the optimum pH.

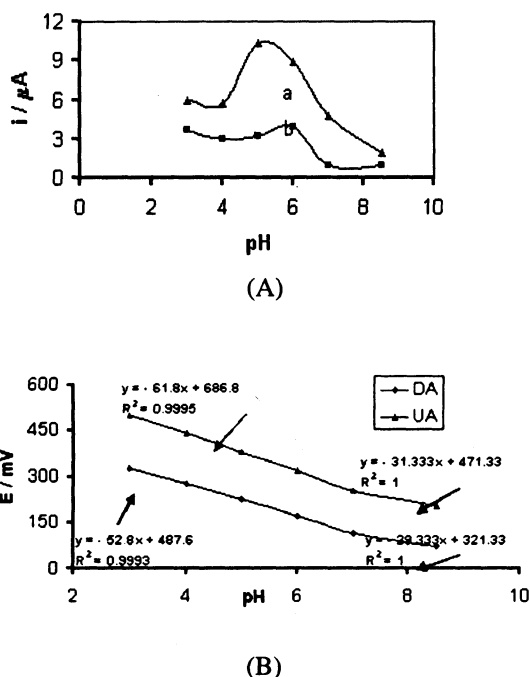
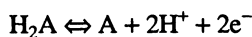


Fig. 4. Dependence of peak currents (A) and peak potentials (B) on pH for 2.5×10^{-3} M UA and 5×10^{-6} M DA at the APLE with 30 cycles in the range of 0.5–2 V

Fig. 4A shows changes of anodic peak potential of DA and UA with change of pH of analyte. As electrode potentials with electrolyte pH are changing, DA and UA can appear in different figures. Fig. 4B shows two linear areas with different slopes in the pH range of 3–8.5. According to the accessed experimental slopes, electrochemical forms of DA and UA in each area can be presented as below:

$$(a) \text{ pH} < 7 \quad \frac{dE^\circ}{dpH} = 61.8 \text{ mV, for UA,} \quad \frac{dE^\circ}{dpH} = 52.8 \text{ mV for DA}$$



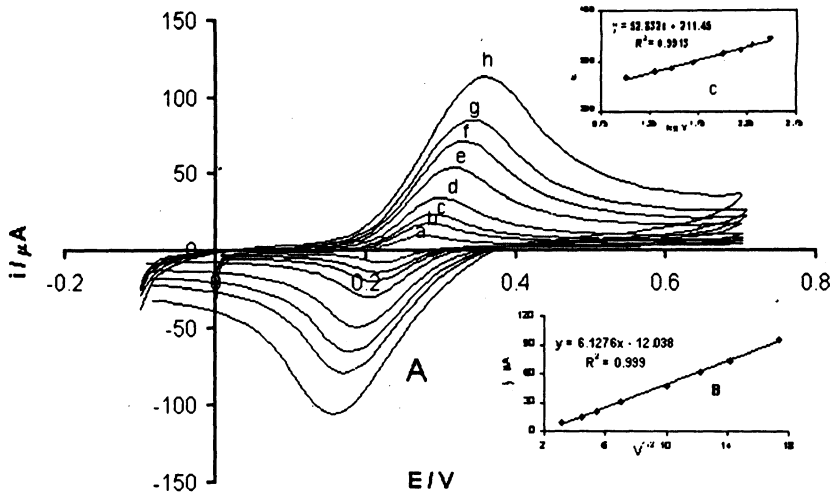
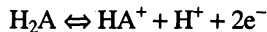


Fig. 5. Cyclic voltammograms of (A) APLE in the UA at various scan rates: (a–h) 10, 20, 30, 50, 100, 150, 200 and 300 mV/s. Insets of (B) and (C) represent the variation of anodic peak currents vs. $V^{1/2}$ and peak potential E_p vs. $\log(v)$ respectively

$$(b) \text{ pH} > 7 \quad \frac{dE^{\circ}}{dpH} = 31.3 \text{ mV}, \quad \text{for UA,} \quad \frac{dE^{\circ}}{dpH} = 29.3 \text{ mV for DA}$$



Effect of scan rate

The cyclic voltammograms APLE in the presence of 1×10^{-3} M DA and 5×10^{-4} M UA at various scan rates are shown in Figs. 5A and 6A, respectively. The peak current increases linearly with root of scan rate and anodic peak potential increases linearly with logarithm of scan rate (Figs. 5C and 6C). These observations suggest oxidation of DA and UA irreversible reaction and mass transfer is controlled by diffusion. The Tafel slope (b) can be obtained by using the following equation valid for totally irreversible diffusion-controlled process²³:

$$E_p = (b/2) \log V + \text{constant} \quad (1)$$

On the basis of equation (1), the slope of E_p vs. $\log V$ plot is $b/2$, where b indicates the Tafel slope. The plot of E_p vs. $\log V$ indicates linear variation for scan rates ranging from 10–300 mV/s (Figs. 5C and 6C). The slope is $\partial E_p / \partial \log V$, which was found to be 0.0528 V for DA and 0.0629 V for UA, respectively; so $b = 2 \times 0.0528 = 0.1056$ V for DA and $b = 2 \times 0.0629 = 0.1258$ V for UA.

