Research Article

Electrochemical Determination of Dopamine on a Glassy Carbon Electrode Modified by Using Nanostructure Ruthenium Oxide Hexacyanoferrate/Ruthenium Hexacyanoferrate Thin Film

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Received 12 February 2011; Revised 5 May 2011; Accepted 5 May 2011

Academic Editor: Kenneth I. Ozoemena

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Application of mixed ruthenium oxide hexacyanoferrate/ruthenium hexacyanoferrate glassy carbon electrode for electrochemical determination of dopamine (DA) is described for the first time. The overlapped voltammetric oxidation potentials of ascorbic acid (AA) and DA are separated and shifted to more facile direction, +170 and +320 mV versus Ag/AgCl, respectively. Voltammetric response of the electrode toward the DA showed a dynamic calibration curve with two linear parts, from 0.50 to 10.00 μM and 25.00 to 550.00 μM DA, and a detection limit of 0.195 μM . The sensitivity (0.2917 $\mu\text{A}/\mu\text{M}$) and detection limit (0.195×10^{-7} μMDA) of this electrode are 21 times higher and 11.5 times lower than those found in our previous paper. The sensor response of 9.95 μMDA was not affected by 5.0 mM of glucose, 4.5 mM of fructose, 0.58 mM of sucrose, 0.28 mM of cystine, 0.25 mM of ascorbic acid, 79.60 μM of cysteine, and 49.70 μM of uric acid and urea. The fabricated sensor was successfully tested for determination of DA in injection medicine and human blood plasma samples.

1. Introduction

Dopamine (DA) is a catecholamine neurotransmitter, which is generated in various parts of central and peripheral nervous system that plays an important role in the function of the central nervous, renal, hormonal, and cardiovascular systems. A knowledge of DA level in biological fluids, like blood and urine, is of paramount importance for diagnosis of a number of diseases like Schizophrenia, Parkinsonism, Alzheimer, and HIV infection [1–3]. One of the most important problems in determination of DA is the interference of the active species, like ascorbic acid (AA), accompanying the DA in biological fluids. Therefore, great deal of researches have been carried out to solve this problem, leading to the development of several methods based on chromatography [4], fluorescence [5], spectrophotometry [6], and electrochemistry [7, 8]. Among these methods, the electrochemical techniques are preferred over others due to their simplicity, rapidity, high sensitivity and specificity, reduced power requirements, and ease of fabrication. These techniques are mainly based on electrodes modified by using

carbon pastes [9, 10], sol-gels [11], polymers [12], and metal oxides [13].

In the previous papers, application of the self-assembled monolayers (SAMs) for determination of DA was reported [1, 14, 15]. In spite of many advantages of SAM-based modified electrodes, like stability and antifouling effects, they suffer from low sensitivity.

Recently, fabrication of the mixed ruthenium oxide hexacyanoferrate/ruthenium hexacyanoferrate (RuOHCF/RuHCF) thin films on the glassy carbon electrodes (GCE) and testing the electrocatalytic activities of these films towards the DA, epinephrine, norepinephrine [16], peroxodisulphate ($\text{S}_2\text{O}_8^{2-}$) [17], deoxyguanosine [18], AA [19, 20], and hydrogen peroxide (H_2O_2) [21] have been reported. Thus, it seems highly promising to modify the surface of the solid electrodes, such as platinum or carbon, using a thin film of RuOHCF/RuHCF and use the electrocatalytic properties of the modified electrode to separate the oxidation potential waves of DA from AA for quantitative determination of DA or AA in the presence of each other.

However, in spite of potentially interesting characteristics of the RuOHCF/RuHCF thin film modified electrode as a DA electrochemical sensor, detailed sensing behavior of this electrode toward quantitative determination of DA has not been reported yet. Besides, there is a lack of information regarding the characterization of this modified electrode by electrochemical impedance spectroscopy (EIS) and X-ray diffraction (XRD).

The present study is intended to prepare, characterize, and apply the GC/RuOHCF/RuHCF thin film modified electrode as a new sensor for selective determination of DA in the presence of AA. Thus, the sensor is characterized by electrochemical and surface techniques, the experimental conditions are studied, and DA is determined in the best conditions. Finally, the sensor is successfully tested for determination of DA in industrial (injection medicine) and biological (human blood plasma) samples.

