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Electrochemically Activation of Pencil Graphite Electrode for Determination of Ascorbic Acid and Uric Acids

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Uric acid and ascorbic acid are very important electroactive compounds inside biological mediums such as blood and urine and their determination has significant topic. The main problem for simultaneous determination of these biological compounds is their analytical signals overlapping. In present study, the assessment of these compounds in samples is considered using electrochemically activated pencil graphite electrode. Optimum conditions to simultaneous determination of these two compounds, has taken as blow: (1) applying cyclic voltammetry method to electroactivation of pencil graphite electrode, (2) adjusting the electrolyte pH value 14 for activation of electrode to (3) applying 30 numbers of cycles to activation of electrode, (4) choosing phthalate buffer (pH = 5) to analyte determination. The obtained results show that in co-present of 9.31×10^{-4} M uric acid the anodic peak current for ascorbic acid is linear for concentration range of 2.33×10^{-5} to 3.66×10^{-3} M having the correlation coefficient of 0.9998. Detection limit for differential pulse voltammetry determination of ascorbic acid has gained 5.74×10^{-5} M.

Key Words: Ascorbic acid, Uric acid, Pencil graphite electrode, Electroactivation.

INTRODUCTION

Uric acid and ascorbic acid are important electroactive compounds inside biological mediums such as blood and urine[1-3]. Their determination has significant topic and is necessary to have a healthy life because of deviation on these compounds' value caused to various diseases[3,4]. Because of co-presenting of uric acid and ascorbic acid at major real samples, the chief problem for simultaneous determination of these biological compounds is their analytical signal overlapping. Several methods are reported to determination one of them beside another one such as electrochemical method[5], spectroscopy[6], separation technique[7], *etc.*

Flow injection analysis method (FIA) was used by Almuaibed and co-author[8] that is mixing of spectrophotometry and amperometry methods that used to simultaneous determination of these compounds. This method basically organized on uric acid absorption at the wave length of 293 nm and consequently amperometric determination of two analyt at 0.6 volt. Relative error for uric acid and ascorbic acid was achieved 3 and 5 %, respectively. Reversed phase-HPLC was used to

simultaneous determination of uric acid and ascorbic acid[9,10] using uric acid absorption assaying at the wave length of 293 nm. In this manner, detection limits gained 80 and 15 mg/mL for ascorbic acid and uric acid, respectively. Recently, it has done substantial efforts to development the voltammetry methods for determination of ascorbic acid and uric acid in biological samples[11].

Capability of simultaneous determination of ascorbic acid and uric acid in real samples is one of the electrochemically analysis research's targets. The basic concentration of ascorbic acid and uric acid in various biological samples is varied from 1×10^{-7} M to 1×10^{-3} M[12]. Both selectivity and sensitivity are the important parameters to development of voltammetry methods.

It is well known that the redox reaction of these two compounds is irreversible on bare electrodes, so high potential is required. Therefore, the direct redox reaction of these two components on bare electrodes be fall at similar potentials, that lead to lack of selectivity, moreover lead to lack of repetition responses because the adsorption of oxidation products on bare electrode surface that caused to ruin the electrode surface. To solve these problems, it has been used various recourses such as utilizing modified electrodes and activated electrodes[5,13,14].

In present study, conditions to achievement the best results is optimized by various parameters to tender the assessment of ascorbic acid and uric acid beside each other in samples using electrochemically activated pencil graphite electrode.

EXPERIMENTAL

The used materials are analytical grade and purchased from Merck Company (Germany) and shown in Table-1.

TABLE-1
THE USED MATERIALS

Usage	Chemical name
For buffer preparation	Potassium dihydrogen phthalate
For buffer preparation	Potassium dihydrogen phosphate
For buffer preparation	Dipotassium phosphate
For buffer preparation	Sodium bicarbonate
For buffer preparation	Ammonia
For buffer preparation	Hydrochloride
For buffer preparation	Sodium hydroxide
For assaying electrocatalytic property	Uric acid, Ascorbic acid
For polishing the surface of electrode	Alumina powder

All chemical materials has been used without further purification. Deionized water produced in the water treatment unit of Shahid Qazi Pharmaceutical Company (Tabriz, Iran) and was used to preparation of solutions. The ascorbic acid and uric acid solutions freshly prepared and utilized on time.

