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Cu/PDA/CCE as a Sensitive Electrode for Concurrent Determination of Ascorbic Acid and Folic Acid

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Abstract
Herein, the electrooxidation of ascorbic acid and folic acid, as two essential vitamins, on the surface of the carbon ceramic electrode modified by polydopamine and copper (Cu/PDA/CCE) was investigated. Poly dopamine was fabricated by applying electro deposition conditions. Initial electrochemical characteristics were performed to study the behavior of the fabricated electrode for simultaneous detection of two biomolecules. From voltammetric studies using the developed electrode, two separated anodic peaks for folic acid and ascorbic acid were found promisingly for concurrent detection of the compounds. Linear calibration diagrams were obtained in the range of 0.5 to 360 μM and 0.83 to 380 μM with detection limits of about 0.031 and 0.057 μM for folic acid and ascorbic acid, respectively. The developed electrode was applied in human urine sample analysis with satisfying results.

Keywords
Folic Acid; Ascorbic Acid; Carbon Ceramic; Concurrent Determination; Polydopamine.

1. INTRODUCTION

Folic acid is known as a type of a water-soluble vitamin B produced by microorganisms and plants. It plays an important role in production of protein and nucleic acids in the body and also prevents disorders in the neural tube of the fetus [1]. Also, folic acid is very important for the preservation and health of living organisms and the growth of cell division. It converts to the bioactive form of tetrahydrofolate by the enzyme tetrahydrofolate dehydrogenase in the intestine. On the other hand, the studies show that a decrease in folate concentration is related to the cancer due to the participation of folic acid with vitamin B12 in the synthesis of nucleotides. This vitamin does not accumulate in the body and is excreted in the urine. As it should be in the daily diet to avoid from anemia, psychosis and heart attack, folic acid detection in body fluids is of great clinical importance [2-4]. Various techniques have been reported for determination of folic acid in pharmaceutical and biological samples, such as: flow injection luminescence [5], photochemical fluorimetry [6], ion pair chromatography [7], capillary electrophoresis [8] and high-performance liquid chromatography (HPLC) [9,10].

The above methods have disadvantages such as high cost, time consuming performance, complicated sample preparation, low sensitivity and selectivity. It seems that a suitable, high-sensitivity, simple, and efficient method for measuring this drug is a challenge and electrochemical methods associated with modified electrodes are powerful alternatives to these methods [11-13].

Ascorbic acid as a natural water-soluble vitamin with antioxidant properties known as L-hexuronic acid. Two chemicals are considered for ascorbic acid, D-ascorbic acid and L-ascorbic acid, the type D of which does not exist in nature and is made artificially; But its L type is found naturally and has antioxidant properties in enzymatic reactions.

Many mammals are able to make L-ascorbic acid while the human body cannot synthesize it and getting it in its daily diet in necessary [14,15]. The role of ascorbic acid as an antioxidant, antihistamine, pre-oxidant, muscle growth and etc have been revealed for many years ago. Ascorbic acid has been detected by various methods such as HPLC [16,17], UV-Vis spectrophotometry [18] and electrochemical techniques [19-23].

Carbon ceramic electrodes (CCEs) were firstly introduced by Leo in 1994 [24]. These electrodes are formed by dispersing graphite powder in modified/unmodified silicate matrices and are made by mixing an appropriate amount of graphite powder with a sol-gel precursor followed by drying in the porous matrix form with the special properties. High electrical conductivity and refreshment capability are the main advantages of CCEs [25-28].

In the present work, a modified carbon ceramic electrode was fabricated and modified with polydopamine and copper and used to simultaneous determination of ascorbic acid and folic acid. The simple low-cost developed

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electrochemical sensor was applied successfully to detect two essential vitamins in real samples. As the role of the both ascorbic acid and folic acid is essential in metabolism, simultaneous determination of the mentioned vitamins should be noteworthy.

2. EXPERIMENTAL

2.1. Chemicals and Instrumentals

Highly pure graphite powder and methyl trimethoxy silane (MTMOS) were prepared from Fluka.

Dopamine hydrochloride and methanol were purchased from Merck. Phosphate buffer saline (PBS) was prepared and the desired pH was adjusted by adding 0.1 M of NaOH to the 0.1 M of phosphoric acid.

Metrohm Computrace Voltammetric Analyzer model 797 equipped with a three-electrode setup: a 3mm diameter carbon ceramic electrode (CCE) and a platinum wire and a silver- silver chloride electrode were used as the working, auxiliary and reference electrodes, respectively.