2. Experimental

2.1. Chemical and Materials. Potassium ferrocyanide, ruthenium(III) chloride, HCl, DA, AA, and other materials used in this work were of analytical grade obtained from "Merck and Sigma" and used without further purification. All of the solutions were prepared by double-distilled water. Britton-Robinson buffer solutions, in the range of pH 2.0 to 8.0, were prepared using 0.04 M boric acid, 0.04 M acetic acid, and 0.04 M phosphoric acid; and the pH of the solutions was adjusted with concentrated NH_3 solution (25% W/W). The supporting electrolyte in determination of DA was 0.1 M NH_4NO_3 . DOPADIC ampoule (Iran Pharmaceutical Development Investment Co., IPDIC, Rasht, I.R. Iran) containing 5 mL of 200 mg/mL DA hydrochloride was used as DA source of pharmaceutical sample.

Blood plasma samples were collected from volunteers in Dr. Naser Ekramian's Medical Diagnostic Lab (Isfahan, Najafabad, I.R. Iran). Two mL of 0.125 M sodium citrate was added to the 18 mL of fresh blood sample (collected from volunteers, see part 2.1) and mixed. Then, the solution was centrifuged at the 3000 rpm. After 5 min, three phases were appeared. The above phase (blood plasma) was separated and used for the next step.

2.2. Electrodes and Instrumentation. A conventional three-electrode cell, including the modified GC disk ($A = 0.0314 \text{ cm}^2$, Azar Electrode Co., Urmia, I.R. IRAN) as working electrode, a large surface area Pt plate (99.99%, 5 cm^2) as auxiliary electrode, and an Ag/AgCl (saturated KCl) electrode as reference, was used for electrochemical measurements. Electrochemical measurements, including EIS, differential pulse, and cyclic voltammetry methods (DPV and CV), were carried out on Potentiostat/Galvanostat EG&G273A and Frequency Response Analyzer EG&G M1025 interfaced with a PC-IV 800 MHz through GPIB-IEEE-NI-488II card and Metrohm 797, respectively. Acquisition of the EIS data was performed using EG&GPowerSine software. Analysis of the EIS data was performed using ZView2.3 (Scribner Associates, Inc.) on the basis of Macdonald's algorithm (LEVIM) using

a complex nonlinear least square (CNLS) approximation method [22].

Surface morphology and elemental composition of the electrodeposited film were investigated by scanning electron microscopy (SEM) and energy dispersive X-ray diffraction microanalysis (EDX) (Seron-AIS2100). Crystal spectrum of the film was monitored by XRD analysis (Bruker-D8ADVANCE) using $\text{Cu K}\alpha$ wavelength, $1.5406 \text{ \AA}$.

2.3. Modification of the Glassy Carbon Electrode. The surface of the GCE is polished by alumina suspension ($5 \mu\text{m}$) on a microcloth polishing pad (Buehler), rinsed with water, and sonicated for 5 min in distilled water. Deposition of the RuOHCF/RuHCF thin film was accomplished by potentiodynamic cycling of the GC electrode between potential limits of -0.5 and $+1.3 \text{ V}$ at 100 mV/s in supporting electrolyte ($0.5 \text{ M NaNO}_3 + 0.05 \text{ M HCl}$) aqueous solution containing $1 \text{ mM K}_3\text{Fe}(\text{CN})_6$, and 1 mM RuCl_3 . After film formation, the electrode was thoroughly rinsed with deionized water. The formation of the RuOHCF/RuHCF thin film on the GC surface was verified by immersing the modified electrode in a supporting electrolyte solution containing $0.5 \text{ M NaNO}_3 + 0.05 \text{ M HCl}$ and performing repetitive potentiodynamic cycling between preset potential limits according to the literature [18]. The amount of immobilized material on the electrode surface, after potentiodynamic experiments, was controlled by the number of potential cycles, and the number of the active places involving in the heterogeneous charge transfer in this electrocatalytic process (Γ) was determined from the charge under the voltammetric peak for the Ru(III/II) redox process (Q_{cv}) using $\Gamma = Q_{\text{cv}}/nFA$, where F is Faraday constant, n is the number of electrons transferred per molecule of redox active species, and A is the geometric surface area of the electrode [19]. A value was also calculated for Γ from EIS measurements based on $\Gamma = 4RTC_\phi/n2F2A$ according to the literature [23, 24], where T is absolute temperature, R is universal gas constant, and C_ϕ is pseudocapacitance.