Autolab apparatus (PGSTAT20 model, Netherlands) was used in voltammetry measurements as a source of potential supply and its stabilizing. The apparatus was connected to a Pentium S computer (200MHz) equipped with a GPES software

(level 4 and 5). Metrohm pH-meter (691 models, Switzerland) was used to measure pH values.

Applied electrodes: In order to carry out the voltammetry technique, a standard three electrodes system was used to decrease the level of ohmic drop. The used cell volume is 20 mL. A pencil graphite electrode and a platinum bar electrode were used as the working and counter electrodes, respectively. A saturated calomel electrode was used as the reference electrode, as well. Both reference and counter electrodes are made by Azar Electrode Company (Tabriz, Iran).

Preparation of pencil graphite electrode: In order to obtain a constant cross sectional area of the pencil graphite electrode, the side area of the pencil is completely covered by wrapping a teflon tape on such a way that only the cross sectional area of the pencil in one end is uncovered and this side is immersed inside the solution while the other end is connected to the electrode wire. Using differential pulse voltammetry technique, the response of ascorbic acid on the surface of all the mentioned electrodes is studied.

RESULTS AND DISCUSSION

The available method for activation of pencil graphite electrode is cited by author in present study: (1) potentiostate by applying constant potentials (step potential) and (2) cyclic votametry. These methods were used to electroactivation of pencil graphite electrode. For appropriate comparison, it is required to gain information about electrochemically activation responses by each of them at optimum conditions.

Activation using potentiostate method: For electrochemically activation of electrode using potentiostate method two parameters have been considered and optimized: (a) the most appropriate pH value and (b) the most appropriate step potential value.

Optimizing the pH value: To achieve the optimum pH value to electro activation of electrode using potentiostate, the pencil graphite electrodes were immersed in solutions of : NaOH (0.01, 0.10 and 1.00 M), bicarbonate buffer (pH = 10), phosphate buffer (pH = 7), phthalate buffer (pH = 5) and HCl 0.1M and had activated with applying the 1.5 V as constant potential for 4 min. Then the response of the electrodes were compared to each other basically on their anodic peak distance of ascorbic acid and uric acid and their anodic current intensity. The results were shown in Fig. 3. Each measurements has been done in phthalate buffer (pH = 5) containing 2 mM ascorbic acid and 0.2 mM uric acid.

