



Electrochemical detection of carbidopa using a ferrocene-modified carbon nanotube paste electrode

HALIMEH YAGHOUBIAN¹, HASSAN KARIMI-MALEH^{2*},
MOHAMMAD ALI KHALILZADEH² and FATEMEH KARIMI²

¹Islamic Azad University, Branch of Bam, Bam and ²Department of Chemistry,
Islamic Azad University, Qaemshahr, Iran

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Abstract: A chemically modified carbon paste electrode (MCPE) containing ferrocene (FC) and carbon nanotubes (CNT) was constructed. The electrochemical behavior and stability of the MCPE were investigated by cyclic voltammetry. The electrocatalytic activity of the MCPE was investigated and it showed good characteristics for the oxidation of carbidopa (CD) in phosphate buffer solution (PBS). A linear concentration range of 5 to 600 μM CD, with a detection limit of $3.6 \pm 0.17 \mu\text{M}$ CD, was obtained. The diffusion coefficient of CD and the transfer coefficient (α) were also determined. The MCPE showed good reproducibility, remarkable long-term stability and especially good surface renewability by simple mechanical polishing. The results showed that this electrode could be used as an electrochemical sensor for the determination of CD in real samples, such as urine samples.

Keywords: carbidopa; ferrocene; carbon nanotube; modified electrode; voltammetry.

INTRODUCTION

Carbon nanotubes (CNTs) are new kinds of porous nanostructured carbon materials, which are promising as immobilization substances because of their significant mechanical strength, excellent electrical conductivity, high surface area and good chemical stability.¹ CNTs can be used to promote electron transfer reactions when used as electrode materials in electrochemical devices, in electrocatalysis and electroanalysis processes due to their significant mechanical strength, high electrical conductivity, high surface area, good chemical stability, as well as relative chemical inertness in most electrolyte solutions and a wide operation potential window.^{2–5} Both redox mediators and CNTs exhibited excellent electrochemical performance for the fabrication of sensors or biosensors. Synergistic ef-

* Corresponding author. E-mail: h.karimi.maleh@gmail.com

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fects in the enhanced current response were observed when both CNTs and redox mediators were employed.^{6,7}

Parkinson's disease victims show a significant depletion of dopamine in the brain. Since this neurotransmitter can not cross the blood–brain barrier into the central nervous system and it can not be employed to restore its normal level, levodopa (LD) (a precursor of dopamine) has been successfully used and is the most widely prescribed drug for the treatment of such patients.⁸ After its administration, LD is converted into dopamine *via* an enzymatic reaction catalyzed by dopa-decarboxylase.

However, since the metabolism of LD is also extra cerebral, several side effects of systemic dopamine can arise if LD is administered in high dosages. In order to achieve better a therapeutic effect and lower toxicity, CD is administered in association with LD in pharmaceutical preparations, which contain 10–25 % CD. This catecholamine acts as an inhibitor for the decarboxylase activity.⁸ Hence, a combination of LD with CD leads to a control of the dopamine concentration at suitable levels, reducing the side effects and improving the efficiency of the therapy.

Accordingly, the development of an analytical method is very important to control the content of these catecholamines in pharmaceuticals. Different techniques have been employed for the determination of CD in pharmaceutical formulations.^{9–15} Long analysis times, the use of organic solvents and high costs are some of the drawbacks associated with these techniques. Voltammetry is considered an important electrochemical technique utilized in electroanalytical chemistry because it provides low cost, sensitivity, precision, accuracy, simplicity and rapidity.^{16–18}

To the best of our knowledge, no study has reported the electrocatalytic determination of CD using carbon nanotube paste electrodes. In addition, no paper has reported FC as a catalyst for the electrocatalysis of CD. Thus, in continuation of studies concerning the preparation of chemically modified electrodes,^{19–22} in this paper, initially the preparation and suitability of a FC modified carbon nanotube paste electrode (FCMCNPE) as a new electrocatalyst in electrocatalysis and the determination of CD in an aqueous buffer solution are described. Finally, in order to demonstrate the catalytic ability of the modified electrode in the electro-oxidation of CD in real samples, this method was examined for the voltammetric determination of CD in urine samples.