3. Results and Discussion

3.1. Construction and Characterization of the Nanostructure RuOHCF/RuHCF Active Thin Film. Formation of RuOHCF/RuHCF active thin film monitored by electrochemical methods and surface techniques is described below.

3.1.1. Cyclic Voltammetry. Repetitive cyclic voltammograms recorded during electrodeposition of mixed RuOHCF/RuHCF thin film from a solution containing Ru(III), $[\text{Fe}(\text{CN})_6]^{3-}$ at pH 1.5 on the bare GCE (Figure 1(A)) showed four pairs of redox peaks around (a) -0.1 , (b) 0.6 , (c) 0.9 , and (d) 1.1 V . These peaks are attributed to the following electrode processes according to the literature [18]:

FIGURE 1: (A) Repeated cyclic voltammograms recorded on GCE in an aqueous solution containing 0.05 M HCl, 0.5 M NaNO₃, 1 mM K₃FeCN₆, and 1 mM RuCl₃ at pH 1.5 to obtain GC/RuOHCF/RuHCF modified electrode. (B) Consecutive cyclic voltammograms recorded on GC/RuOHCF/RuHCF modified electrode in a solution containing *only* 0.05 M HCl and 0.5 M NaNO₃. Scan rate: 100 mV/s. (C) EIS complex plane plots obtained on (a) the bare GCE and (b) GC/RuOHCF/RuHCF modified electrode in a solution containing *only* 0.5 M NaNO₃ at pH 1.5, $E_{DC} = +0.5$ versus Ag/AgCl, $E_{ac} = 15$ mV, and frequency range: 10 kHz to 10 mHz.

Wave (e) is attributed to the background. A comparison between these results (Figure 1(A)), and the repetitive cyclic voltammograms obtained on the modified electrode in a solution containing *only* supporting electrolyte solution at pH 1.5 (Figure 1(B)), indicates an additional reversible wave (b_0) around +0.32 V (Figure 1(A)) that could be attributed to the presence of K₃Fe(CN)₆ in deposit solution (i.e., the reaction of $[\text{Fe}(\text{CN})_6]^{4-} \longleftrightarrow [\text{Fe}(\text{CN})_6]^{3-} + e^-$). A comparison between the CV recorded on the bare GCE in a solution containing 0.05 M HCl, 0.5 M NaNO₃, and 1 mM K₃FeCN₆ and the background recorded in these conditions but in the absence of K₃FeCN₆ supported this ascription (data not shown). These behaviors demonstrate formation of RuOHCF/RuHCF thin film on GCE.

3.1.2. Electrochemical Impedance Spectroscopy. EIS is a powerful, nondestructive, and informative technique that has played an essential role in kinetic characterization and diagnosis of the events occurring at the different types of electrode/solution interfaces in a wide range of applied DC potentials [22–28]. EIS studies of RuOHCF/RuHCF thin film have not been reported till now.

In this step of the work, EIS measurements are performed in different conditions (including pH, AC, and DC potentials, and external redox probes) to trace and support formation of RuOHCF/RuHCF thin film on the GC electrode. After testing the conditions, a sensitive and reproducible behavior was found by using 15 mV peaktopeak

AC potential superimposed on +0.5 V DC potential, in the frequency range of 10 kHz to 10 mHz, at pH 1.5 based on the confined redox reaction of RuHCF (i.e., in the *absence* of external probe). Then, the impedances recorded in these conditions were analyzed to find the kinetics of the modified electrode.