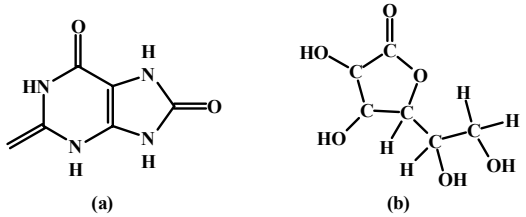

Fig. 1. Molecular structure of (a) uric acid and (b) ascorbic acid

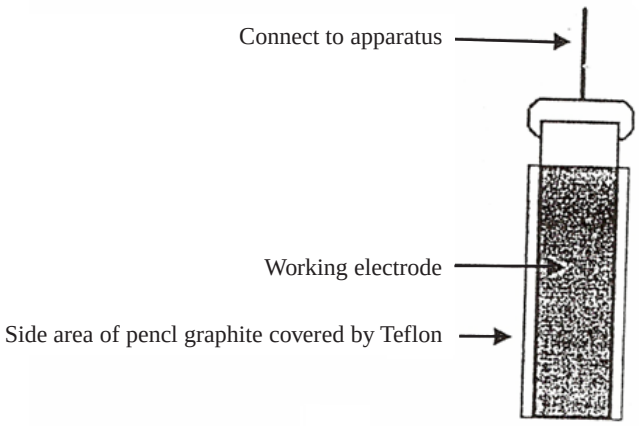

Fig. 2. Schematic structure of pencil graphite electrode

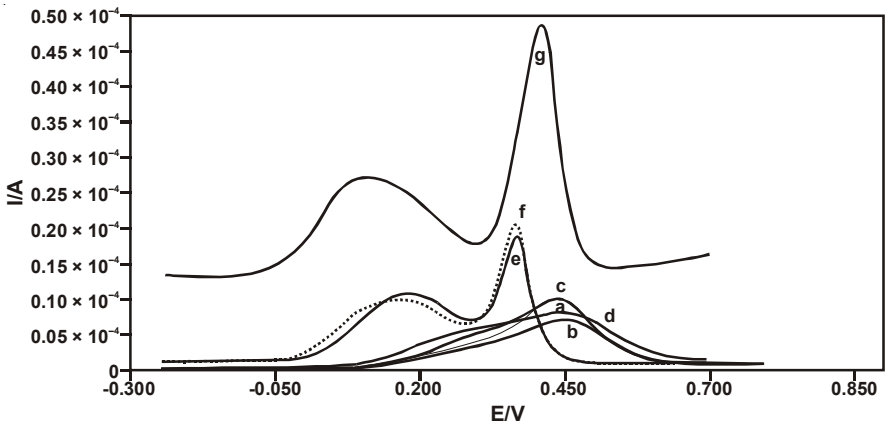

Fig. 3. Differential response related to step potential activated electrodes at various pH values in ascorbic acid and uric acid solution; (a) HCl (pH = 1), (b) phthalate buffer (pH=5), (c) phosphate buffer (pH = 7), (d) bicarbonate buffer (pH = 10), (e) NaOH (pH = 12), (f) NaOH (pH =13), (g) NaOH (pH = 14)

As shown in Fig. 3, electroactivation of electrode with applying constant potential in buffers with the pH between 1 to 10, no anodic potential peak segregation (ΔE_p) can be observed. But at pHs higher than 12, two segregated anodic potential peaks have been observed. The maximum peak distance is observed at the pH value 14. Thus, at this pH value to electroactivation of electrode caused to notable segregation of ascorbic acid and uric acid (ΔE_p = 261 mV).

Optimizing the potential step value: To achieve the best potential step value, it must be considered applied potential and the time period of application. For selection of optimum condition, it must be considered, maximum anodic potential peak segregation and maximum sensitivity of obtained electrode. So step potentials

of 1, 1.25, 1.5, 1.75 and 2 volt were applied with applying various times (1, 2, 4 and 6 min) in NaOH buffer solution. The obtained electroactivated electrode in each case was immersed in solution containing 2 mM ascorbic acid and 0.2 mM uric acid. The DPV responses due to each electrode has shown in Table-2.

TABLE-2
DIFFERENTIAL PULSE VOLTAMMETRY RESPONSES DUE TO ELECTRODE ACTIVATION AT VARIOUS POTENTIAL STEPS AND VARIOUS POTENTIAL APPLYING TIMES

Potential step values	Applying time	ΔE/mV	i (Ascorbic acid)/μA	i (Uric acid)/μA
1.25	1	–	–	7.86
	2	–	–	8.45
	4	156	0.80	9.67
	6	236	1.52	15.50
1.50	1	221	3.73	13.70
	2	241	3.69	13.90
	4	311	11.50	32.60
	6	216	0.27	19.20
1.75	1	236	3.40	18.80
	2	261	4.18	24.10
	4	256	1.22	21.80
	6	302	2.46	17.90
2.00	1	251	6.18	23.00
	2	266	4.78	20.30
	4	282	4.14	16.10
	6	252	2.98	7.90

The electroactivation of pencil graphite electrode with applying 1.5 volt as step potential value for 4 min, caused to achieve maximum anodic potential peak segregation (ΔE_p = 313 mV) and maximum sensitivity to ascorbic acid and uric acid (Table-2).