EXPERIMENTAL

Apparatus and reagents

All the cyclic voltammetric measurements were performed using a BHP 2063⁺ electrochemical analysis system, Behpajoo, Iran, comprising the potentiostat/galvanostat coupled with a Pentium IV personal computer connected to a HP laser jet 6L printer.

An Ag/AgCl/3 M KCl, a platinum wire, and a FCMCNPE were used as the reference, auxiliary and working electrodes, respectively. A digital pH/mV-meter (Metrohm model 710)

was applied for pH measurements. Graphite fine powder, paraffin oil and reagents were analytical grade from Merck. CD was purchased from Merck. Multi-walled carbon nanotubes (purity more than 95 %) with *o.d.* between 10 and 20 nm, *i.d.* between 5 and 10 nm and tube length from 0.5 to 200 nm were prepared by Nanostructured & Amorphous Materials (USA). The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range 2.0–11.0.

Preparation of the electrode

Modified carbon nanotube paste electrodes were prepared by dissolving 0.010 g of FC in diethyl ether and hand mixing with 89-times its weight of graphite powder and 10-times its weight of carbon nanotubes with a pestle and mortar. The solvent was evaporated by stirring. A 70:30 (w/w) mixture of FC spiked carbon nanotube powder and paraffin oil was blended by hand mixing for 20 min until a uniformly-wetted paste was obtained. The paste was then packed into the end of a glass tube (*ca.* 2 mm *i.d.* and 10 cm long). Electrical contact was made by inserting a copper wire into the glass tube at the back of the mixture. When necessary, a new surface was obtained by pushing an excess of paste out of the tube and polishing it on a weighing paper. Unmodified carbon paste, prepared in the same way but without the addition of FC and carbon nanotubes to the mixture, was used for comparison purposes.

Preparation of a real sample

The urine sample was stored in a refrigerator immediately after collection. Ten milliliters of the sample was centrifuged for 10 min at 1000 rpm. The supernatant was filtered through a 0.45 μm filter and then diluted 10-times with 0.10 M phosphate buffer solution (pH 7.0). The solution was transferred into a voltammetric cell to be analyzed without any further pretreatment. The standard addition method was used for the determination of CD in real samples.

RESULTS AND DISCUSSION

Electrochemical behavior of the FCMCNPE

Cyclic voltammetry was employed for the investigation of the electrochemical properties of the FCMCNPE in a pure buffered aqueous solution (pH 7.0). The cyclic voltammogram (Fig. 1) exhibits an anodic and corresponding cathodic peaks with $E_{\text{pa}} = 0.370$ V and $E_{\text{pc}} = 0.265$ V *vs.* Ag/AgCl/3 M KCl. The experimental results showed well-defined and reproducible anodic and cathodic peaks related to the FC/FC⁺ redox couple with quasi-reversible behavior, because the peak separation potential, $\Delta E_{\text{p}} = (E_{\text{pa}} - E_{\text{pc}})$, was greater than the $59/n$ mV expected for a reversible system. In addition, the result obtained from cyclic voltammetry of this modified electrode in various buffered solutions did not show any shift in the anodic and cathodic peak potentials. Therefore, the electrochemical behavior of the redox process of FC/FC⁺ in the FCMCNPE is independent of the pH of the aqueous solution.

The capability of the electrode for the generation of a reproducible surface was examined by cyclic voltammetric data obtained in the optimum solution pH from five separately prepared FCMCNPEs (Table I). The calculated RSDs for various parameters accepted as the criteria for a satisfactory surface reproduce-

bility were 1–4 %. This degree of reproducibility is virtually the same as that expected for a renewed or ordinary carbon paste surface.