Typical EIS complex plane plots ($-Z_{im}$ versus Z_{re}) obtained on the bare GC and GC/RuOHCF/RuHCF thin film electrodes are presented in Figure 1(C). The bare GCE in background electrolyte exhibits almost a straight line (curve a), which is characteristic of a pure capacitive effect in series with a pure resistant [22, 25–31]. However, the deposited film shows a faradic charge transfer behavior, which is reflected by the appearance of the semicircle part on the spectrum (curve b). Since the experiments have been performed in the background electrolyte solution in the absence of any external redox probe, this behavior is attributed to the confined faradaic redox reaction on the GC/RuOHCF/RuHCF electrode surface.

A detailed theoretical study regarding EIS of the electroactive thin films has already been reported [23, 24, 32–36]. Accordingly, we assumed (i) a pseudocapacitance (C_ϕ) and (ii) a double-layer capacitance (C_{dl}) effect, which are originating, respectively, from the (i) fast reversible faradaic reaction of the confined redox centers occurring on the GC/RuOHCF/RuHCF electrode surface and (ii) charge separation at the electrode/electrolyte interface. Then, the contribution of these elements to the total impedance of the electrode is estimated using *appropriate* equivalent circuit models.

Several equivalent circuit models are applied, EIS data are approximated, and finally, more *appropriate* models, considering *criteria* of the fit [22] like the number of

model parameters, their physical meaning, errors of the fit (χ^2), are selected (Figure 2, models (a) and (b) for the bare and modified electrodes, respectively, and the electrodes kinetic parameters such as solution resistance (R_s), charge transfer resistance (R_{ct}), C_ϕ , and C_{dl} , are extracted (Table 1).

The C_ϕ , which is an indicative of the active sites formed on the electrode surface by modification process, can help to estimate Γ , where $\Gamma = 4RTC_\phi/n^2F^2A$ [23, 24]. Accordingly, a value of 8.02×10^{-9} mole/cm² is obtained for Γ via the EIS measurements. This value is in good agreement with that observed by CV, $\Gamma = Q_{cv}/nFA = 8.4 \times 10^{-9}$ mole/cm² (Figure 1(B), peak (a)) with $\Delta\Gamma\% = 4.6$, where $\Delta\Gamma\% = [2(\Gamma_{cv} - \Gamma_{EIS})/(\Gamma_{cv} + \Gamma_{EIS})] \times 100$. These results support the formation of active RuOHCF/RuHCF thin film. The electrocatalytic activity of this thin film modified electrode towards the substrate, that is, DA, is subsequently studied in this work (Section 3.2).

3.1.3. Surface Morphology. Typical SEM micrographs obtained on the surface of the bare GC and GC/RuOHCF/RuHCF electrodes are presented in Figures 3(a) and 3(b), respectively. Particles with diameter below 100 nm are observed in the SEM micrograph (Figure 3(b)), indicating a nanostructure for the RuOHCF/RuHCF deposited thin film on the GCE.

A typical EDX spectrum obtained from surface of the modified electrode is also presented in Figure 3(c). The presence of the Ru, Fe, Na, O, and C elements is shown in the spectrum. The sharp peak of carbon at (0.3 keV) in spectrum is related to the GC base. This information also supports formation of RuOHCF/RuHCF thin film on the surface of GCE.

The XRD spectrum of the modified surface is displayed in Figure 3(d). This spectrum includes a sharp peak and several small ones, indicating a crystalline structure for RuOHCF/RuHCF film. Up to our knowledge, there is no XRD data about structure of RuOHCF/RuHCF film in literature. A systematic investigation of the structure and the phase or phases possibly existing in this compound is not the purpose of this work. However, Scherrer's equation [37] is used to estimate the grain size, and values of 27.8, 21.5, 22.5, and 26.5 nm are obtained for 2θ of 18.224°, 31.657°, 37°, and 41.5°, respectively. The average grain size is about 24.8 nm, indicating a nanostructure for RuOHCF/RuHCF film.