Activation of electrode using cyclic voltammetry program: For electrochemical activation of electrode using cyclic voltammetry method, optimization of number of cycles is one of the most important parameters.

To check the numbers of cycles effect on electrode activation, various cycles was used to activation of electrode at fixed situations such as constant concentration of ascorbic acid and uric acid, constant scanning rate and the same range of potentials. The obtained activated electrode's behaviour was studied in each case. Fig. 4 shows anodic potential peak segregation (ΔE_p) of ascorbic acid and uric acid than the number of cycles used to activation. It is obvious in Fig. 4 that up to 30 cycles, increasing in the number of cycles, induces to increasing the anodic potential peak segregation (ΔE_p); after this point (30) it will be fixed up to 100 cycles. For more than 100 cycles, it is not possible to determining ΔE_p, because of disappearing ascorbic acid anodic peak current.

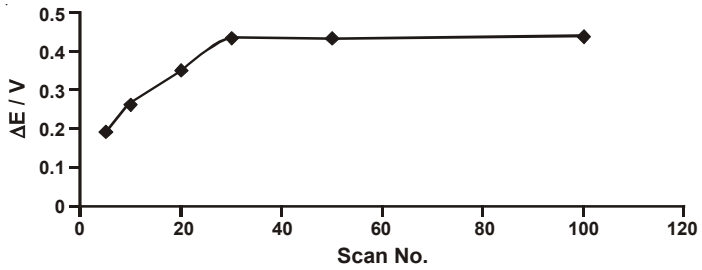

Fig. 4. Number of cyclic voltammetry's effect at activation stage on responsibility of obtained electrode to segregation of ascorbic acid and uric acid anodic potential peak

Another important parameter to cyclic voltammetry activation is the range of used potential that must be optimized. The range of used potential is reported in literatures is in the space of 2600 mV (-0.6 to 2 volt)[15]. For this purpose 2 different experiments was carried out. In one experiment, the initial potential impounded at fixed value but the final potential moved from 1.5 to 2.2 volt. Diverse final potential to activation of electrode induces different responsibility of obtained electrode as shown in Fig. 5. It can be resulted from this figure that the value of 2.2 volt can be selected as final potential to cyclic voltammetric activation of electrode because of its most appropriate responsibility.

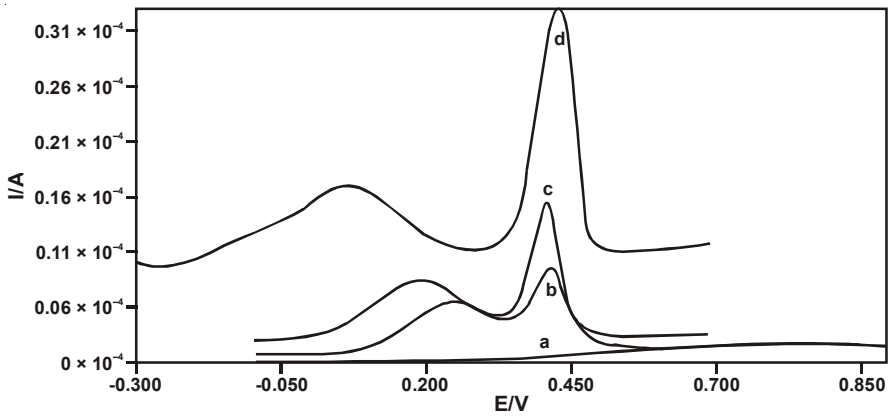

Fig. 5. Differential pulse voltammetry responsibility related to cyclic voltammetry activated electrodes at various final potentials in solution of 2 mM ascorbic acid and 0.2 mM uric acid; (a) 1.5 (b) 1.8 (c) 2 and (d) 2.2 volt with 100 mV/s scanning rate. The initial potential was fixed at -0.6 volt and 10 cycles were used number of used cycle: 30

Experimental studies show that the initial potential for cyclic activation of electrode can effect on electrode response. Similar experiment was carried out to achieving the most appropriate initial potential to cyclic voltammetry activation of electrode.