2.2 Carbon ceramic electrode fabrication

0.5 ml of methyl methoxy silane was mixed with 0.8 ml of methanol 80% and transferred into a clean and dry test tube. Then 0.08 ml of hydrochloric acid (11M) was added and sonicated for 10 min. After that,1.2 grams of graphite powder were added and sonicated again for 10 min to reach a homogeneous suspension.

The resulting homogeneous paste was pressed into a 5 cm glass tube with 3 mm inner diameter and a copper wire passed to the material to establish the electrical connection. Then, the assembled device was dried to make a carbon ceramic electrode. Finally, The fabricated electrode was polished with alumina powder and washed twice with double distilled water.

2.3. Modification of the carbon ceramic electrode with polydopamine and copper

After polishing the as-prepared carbon ceramic electrode (CCE), it was inserted in an electrochemical cell containing a solution of 5 mM of dopamine and acetate buffer (pH=5) and 10 cycles with potential scan rate equal to 0.05V/s were applied through cyclic voltammetry. Consequently, the surface of the carbon ceramic electrode was modified with polydopamine (PDA). In the following, the electrode was inserted in an electrochemical cell containing 1 mM and 10 mM of copper chloride and potassium chloride, respectively and continued by applying 10 cycles in the range of -0.7 to +0.3 V till modification of the electrode with copper was completed (Fig. 1). By this way, the Cu/PDA/CCE electrode was prepared for future experiments.

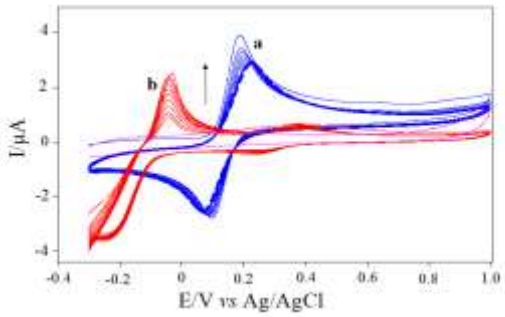

Fig. 1. 10 Cyclic voltammograms of bare CCE in dopamine and acetate buffer solution (a), and CCE/PDA in 1 mM and 10mM of copper chloride and potassium chloride respectively.

3. RESULT AND DISCUSSION

3.1. Study of electrochemical behavior of dopamine on the surface of unmodified CCE and Cu/PDA/CCE

Fig. 2 shows cyclic voltammograms of unmodified carbon ceramic electrode in phosphate buffer solution (pH = 7) and modified carbon ceramic electrode (Cu/PDA/CCE) at the 150 mVs^{-1} potential scan rate, in the presence and absence of ascorbic acid and folic acid. No peak was observed for the unmodified carbon ceramic electrode in absence of the analytes (Fig. 2a). For unmodified carbon ceramic electrode in the presence of 350 μM of ascorbic acid and folic acid, just a weak peak was observed (Fig. 2b). The modified carbon ceramic electrode, Cu/PDA/CCE, inserted in the same solution, showed two separated peaks related to the ascorbic acid and folic acid at the potentials of 0.15 and 0.7 V, respectively (Fig. 2c).

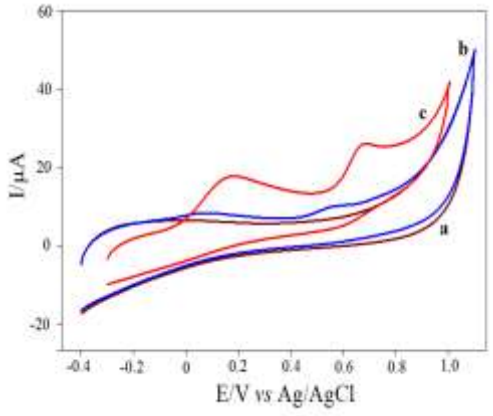

Fig. 2. Cyclic voltammograms of: unmodified carbon ceramic electrode (a), unmodified carbon ceramic electrode in the presence of ascorbic acid and folic acid (350 μM) (b); The Cu/PDA/CCE in the presence of ascorbic acid and folic acid (350 μM) (c).

3.2. Effect of pH

Since the electrochemical behavior of these biomolecules is dependent on pH, the CV responses were anticipated in phosphate buffer solutions (pHs of 4, 5, 7 and 9) through Cu/PDA/CCE. For this purpose, buffered solutions containing ascorbic acid and folic acid at a known concentration (350 µM) were used. According to the recorded voltammograms (Fig. 3), pH = 7 was selected as the optimum pH.