3.2. Electrocatalytic Activities of GC/RuOHCF/RuHCF Electrode towards the Oxidation of DA

3.2.1. Cyclic Voltammetry. The cyclic voltammogram obtained on the bare GCE (Figure 4, curve (a1)) shows sluggish electron transfer kinetics for redox reaction of DA ($E_{pa} = +465$ mV and $E_{pc} = +162$ mV) with a large peak separation ($\Delta E_p \cong 300$ mV), which can be related to nature of DA redox reaction on the unmodified electrode as well as fouling of the bare GCE surface by DA and its oxidation products [38]. However, the anodic and cathodic peak potentials are shifted to more facile direction ($E_{pa} = +320$ mV

FIGURE 2: Equivalent circuits used for approximation of the EIS data. (a) The bare GC and (b) GC/RuOHCF/RuHCF electrodes in the absence of external probe.

and $E_{pc} = +264$ mV) on GC/RuOHCF/RuHCF electrode, indicating an electrocatalytic effect for DA oxidation. Also, a comparison between anodic peak currents indicates that the oxidation peak current of DA is increased by a factor of 8 from the bare to the modified GCE (Figures 4(a1) and 4(a2)). An increase in the current or a shift in the reaction potential to more facile direction for an electrochemical reaction observed on a modified electrode surface is so called the electrocatalytic effect of the modifier. Interestingly, here, both effects are observed for GC/RuOHCF/RuHCF thin film towards the oxidation of DA.

Variations of the oxidation peak current of DA ($I_{p,a}$) as a function of the potential scan rate (ν) depicted a linear behavior in the range of 10 to 120 mV (data not shown) [$I_{p,a}(A) = 1 \times 10^{-5} \nu(Vs^{-1}) + 4 \times 10^{-7}$, $r^2 = 0.999$], indicating that the charge transfer rate at the GC/RuOHCF/RuHCF electrode surface is controlled by the redox reaction of DA adsorbed on the surface. This behavior is used subsequently in this work to develop a method for direct quantitative determination of DA based on anodic adsorptive stripping voltammetry (see Section 3.3).

3.2.2. Electrochemical Impedance Spectroscopy. Oxidation of DA at the bare and GC/RuOHCF/RuHCF electrodes is also studied by the EIS. The complex plane plots obtained for 1 mM DA at the bare (curve a) and modified (curve b) electrodes are presented in Figure 4(B). The data are approximated, based on the argument presented in Section 3.1.2, using appropriate equivalent circuits (Figures 5(a) and 5(b)) for the bare GC and the GC/RuOHCF/RuHCF electrodes, respectively). The values estimated for the model parameters are presented in Table 2. The slow reaction of DA at the unmodified electrode causes only a semicircle in complex plane plot with a negligible effect of Warburg impedance (Figure 4(B)). The formation of RuOHCF/RuHCF thin film on GCE has led to a decrease in R_{ct} by a factor of 29. This behavior can be attributed to the adsorption of DA on GC/RuOHCF/RuHCF thin film, which is revealed by a C_ϕ of 26.7 μF . Since the redox reaction of adsorbed DA is accompanied by diffusion of proton ion (H^+), the low-frequency range line in the impedance spectrum is attributed to the Warburg impedances associated with proton diffusion at the film/electrolyte interface [39, 40] (see Table S1 in Supplementary Material available online at doi: 10.4061/2011/603135).

TABLE 1: Kinetic parameters extracted using EIS data obtained on the bare GC and GC/RuOHCF/RuHCF electrodes in 0.5 M NaNO₃ at pH 1.5, in the absence of any external probe, $A = 0.0314 \text{ cm}^2$, $E_{DC} = +0.5$.

Electrode	$R_s (\Omega)$	$R_{ct} (\Omega)$	$Q_\phi (\mu\text{F})$	$Q_{dl} (\mu\text{F})$	n
Bare GC	21.03 ± 3.00	—	—	19.40 ± 0.20	0.70
GC/RuOHCF/RuHCF	50.75 ± 0.35	$(3.01 \pm 0.23) \times 10^4$	$(2.30 \pm 0.10) \times 10^3$	12.60 ± 0.30	0.80

$\chi^2 = 6.98 \times 10^{-3}$.

FIGURE 3: SEM microphotographs obtained on (a) the bare GC and (b) GC/RuOHCF/RuHCF modified electrodes. (c) EDX microanalysis spectrum and (d) XRD spectra of GC/RuOHCF/RuHCF modified electrode obtained using Cu K_α 1.5406 Å.