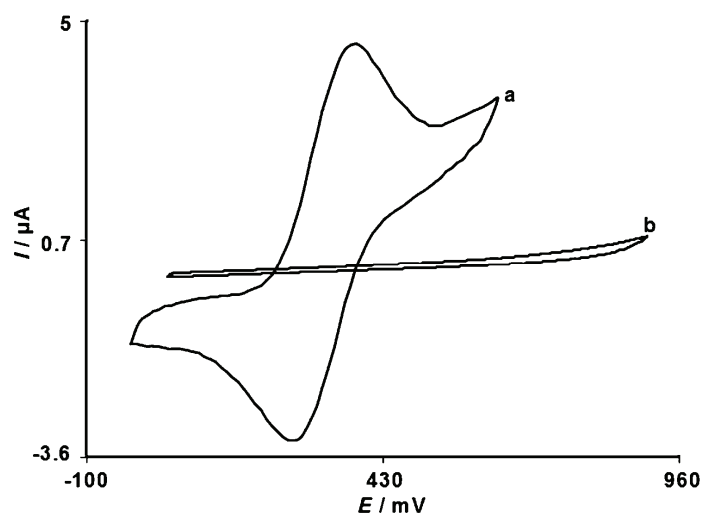


Fig. 1. Cyclic voltammograms of a) FCMCNPE and b) bare CPE in 0.1 M PBS (pH 7.0) at a scan rate 20 mV s^{-1} .

In addition, the long term stability of the FCMCNPE was tested over a three-week period. Cyclic voltammetry of the CD at the surface of the FCMCNPE after the modified electrode had been stored under ambient conditions showed that the oxidation peak potential of the CD was unchanged and the anodic peak current was only decreased by less than 2.1 % of the initial oxidation peak current. The antifouling properties of modified electrode toward CD and its oxidation product were investigated by recording the cyclic voltammograms of this modified electrode before and after using in the presence of CD.

TABLE I. Cyclic voltammetric data obtained for the constructed FCMCNPE in 0.10 M PBS (pH 7.0) at 20 mV s^{-1} ([a]: vs. Ag/AgCl/3 M KCl as the reference electrode; [b]: the values in parenthesis indicate the calculated RSD)

E_{pa} / V [a]	E_{pc} / V [a]	$E_{1/2} / \text{V}$ [a]	$\Delta E_{pa} / \text{V}$ [a]	$I_{pa} / \mu\text{A}$	$I_{pc} / \mu\text{A}$	$\Gamma_a / \text{mol cm}^{-2}$	$\Gamma_c / \text{mol cm}^{-2}$
0.370	0.265	0.3175	0.105	4.55	3.27	1.85×10^{-9}	1.45×10^{-9}
(0.75) [b]	(0.67) [b]	(0.78) [b]	(0.81) [b]	(2.45) [b]	(2.12) [b]	(3.4) [b]	(2.8) [b]

The cyclic voltammetry of the CD at the surface of the FCMCNPE after 10 repetition cycles at a scan rate of 20 mV s^{-1} showed that the oxidation peak potential of CD was not changed and the anodic peak current was decreased by less than 3.3 %. However, it should be emphasized that the surface of the FCMCNPE was regenerated before each experiment.

Optimization of the pH of the solution

The electrochemical behavior of CD is dependent on the pH value of the aqueous solution, whereas the electrochemical properties of the FC/FC⁺ redox couple are independent of pH. Therefore, pH optimization of the solution seemed to be necessary in order to realize the electrocatalytic oxidation of CD. Thus, the electrochemical behavior of CD in 0.10 M phosphate buffer solutions of different pH values ($2.0 < \text{pH} < 11.0$) at the surface of the FCMCNPE by cyclic voltammetry was examined. It was found that the electrocatalytic oxidation of CD at the surface of the FCMCNPE was more favored under neutral conditions than in acidic media. This appears as a gradual growth in the anodic peak current and a simultaneous decrease in the cathodic peak current in the cyclic voltammograms recorded at the surface of the FCMCNPE. The results showed that the anodic peak potential of CD at the surface of the FCMCNPE was shifted to a less-positive potential. In addition, the anodic peak current and the shifted potential value for the electro-oxidation of CD are high at physiological pH values. Thus, pH 7.0 was chosen as the optimum pH for electrocatalysis of CD oxidation at the surface of the FCMCNPE.