It has done by fixing final potential at 2.2 volt. As shown in Fig. 6, applying the value 1.5 volt as initial potential to cyclic voltammetry activation of electrode cause to maximum segregation of ascorbic acid and uric acid anodic peak potential (ΔE_p = 0.26 V).

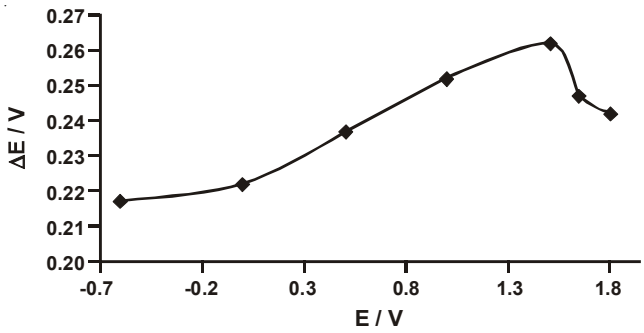

Fig. 6. Condition of ΔE_p at various initial potential

As a result the potential range of 1.5-2.2 V as initial and final potentials respectively, was selected as optimized range to cyclic voltammetry activation of electrode.

Comparison of potentiostate and cyclic voltammetry to activation of electrode: Optimized conditions for step potential activation of electrode resulted more segregation of ascorbic acid and uric acid potential peak (ΔE_p = 313 mV) than cyclic voltammetry activation of electrode (ΔE_p = 0.26 V). At first glance it seems that applying step potential to activation of electrode is more appropriate than cyclic voltammetry. But in spite of this matter, the first manner is not applicable than second because calibration curve for simultaneous determination of ascorbic acid and uric acid are not good straight on electrode obtained from step potential activation as shown in Fig. 7. This topic can be due to instability of active groups produced on surface of electrode[1]. As a result, cyclic voltammetry method was selected to activation of pencil graphite electrode because of its calibration curve has suitable straight line.

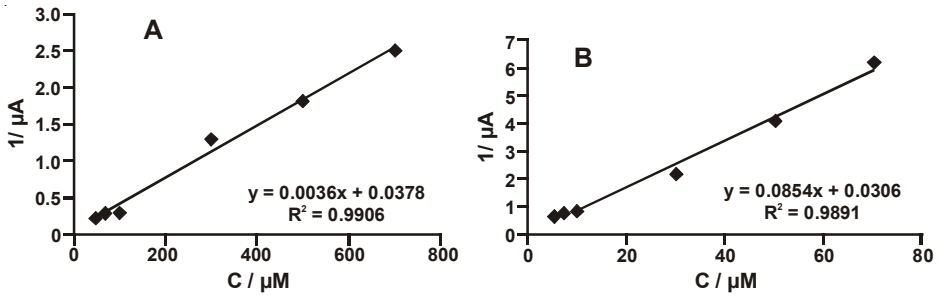

Fig. 7. Standard's line related to differential pulse voltammetry responses of obtained electrode from step potential activation; (A) ascorbic acid and (B) uric acid

Optimization of buffer pH contained ascorbic acid and uric acid: To check the pH effect in analyt solution on response of electrode, the electrode obtained at optimized condition (as mentioned in section 3-3) was immersed in solutions containing 2 mM ascorbic acid and 0.2 mM uric acid at various buffer pH values: 3, 5, 7, 8 and 10.

For this purpose it was studied anodic current peak vicissitudes and also the amount of ΔE_p than commutation of pH.

Fig. 8 shows differential pulse voltammetry responses of activated pencil graphite electrode in different pHs of buffer solution containing 2 mM ascorbic acid and 0.2 mM uric acid.