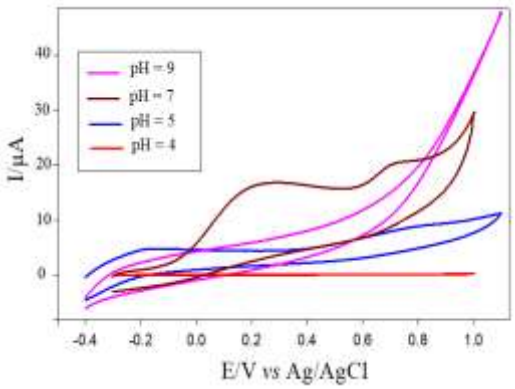

Fig. 3. Effects of solution pH on the peak currents of ascorbic acid and folic acid (350 µM) at the Cu/PDA/CCE

3.3. Effect of potential scan rate

Cyclic voltammograms shown in fig. 4A are related to a solution of 80 µM of folic acid dissolved in phosphate buffer solution (pH=7) at the potential scan rates of 10 to 100 mV/s, which confirms that the increases in anodic peak current with increase in potential scan rates. The plot of anodic peak current ($I_{p,a}$) versus root of the scan rates ($v^{1/2}$) confirms the diffusion process of folic acid on the surface of the Cu/PDA/CCE**.**

As well, cyclic voltammograms of 100 µM of ascorbic acid buffered solution (pH = 7) in the range of potential scan rates (10 to 100 mV/s) are shown in Fig. 4B. Herein, the anodic peak current increases with increasing potential scan rate. The plot obtained from $I_{p,a}$ versus $v^{1/2}$ confirms the diffusion manner of the folic acid on the surface of the Cu/PDA/CCE too.

3.4. Simultaneous determination of folic acid and ascorbic acid by Cu/PDA/CCE

Quantitative analysis of concurrent detection of two vitamins by Cu/PDA/CCE was performed by differential pulse voltammetry (DPV) in phosphate buffer solution (pH=7). The obtained DPVs at the concentrations of 0.5, 60, 130, 200, 250, 300, 360 µM of folic acid in the presence of a constant concentration of ascorbic acid (230 µM) are shown in Fig. 5A Accordingly, with increasing folic acid concentration, a linear range was observed in the range of 0.5-360µM with the significant correlation coefficient (r^2 = 0.9997).

On the other hand, to measure the concentration range of ascorbic acid in the presence of a constant amount of folic acid, DPVs in Fig. 5B of the Cu/PDA/CCE were obtained in the concentrations of 0.83, 70, 150, 200, 250, 300, 340 and 380 µM of ascorbic acid in the presence of a constant concentration of folic acid (250 µM). As it is shown, with increasing ascorbic acid concentration, linear range was observed in the range of 0.83-380 µM with the excellent correlation coefficient (r^2 = 0.9992).

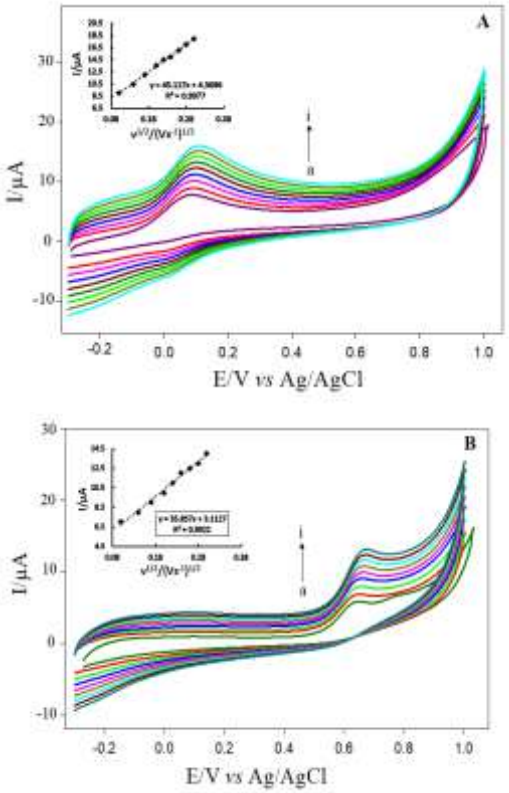

Fig. 4. Cyclic voltammograms of the folic acid (80 µM) (A) and ascorbic acid (100 µM) (B) at the Cu/PDA/CCE at different scan rates: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 mV s^{-1} (a to i) in 0.1 M phosphate buffer solution (pH 7).