So, I_p of voltammetry and also $1/R_{ct}$ of EIS measurements are found to be proportional to the DA concentration in solution phase [DA]. As a conclusion, an adsorption/oxidation mechanism may be assumed for DA via RuOHCF/RuHCF thin film active sites, in which adsorption step is relatively fast, under equilibrium, and proportional to the DA concentration in solution phase. This object is studied in Section 3.3.

3.2.3. Effect of Operational Parameters. Effect of pH, supporting electrolyte, and ionic strength of the working solution on the electrocatalytic oxidation of the DA at

GC/RuOHCF/RuHCF are studied by monitoring the anodic stripping differential pulse voltammetry peak current ($I_{p,a}$) of adsorbed DA in a BrittonRobinson buffer solution containing a fixed concentration of DA ($20 \mu\text{M}$ DA), and varying each one of the parameters. Data are not shown to save the space. The $I_{p,a}$ of DA showed a maximum value around pH 5.0 in BrittonRobinson buffer solution containing $0.10 \text{ M NH}_4\text{NO}_3$ as an electrolyte. Accumulation potential is examined in the range of $+150$ to -500 mV at a constant time (60 s) and pH (5.0) in BrittonRobinson buffer solution containing $0.10 \text{ M NH}_4\text{NO}_3$ and $20 \mu\text{M}$ DA. A maximum of $I_{p,a}$ was found around -500 mV versus Ag/AgCl and used as optimized potential in the next step

FIGURE 4: (A) Cyclic voltammograms obtained on the bare GC (a1) and GC/RuOHCF/RuHCF modified (a2) electrodes in the presence of 0.2 mM DA at pH 5. Scan rate 100 mV/s. (B) Complex plane plots obtained in 0.1 M NH_4NO_3 in the presence of 1 mM DA at pH 5 on (a) the bare GC, and (b) GC/RuOHCF/RuHCF modified electrodes. $E_{\text{DC}} = +0.260$ V versus Ag/AgCl, $E_{\text{ac}} = 15$ mV, and frequency range: 10 kHz to 10 mHz.

TABLE 2: Kinetic parameters extracted using the EIS data obtained on the bare GCE and modified GC/RuOHCF/RuHCF electrodes in 0.1 M NH_4NO_3 at pH 5 in the presence of 1 mM DA, $A = 0.0314$ cm^2 , $E_{\text{DC}} = +0.260$ V.

Electrode	$R_s(\Omega)$	$R_{\text{ct}}(\Omega)$	$Q_\phi(\mu\text{F})$	$Q_{\text{dl}}(\mu\text{F})$	n
Bare GC	62.90 ± 13.00	$(2.54 \pm 0.45) \times 10^4$	—	2.20 ± 0.01	0.80
GC/RuOHCF/RuHCF	109.10 ± 0.50	874.90 ± 92.30	98.87 ± 19.47	17.94 ± 6.06	0.80

$\chi^2 = 8.67 \times 10^{-5}$.

to study the accumulation time. A plot of $I_{\text{p,a}}$ of DA versus accumulation time showed that after 30 s, the oxidation peak current was almost invariant. So, 30 s is selected as optimum time. Finally, the instrumental parameters were optimized. Accordingly, the best conditions for determination of DA by DPV are summarized as follows: pH (5.0), supporting

electrolyte and ionic strength (BrittonRobison buffer, containing 0.1 M NH_4NO_3), accumulation potential (-0.5 V), accumulation time (30 s), scan rate (14 mV/s), and pulse amplitude (65 mV). After recording each DPV, the electrode surface was recovered by keeping the electrode at $+0.7$ V for 30 s in blank solution.

FIGURE 5: Equivalent circuits used for approximation of the EIS data: (a) the bare GC and (b) GC/RuOHCF/RuHCF electrodes in the presence of DA (external probe). Warburg element considers the possible contribution of DA in the solution phase at very low frequency. The double-layer capacitance is replaced by constant phase element CPE.