Assuming the number of electrons transferred in the rate-limiting step is equal to 1, a transfer coefficient of α was estimated as 0.46 for DA and 0.53 for UA, respectively. If we assumed $n = 2$, it would then be equal to 0.73 for DA and 0.76 for UA which are not common values, because for most electrode processes a ranges between 0.3 and 0.7²⁴.

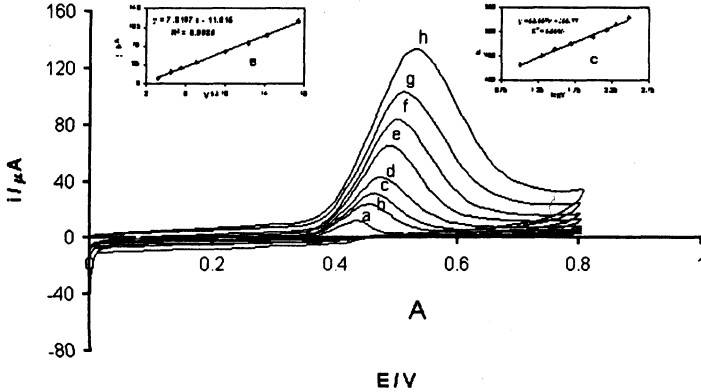


Fig. 6. Cyclic voltammograms of (A) APLE in the DA at various scan rates: (a-h) 10, 20, 30, 50, 100, 150, 200 and 300 mV/s. Insets of (B) and (C) represent the variation of anodic peak currents vs. $V^{1/2}$ and peak potential E_p vs. $\log(v)$ respectively

These results obtained from cyclic voltammetry lead to the conclusion that the overall electrochemical oxidation of DA and UA under these conditions is controlled by the diffusion of DA and UA in the solution.

Analytical applications

Determination of dopamine in the presence of uric acid: The possibility of using the activated PLE for the determination of DA was tested. Differential pulse voltammetry was adopted in experiments and oxidation peak current of UA was used as analytical signal in presence of 4.6×10^{-4} M UA (Fig. 7A). Experiments indicated that there was a linear relationship between the oxidation peak current of DA and its concentration over the range from 6.8×10^{-6} – 1.8×10^{-5} M. The detection limit, calculated from the calibration curve for DA, was 8.6×10^{-7} with correlation coefficient of 0.9958 (Fig. 7B).

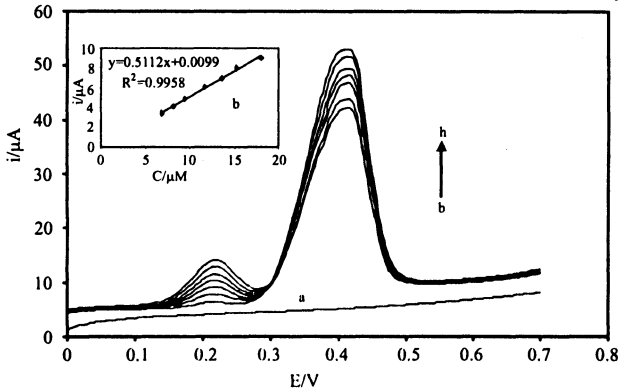


Fig. 7. Differential pulse voltammograms obtained for APLE in the absence (a) and the presence (b-i) of different concentrations of DA: (b-h) 6.81, 8.16, 9.41, 11.6, 13.6, 15.2 and 18 μ M. The initial concentration of UA is 4.6×10^{-4} M. Inset represents the variations of anodic peak currents vs. DA concentration. Supporting electrolyte: phosphate buffer, pH = 5

Determination of uric acid in the presence of dopamine: Fig. 8A shows the differential pulse voltammograms obtained for the mixture of UA and DA at activated PLE. The concentration of DA is 1×10^{-5} M and voltammograms obtained by increasing of standard solution of UA. The peak currents for UA linearly increase with the increase its concentration. The calibration plot is linear for a range of concentration. 5.0×10^{-6} – 1.1×10^{-5} M and correlation coefficient is 0.9992. The detection limit for UA in the presence of DA was found to be 3.2×10^{-6} M.