Electrochemistry of CD at the FCMCNPE

The cyclic voltammetric responses from the electrochemical oxidation of 250 μM CD at the FCMCNPE (curve f), the FC modified CPE (FCMCPE) (curve e), CNPE (curve d) and the bare CPE (curve a) are depicted in Fig. 2. As can be seen, the anodic peak potentials for the oxidation of CD at the FCMCNPE (curve f) and the FCMCPE (curve e) are about 370 mV, while at the CNPE (curve d), the peak potential is about 720 mV and at the bare CPE (curve b), the peak potential is about 750 mV for CD. From these results, it was concluded that the best electrocatalytic effect for CD oxidation was observed at the FCMCNPE (curve f). For example, the results showed that the peak potential of CD oxidation at the FCMCNPE (curve f) shifted by about 350 and 380 mV toward negative values compared with that at the CNPE (curve d) and the bare CPE (curve b), respectively. Similarly, when the oxidation of CD at the FCMCPE (curve e) and the FCMCNPE (curve f) are compared there is a dramatic enhancement of the anodic peak current at the FCMCNPE relative to the value obtained at the FCMCPE. In the other words, the obtained data clearly showed that the combination of carbon nanotubes and mediator (FC) definitely improve the characteristics of CD oxidation. The FCMCNPE in 0.10 M phosphate buffer (pH 7.0) without CD in the solution exhibited a well-behaved redox reaction (curve c) upon the addition of 250 μM CD, the anodic peak current of mediator was greatly increased, while the corresponding cathodic peak disappeared on the reverse scan of the potential (curve f). This behavior is typical of that expected for electrocatalysis at chemically modified electrodes.²³

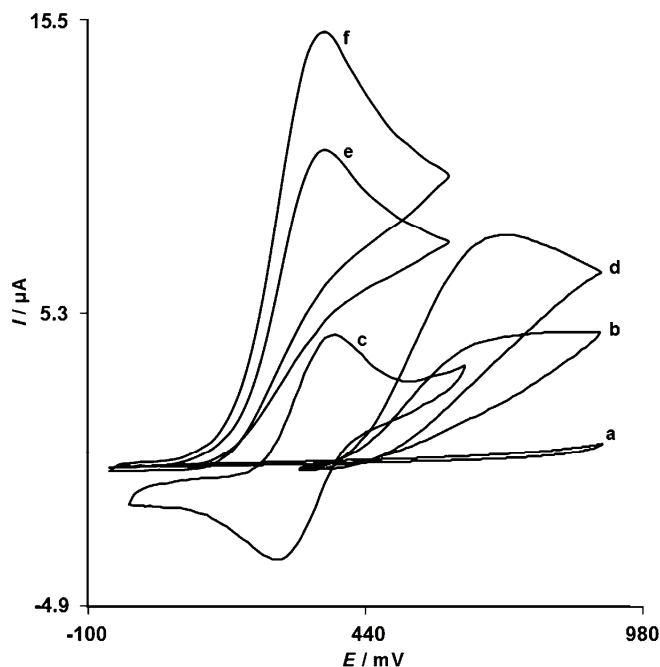


Fig. 2. Cyclic voltammograms of a) CPE in 0.1M PBS (pH 7.0) at a scan rate 20 mV s^{-1} and b) as a) + $250 \mu\text{M}$ CD; c) as a) and d) as b) at the surface of the FCMCNPE and CNPE, respectively; e) and f) as b) at the surface of the FCMCPE and FCMCNPE, respectively.