The range of simultaneous changes in ascorbic acid and folic acid concentrations was recorded by DPV in the concentration ranges of 0.5-360 µM and 0.83-380 µM for folic acid and ascorbic acid, respectively. Fig. 5C shows that peak anodic current increases with increasing concentrations of ascorbic acid and folic acid and also a linear relationship was observed at the used concentration ranges.

The limit of detection (L.O.D) indicates the concentration of the analyte that produces a signal

three times greater than the background signal. Accordingly, the L.O.D was obtained using the following equation: $DL=3S_b/m$

In this regard, S_b is the standard deviation of blank response (µA) for 7 detections and *m* is the slope of the calibration curve. The L.O.D of the method was calculated about 0.031 µM and 0.057 µM for folic acid and ascorbic acid respectively by Cu/PDA/CCE.

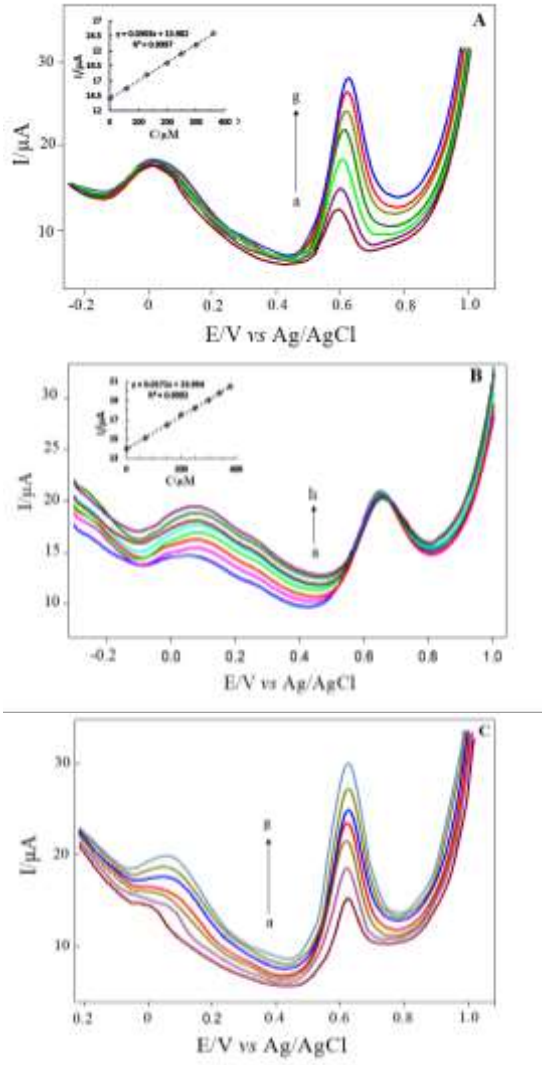

Fig. 5. Differential pulse voltammograms of 230 µM ascorbic acid with different concentrations of folic acid (a to g): 0.5, 60, 130, 200, 250, 300 and 360 µM (A) and differential pulse voltammograms of 250 µM folic acid with different concentrations of ascorbic acid (a to h): 0.83, 70, 150, 200, 250, 300, 340 and 380 µM ascorbic acid (B), simultaneous changes in ascorbic acid and folic acid concentrations (C), at the Cu/ PDA/CCE.

Undoubtedly, one of the important advantages of method is the reproducibility during continuous measurements which is expressed in terms of relative standard deviation (RSD%). For calculating the mentioned parameter, a solution of two analytes with a concentration equal to 150 µM was detected during seven times differential pulse voltammetry and the difference in the peak anodic current of the analytes were determined and the relative standard deviations were estimated using below equation:

$RSD\%=(S/X) \times 100$

Based on results, the RSD% was estimated equal to 2.1% and 1.8% for folic acid and ascorbic acid, respectively. Overall, the results showed that the electrode has good repeatability for simultaneous measurement of folic acid and ascorbic acid.

The stability of Cu/PDA/CCE for 150 µM of folic acid and ascorbic acid in phosphate buffer (pH = 7) was evaluated after 20 consecutive repetitions. The reduction in response of the electrode for two analytes was estimated to be less than 5%. Hence, suitable stability was evaluated for Cu/PDA/CCE.

3.5. Real sample analysis and comparison with other modified electrodes

The fabricated Cu/PDA/CCE was examined to determine folic acid and ascorbic acid in human urine samples. Table 1 shows the outcome results. The known concentrations of folic acid and ascorbic acid were added to the buffered samples after several dilutions. The recovery percentages for folic acid and ascorbic acid were calculated between 98 to 102%. Totally, the results promisingly confirmed the usefulness of the developed Cu/PDA/CCE for determination of folic acid and ascorbic acid in real samples.