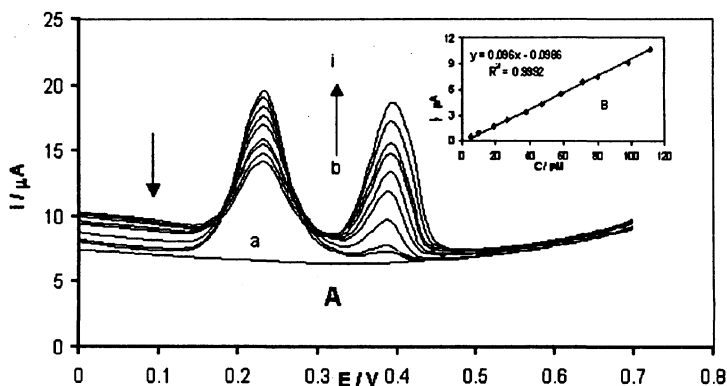


Fig. 8. Differential pulse voltammograms obtained for APLE in the absence (a) and the presence (b-h) of different concentration of UA: 5, 9.76, 26.6, 47, 58.2, 71, 80, 98.1 and 111.7 μ M. The initial concentration of DA is 10 μ M. Inset represents the variation of anodic peak currents vs. UA concentration. Supporting electrolyte: phosphate buffer, pH = 5

The stability and reproducibility of the pencil lead electrode: Well-defined peaks of DA and UA can be obtained after activating PLE and the peak currents remain the same after placing the electrode in the phetalat buffer solution for 1 h. For 10 h placing, the peak currents decrease for DA and for UA. When the electrode was placed in the air, the peak currents decreased very fast after 10 h that indicates the electrode must be placed in the buffer solution after activation and measurements. Also to characterize the reproducibility of the method, repetitive measurement-regeneration cycle was carried out in 5×10^{-6} M DA and 2.5×10^{-6} M UA. The results of 8 successive measurements showed an RSD of < 4.3% for DA and < 3.7% for UA.

Determination of dopamine in pharmaceutical sample: As an example of analytical performance of the APLE the method was applied to the analysis of an injection of DA. In order to fit into the linear range all the samples used for the determination were diluted by 10000 times. The results obtained are shown in Table-1. To ascertain the correctness of the results, the samples were spiked with certain amount of DA in about the same concentration as found in the sample. The recovery rates of the spiked samples were determined and ranged between 94.5 to 96.3% for injection sample.

TABLE-1
DETERMINATION OF DA IN INJECTION OF DOPAMINE HYDROCHLORIDE

No.	Found ($\times 10^{-6}$ M)	RSD. (%)	Added ($\times 10^{-6}$ M)	Found ($\times 10^{-6}$ M)	Recovery (%)
1	1.21		1	2.21	95.9
2	1.22	5.3	2	3.21	96.3
3	1.23		3	4.21	94.5
Average	1.22	—	—	—	95.6

Determination of uric acid in Human serum: The mentioned method was applied for determination of UA in eighteen human serum samples from clinical laboratory. To fit into the linear range all the samples used for the determination were diluted 100 times. The dilution process can actually help in reducing the matrix effect of the urine samples. The results obtained are listed in Table-2. To ascertain the correctness of the results, the samples were spiked with certain amount of UA in about the same concentration as found in the samples themselves. In order to confirm the validity of the present method further, the concentration of UA in the serum sample was tested by standard method²⁵. No significant difference is observed at 95% confidence level between the results of two procedures.

TABLE-2
DETERMINATION OF UA IN HUMAN SERUM SAMPLES WITH
TWO DIFFERENT METHODS, N = 3

Serum samples	Presented method		Standard method	
	\bar{X}^* (mg/dl)	R.S.D. (%)	\bar{X}^* (mg/dl)	R.S.D%
1.	5.34 \pm 0.091	2.66	5.41 \pm 0.25	3.84
2.	5.19 \pm 0.14	3.58	5.22 \pm 0.25	4.95
3.	5.52 \pm 0.26	5.25	6.63 \pm 0.25	4.57
4.	6.38 \pm 0.082	1.36	6.59 \pm 0.25	3.71
5.	6.23 \pm 0.39	3.12	6.34 \pm 0.25	4.15
6.	4.55 \pm 0.16	4.05	4.67 \pm 0.25	5.25
7.	2.73 \pm 0.12	0.92	2.82 \pm 0.25	4.41
8.	7.46 \pm 0.061	3.80	7.51 \pm 0.25	4.64
9.	7.33 \pm 0.13	2.11	7.79 \pm 0.25	3.78
10.	4.18 \pm 0.31	0.76	4.25 \pm 0.25	2.36
11.	5.19 \pm 0.043	4.28	6.03 \pm 0.25	3.24
12.	6.34 \pm 0.46	1.25	6.66 \pm 0.25	5.92
13.	4.61 \pm 0.28	8.85	4.87 \pm 0.25	3.55
14.	5.54 \pm 0.19	2.32	5.48 \pm 0.25	4.06
15.	4.06 \pm 0.31	3.54	4.11 \pm 0.25	5.38
16.	6.52 \pm 0.14	3.64	6.63 \pm 0.25	4.33
17.	6.11 \pm 0.054	9.65	6.26 \pm 0.25	5.25
18.	2.35 \pm 0.11	1.65	2.51 \pm 0.25	4.69

Conclusion

In summary, the experimental results reported here demonstrate the preparation of activated pencil lead electrode that can improve the electrochemical monitoring of biological analytes such as dopamine and uric acid. Optimum conditions for electrode activation using cyclic voltammetric method were found: potential windows between 0.5–2 V, phetalat buffer pH = 5 and scan number 30. The method was used for the determination of dopamine in injection samples and uric acid in serum samples.

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