The effect of scan rate on the electrocatalytic oxidation of $250 \mu\text{M}$ CD at the FCMCNPE was investigated by cyclic voltammetry. The oxidation peak potential shifted with increasing scan rate towards more positive potentials, confirming the kinetic limitation of the electrochemical reaction. In addition, a plot of peak height (I_p) against the square root of the scan rate ($v^{1/2}$) was linear in range of $10\text{--}90 \text{ mV s}^{-1}$ (Fig. 3A, curve a), suggesting that at a sufficient overpotential, the process is diffusion rather than surface controlled. A plot of the sweep rate normalized current ($I_p/v^{1/2}$) vs. sweep rate (Fig. 3A, curve b) exhibited the characteristic shape typical of an EC_{cat} process.

From the slope of the E_p vs. $\log v$ curve, as shown in Fig. 3B, curve b, the Tafel slope can also be obtained from the following equation:²⁴

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

The slope of E_p vs. $\log v$ plot is $b/2$, where b indicates the Tafel slope. The slope of the E_p vs. $\log v$ plot is $\partial E_p / \partial (\log v)$, which was found to be 0.050 V in this work; hence, $b = 2 \times 0.050 = 0.100 \text{ V}$. The value of Tafel slope indicates that a one-electron transfer process is the rate limiting step assuming a transfer coefficient, α , of about 0.41.

A Tafel plot constructed from data of the rising part of the current–voltage curve recorded at a scan rate of 20 mV s^{-1} is shown in Fig. 3B, curve a. This part of the voltammogram, known as the Tafel region, is affected by the kinetics of the electron transfer between CD and FC, assuming deprotonation of the substrate is a sufficiently fast step. Under this condition, the number of electrons involved in the rate determining step can be estimated from the slope of the Tafel plot. A slope of 0.100 V dec^{-1} was obtained, indicating that a one electron transfer is rate limiting assuming a transfer coefficient of $\alpha = 0.41$.

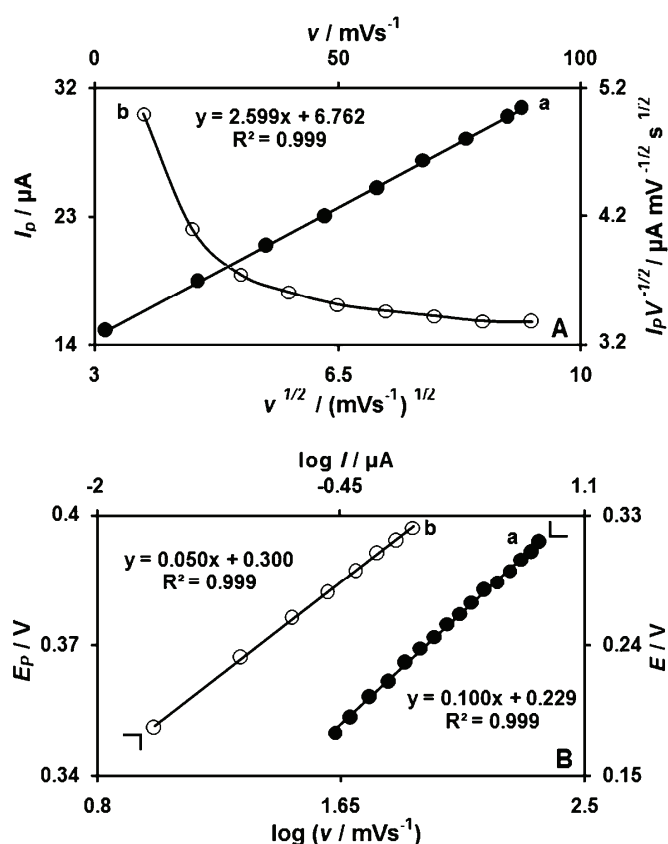


Fig. 3. A) Curve a, variation of the electrocatalytic current (I_p) with the square root of scan rate and curve b, variation of the scan rate-normalized current ($I_p/v^{1/2}$) with scan rate; B) curve a, Tafel plot derived from the rising part of the voltammogram recorded at a scan rate 20 mV s^{-1} and curve b, plot of E_p vs. $\log v$.