Also, the results obtained by the fabricated electrode were compared with other electrochemical methods reported for the determination of folic acid and ascorbic acid. According to table 2, the performance of the PDA/Cu/CCE seem to be considerable in comparison to other similar works.

Table 1. Determination of folic acid and ascorbic acid in human urine sample by Cu/PDA/CCE.

sample	Added(µM)		found(µM)		RSD		%Recovery	
	folic acid	ascorbic acid	folic acid	ascorbic acid	folic acid	ascorbic acid	folic acid	Ascorbic acid
Urine	0	0	0.26	2.5	------	-------	-------	-------
	10	30	10.43	32.1	±1.9	±2.1	101.66	98.2
	20	40	20.48	43.11	±2.1	±1.8	101.08	101.43
	30	50	29.9	52.1	±2.4	±2.2	98.8	99.2

Table 2. Comparison of the recently reported results with the present work.

Modified electrode	L.O. D	Concentration range	Ref.
CPE-PANI/TPA	3×10^{-6} M (FA)	2×10^{-6}-2.1×10^{-3} M	[11]
Modified multiwall carbon nanotubes paste electrode	1.1×10^{-6} M (FA)	4.6×10^{-6} -1.52×10^{-4} M	[12]
Carbon Paste Electrode Modified by Nickel Ions Dispersed into Poly(*o*-anisidine) Film	0.091mM (FA)	0.1-5 mM	[13]
Graphite zeolite-modified electrode (Cu^{2+}A/ZCME)	2.76×10^{-7}M (AA)	0.003-6 mM	[21]
AsOx/c-MWCNT/PANI/Au electrode	0.9 μM (AA)	2-206 μM	[22]
poly(bromocresol purple) film modified glassy carbo electrode(poly(BCP)/GCE)	6.5×10^{-6}M (AA)	2×10^{-5}-7×10^{-4} M	[23]
Cu/PDA/CCE	0.031μM (FA) 0.057 μM (AA)	0.5-360 μM 0.83-380 μM	Present work

4. CONCLUSION

The biomolecules, folic acid and ascorbic acid, as important vitamins play principal roles in many activities of the human body. Increasing or decreasing in the concentration of these two molecules can cause defects in the human body and consequently concurrent detection of the mentioned vitamins are necessary for metabolism aspects. The optimal conditions for detection of two compounds by the Cu/PDA/CCE were obtained. Simultaneous measurement of two types of electroactive ascorbic acid and folic acid with low detection limits by the fabricated electrode as a simple, stable and low-cost electrochemical sensor are the main characteristic of the developed sensor.

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الکترود کربن سرامیک اصلاح شده با پلی دوپامین و مس به عنوان الکترودی حساس برای تعیین همزمان آسکوربیک اسید و فولیک اسید

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درکار حاضر، الکترو اکسیداسیون اسید اسکوربیک و اسید فولیک، به عنوان دو ویتامین ضروری، در سطح الکترود کربن سرامیک اصلاح شده توسط پلی دوپامین و مس (Cu/PDA/CCE) بررسی شد. الکترود اصلاح شده با پلی دوپامین با استفاده از شرایط رسوبگذاری الکتروشیمیایی تهیه گردید. در مرحله بعد الکترود حاصل با مس اصلاح شد. مشخصه یابی مقدماتی الکتروشیمیایی برای مطالعه رفتار الکترود اصلاح شده برای اندازه گیری همزمان اسید اسکوربیک و اسید فولیک انجام شد. با مطالعات ولتامتری الکترود اصلاح شده، دو پیک آندی مجزا برای اندازه گیری همزمان اسید فولیک و اسید اسکوربیک حاصل شد. از رسم نمودارهای کالیبراسیون محدوده های خطی به ترتیب در دامنه ۰,۵ تا ۳۶۰ میکرومولار و ۰,۸۳ تا ۳۸۰ میکرومولار با حد تشخیص های به ترتیب ۰,۰۳۱ و ۰,۰۵۷ میکرومولاربرای اسیدفولیک و اسید اسکوربیک بدست آمد. از کاربرد الکترواصلاح شده نتایج رضایت بخشی در آنالیزنمونه ادرار حاصل شد.

واژه‌های کلیدی

فولیک اسید؛ آسکوربیک اسید؛ کربن سرامیک؛ اندازه گیری همزمان؛ پلی دوپامین.