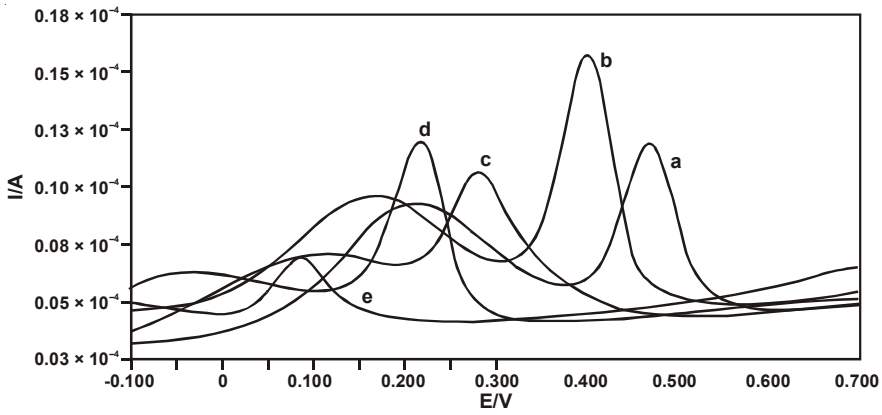

Fig. 8. Differential pulse voltammetry responses of activated pencil graphite electrode in solution contained 2 mM ascorbic acid and 0.2 mM uric acid with different pHs of buffer; (a) phthalate (pH = 3), (b) phthalate (pH = 5), (c) phosphate (pH = 7), (d) phosphate (pH = 8) and (e) bicarbonate (pH = 10)

As shown in Fig. 8, increasing in pH value of analyt solution induces shifting of peak potential for both ascorbic acid and uric acid to less positive values. It is also shown in this figure that, at pH over 10, uric acid peak current be eliminated that means electrode's insensitivity than uric acid. The best function of activated electrode's response observed in phthalate buffer (pH = 5).

Ascorbic acid detection limit beside uric acid: As mentioned before, it is not possible to determining ascorbic acid and uric acid in real samples on plane electrode and inactivated electrode because of overlapping the peak currents. Activation of pencil graphite electrode was carried out to solving this problem. It is portrayed calibration curve for activated electrode to achieving the range of concentration that current condition is linear. For this purpose it was prepared some solutions containing different concentration of ascorbic acid in the range of 23 to 100 μM but fixed concentration of uric acid (9.31×10^{-4} M). Using differential pulse voltammetry, the currents was determined. It is obvious in Fig. 9 that in presence of fixed concentration of uric acid, the anodic peak current for ascorbic acid is linear for the concentration range of 2.33×10^{-5} to 3.66×10^{-3} M.

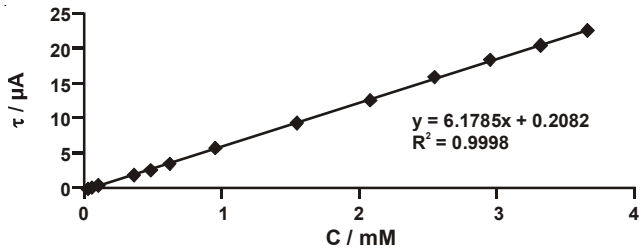

Fig. 9. Calibration curve related to differential pulse voltammetry responses of various ascorbic acid concentrations beside fixed concentration of uric acid

$$Y = 0.0062x - 2\times10^{-7}$$

$$\sum(Y_i - \dot{Y}_i)^2 = 2.25\times10^{-13}$$

$$S_{Y/X} = \left(\frac{\sum_i (Y_i - \hat{Y}_i)^2}{n-2}\right)^{1/2} = 1.19\times10^{-7}$$

$$Y_{LOD} = 3S_{Y/X} + a = 1.56\times10^{-7}$$

$$X_{LOD} = 5.74\times10^{-5}$$

Using some easy statically calculations as shown below detection limit for ascorbic acid in presence of uric acid was determined 5.74×10^{-5} M.

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