Chronoamperometric studies

The catalytic oxidation of CD by a FCMCNPE was also studied by chronoamperometry. Chronoamperometric measurements of different concentrations of

CD at the FCMCNPE were performed by setting the working electrode potential at 450 mV. From the chronoamperometric studies, we have determined the diffusion coefficient, D , of CD was determined. The experimental plots of I vs. $t^{-1/2}$ with the best fits for different concentrations of CD were employed. The slopes of the resulting straight lines were then plotted vs. the CD concentration. From the slope of the resulting plot and using the Cottrell Equation:²⁰

$$I = nFAD^{1/2}c_b\pi^{-1/2}t^{-1/2} \quad (2)$$

a diffusion coefficient of $(2.65 \pm 0.4) \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ was determined for CD.

Calibration plot and limit of detection

Differential pulse voltammetry was used to determine the concentration of CD (Fig. 4A). The responses were linear with CD concentration in the range from 5.0×10^{-6} to 6.0×10^{-4} M and the current sensitivity was $0.0414 \mu\text{A}/\mu\text{M}$ (Fig. 4B). The detection limit (3σ) was $3.6 \pm 0.17 \mu\text{M}$.

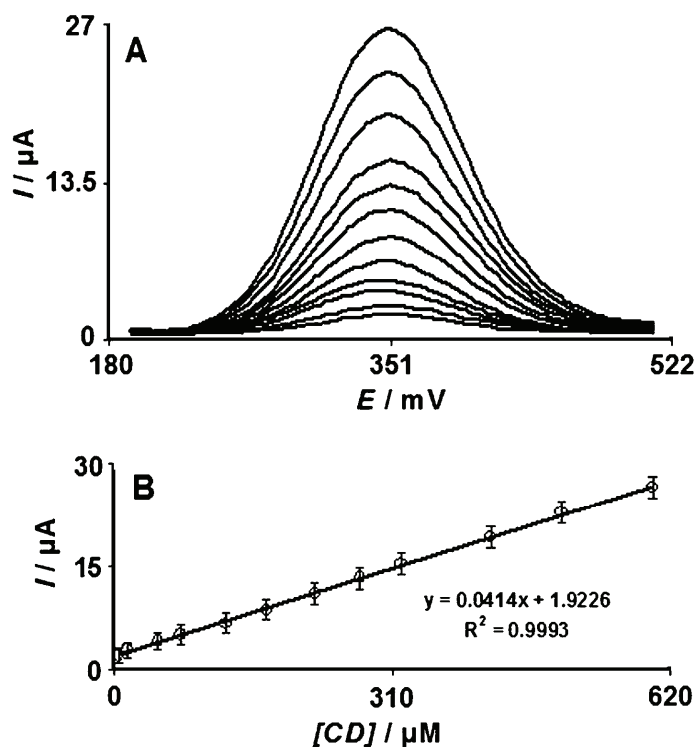


Fig. 4. A) Differential pulse voltammograms of the FCMCNPE in 0.10 M PBS (pH 7.0) containing different concentrations of CD, from inner to outer corresponding to 5, 15, 50, 75, 125, 170, 225, 275, 320, 420, 500 and 600 μM of CD; B) plots of the electrocatalytic peak current as a function of CD concentration.

Interference studies

The influence of various substances as compounds potentially interfering with the determination of CD was studied under optimum conditions with 10 μM CD at pH 7.0. The potentially interfering substances were chosen from the group of substances commonly found with CD in pharmaceuticals and/or in biological fluids. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error of less than $\pm 5\%$ in the determination of CD. According to the results, neither an 800-fold excess of glucose, sucrose, lactose, fructose or citric acid, nor a 600-fold excess of methanol, ethanol, Ca^{2+} , Mg^{2+} , SO_4^{2-} , Al^{3+} , NH_4^+ , Fe^{2+} , Fe^{3+} , CO_3^{2-} , Cl^- or F^- , nor a 200-fold excess of alanine, methionine, phenylalanine, glycine, or folic acid (vitamin B₉) affected the selectivity. In addition, neither a saturated starch solution nor a 50-fold excess of urea interfered with the determination of CD. Although ascorbic acid showed interference, this interference could be minimized, if necessary, by using ascorbic oxidase enzyme, which exhibits a high selectivity to the oxidation of ascorbic acid. Also, LD interfered with the determination of CD.