3.3. Analytical Application for Determination of DA

3.3.1. Merits of the Method. Under the optimum conditions, the calibration curve is constructed by systematically increasing the concentration of DA, from 5.0×10^{-7} to 5.50×10^{-4} M, in the test solution and monitoring the response of GC/RuOHCF/RuHCF electrode by anodic stripping of DA (Figure 6). The peak currents are extracted and plotted versus concentration. A dynamic calibration curve with two linear ranges, from 5.0×10^{-7} to 1.0×10^{-5} and 25.0×10^{-5} to 5.50×10^{-4} M DA, is observed as $[I_p(A) = 0.2917 (\pm 0.0023) [DA] - 0.100 \times 10^{-6} (\pm 0.001 \times 10^{-6})]$, $r^2 = 0.999$ and $[I_p(A) = 0.061 (\pm 0.004) [DA] + 5.000 \times 10^{-6} (\pm 0.001 \times 10^{-6})]$, $r^2 = 0.991$], respectively (Figure 6, insets). The detection limit of this electrode, calculated from the standard deviation (S) of the background (for signal equals $3S$, where $S = 1.897 \times 10^{-8}$ A for 8 measurements [3]), is 1.95×10^{-7} M DA, and the relative standard deviation (RSD) for 4 measurements at 9.95×10^{-6} M DA is 2.36%. These results indicate that the GC/RuOHCF/RuHCF electrode is a sensitive sensor for detection of DA at low concentrations. The sensitivity ($0.2917 \mu A/\mu M$) and detection limit (1.95×10^{-7} M DA) of this electrode are, respectively, 21 times higher and 11.5 times lower than those found in our previous report [15]. In addition, these results indicate that the sensitivity and detection limit of this electrode are, respectively, 1.6 time higher and 2.1 times lower than those found on thick film chemically modified electrodes in the best conditions (see Supporting Information, Table S2, row 2).

3.3.2. Effect of Interferences on Determination of DA. The main interference in determination of DA in biological fluids is AA. On the bare electrode, AA is oxidized almost at the same potential as DA, in a very similar mechanism. Therefore, overlapped voltammetric responses for the oxidation of DA and AA are usually observed on the bare electrodes. Also,

FIGURE 6: Differential pulse voltammograms recorded in the presence of different concentration of DA in 0.1 M NH_4NO_3 at pH 5. The insets show the two linear range calibration curves extracted from the voltammograms; (a) lower range and (b) upper range. See the text for details.

large overpotentials for such oxidations and fouling of the electrode surface by the electrochemical oxidation products of DA and AA impose difficulties in the voltammetric determination of DA [41]. By using this modified electrode, GC/RuOHCF/RuHCF, the overlapped oxidation potentials of AA and DA (+512 mV) on the bare GCE are separated by 150 mV and shifted to more facile direction (i.e., to less positive values), +170 and +320 mV versus Ag/AgCl, respectively (Figure 7). This behavior allows elimination of the interferences of several of biological species, in particular, the effect of AA.

Overall shift in oxidation potentials of AA and DA is attributed to the electrocatalytic activity of the film toward these species. Separation of AA and DA oxidation peak potentials could be due to different mechanisms that they follow in this process. While AA is not adsorbed on the electrode, DA is expected to follow an adsorption-oxidation mechanism. Thus, oxidation potential of AA is shifted from 500 mV to +170 mV versus Ag/AgCl in comparison with that of DA, which is shifted to +320 mV versus Ag/AgCl.

Thus, we examined possible interferences in the determination of DA under the optimum experimental conditions. For this purpose, the $9.95 \mu\text{M}$ DA samples were taken, different amounts of each interferent were added, and their voltammograms were recorded. The interference effect is defined as the concentration of an interfering species that can change the electrode response towards the analyte by a value more than that predicted by t -test. The results show that the peak current of $9.95 \mu\text{M}$ DA in the confidence intervals, determined at 95% for $n = 8$, is not affected by 5.0 mM of glucose, 4.5 mM of fructose, 0.58 mM of sucrose, 0.28 mM of

FIGURE 7: Cyclic voltammograms obtained on (A) the bare GCE in different solutions, blank (curve a), 100 μM AA (curve b), 120 μM DA (curve c), and in a mixture solution of 100 μM AA and 100 μM DA (curve d) and (B) GC/RuOHCF/RuHCF electrode in a mixture solution of 1.5 mM AA and 50 μM DA, pH 5, scan rate: 100 mV/s.

cystine, 0.25 mM of ascorbic acid, 79.6 μM of cysteine, and 49.7 μM of uric acid or urea. Further studies indicated that effect of interferences did not depend on DA concentration. Therefore, the standard addition methods can be efficiently used for analysis of the DA in real samples.