Determination of CD in a real sample

To evaluate the applicability of the proposed method to real samples, it was applied to the determination of CD in urine samples. The CD contents were measured after sample preparation using the standard addition method. The results are given in Table II.

TABLE II. Concentration values obtained from the proposed and the reference method (spectrophotometric method) for CD analysis of urine samples using the proposed method under optimum conditions ($n = 3$)

Sample	Added, μM	Proposed method ^a , μM	Standard method ^a , μM	F_{ex}	$F_{\text{tab}}^{\text{b}}$	t_{ex}	$t_{\text{tab}(98\%)}$
Urine ^c	10.0	10.1 \pm 1.2	10.3 \pm 1.5	4.2	19	2.8	3.3
Urine ^c	10.0	10.2 \pm 1.4	10.5 \pm 1.8	3.7	19	2.5	3.3
Urine ^d	10.0	10.6 \pm 1.5	10.8 \pm 2.1	4.3	19	2.9	3.3
Urine ^d	10.0	10.4 \pm 2.1	10.5 \pm 2.2	3.9	19	2.7	3.3

^a \pm Standard deviation; ^b $F_{\text{tab}(0.05),(2,2)}$; ^ca man who is safe; ^da woman who is safe

CONCLUSIONS

This work demonstrates the construction of an FCMCNPE and its application in the determination of CD. The results showed that the oxidation of CD was catalyzed at pH 7.0, whereas the peak potential of CD was shifted by 380 mV to a less positive potential at the surface of the FCMCNPE. The catalytic peak currents obtained using DPV, were linearly dependent on the CD concentrations and the detection limit for CD was 3.6 \pm 0.17 μM . The high current sensitivity, low detection limit and high selectivity of the FCMCNPE for the detection of CD

proved its potential as a sensor. In addition, the FCMCNPE was employed for the determination of CD in some urine samples.

ИЗВОД

ЕЛЕКТРОХЕМИЈСКА ДЕТЕКЦИЈА КАРБИДОПЕ КОРИШЋЕЊЕМ ЕЛЕКТРОДЕ ОД
ПАСТЕ УГЉЕНИЧНИХ НАНОЦЕВИ МОДИФИКОВАНИХ ФЕРОЦЕНОМ

HALIMEN YAGHOUBIAN¹, HASSAN KARIMI-MALEH², MOHAMMAD ALI KHALILZADEH² и FATEMEH KARIMI²

¹Islamic Azad University, Branch of Bam, Bam и ²Department of Chemistry,
Islamic Azad University, Qaemshahr, Iran

Направљена је хемијски модификована електрода на бази угљеничне пасте која садржи угљеничне наноцеве и фероцен. Електрохемијско понашање и стабилност ове електроде су испитивани цикличном волтаметријом. Показана је добра активност електроде за електрохемијску оксидацију карбидопе у фосфатном пуферу. Добијена је линеарна зависност у опсегу концентрација од 5 до 600 μM карбидопе са границом осетљивости од $3,6 \pm 0,17 \mu\text{M}$. Такође су одређени коефицијент дифузије карбидопе и коефицијент прелаза (α) за његову оксидацију. Електрода је показала добру репродуктивност, изузетну стабилност током дугих времена и посебно добру репродуктивност површине након механичког полирања. Резултати су показали да се испитивана електрода може користити као електрохемијски сензор за одређивање карбидопе у реалним системима, као што су узорци урина.

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