3.4. Application for Analysis of the Real Samples

3.4.1. Determination of Dopamine in Injection Medicines. Four pharmaceutical samples were prepared, and their DA concentration was adjusted by dilution of DOPADIC ampoule contents (5 mL of 200 mg/mL DA hydrochloride injection solution) in buffer solution, pH 5.0. Then, each sample was transferred to electrochemical cell for determination of its DA content (Table 3). The results show that the proposed method can be effectively used for the determination of DA in industrial (injection medicine) samples.

3.4.2. Determination of Dopamine in Human Blood Plasma Samples. Five portions of 1.0 mL of this phase were used for quantitative analysis by electrochemical methods. Because the amount of DA in blood sample was below the detection limit of the method (1.95×10^{-7} M DA), a constant volume of the 2 mM DA (considered as unknown, V_x) was spiked into a number of blood plasma samples and, then, diluted in volumetric vessels with buffer solution, pH 5, so that, each one of the samples contained 7.5×10^{-7} M DA (above

TABLE 3: Results of the recovery tests obtained for determination of DA in real sample (DOPADIC ampoule) after dilution to different concentrations. Results are average of four measurements on each sample.

$C_{\text{DA}}/\mu\text{M}$ (Expected)	$C_{\text{DA}}/\mu\text{M}$ (Recovered)	Recovery %	% RSD (Recovered)
4.98	5.03	101.03	1.50
7.47	7.37	98.70	1.30
9.95	10.00	100.50	2.36
66.60	67.50	101.35	3.89

the detection limit). Then, these solutions were applied for determination of DA by standard addition method using different volumes of 2 mM DA (considered as V_s), similar to the method reported in literature for adenosine [2]. A linear relation is observed (Figure 8) for $I_{p,a}$ versus DA concentration (V_s) as $[I_p(\mu\text{A}) = 10.0 (\pm 0.7) V_s + 0.0040 (\pm 0.0009)]$, $r^2 = 9913$. Using this equation, one can find the concentration of spiked solution, C_x . Accordingly, the recovery values are found to be 106.6% for the first recovery and 98.7% and 102.9% for the subsequent tests.

4. Conclusions

Layers of RuOHCF/RuHCF were successfully electrodeposited onto the surface of GCE and characterized by

FIGURE 8: Differential pulse voltammograms obtained on GC/RuOHCF/RuHCF electrode. Solutions prepared by standard addition into the blood plasma as follows: (a) 0.00, (b) 5.0, (c) 12.5, (d) 25.0, (e) 37.5, and (f) 50.0 μL of 2 mM DA solution. Inset shows calibration curve obtained from variations of the stripping peak current as a function of DA concentration (see the text for details).

using surface techniques and electrochemical methods such as SEM, EDX, XRD, EIS, DPV, and CV. The GC/RuOHCF/RuHCF electrode was applied for quantitative detection of DA at pH 5.0 for the first time. The electrode exhibited high electrocatalytic sensitivity and selectivity towards the oxidation of DA; $I_{\text{p,DA}}$ of DA was increased by a factor of 8, and the overlapped oxidation potentials of AA and DA appeared around +512 mV on the bare GCE, were separated by 150 mV, and shifted to more facile direction, +170 and +320 mV versus Ag/AgCl, respectively. This behavior allowed elimination of the interference effects of several biological species, so that 5 mM of glucose, 4.5 mM of fructose, 0.58 mM of sucrose, 0.28 mM of cystine, 0.25 mM of ascorbic acid, 79.6 mM of cysteine, and 49.7 of uric acid or urea did not interfere significantly. Therefore, the proposed method was successfully used for quantitative determination of DA in industrial (injection medicine) and biological fluids (blood plasma) real samples with satisfactory results.

Acknowledgments

The authors acknowledge the University of Isfahan providing research facilities for this work. Special thanks are due to Dr. Naser Ekramian's Medical Diagnostic Lab (Isfahan, Najafabad, I.R. Iran) for free sharing his Lab facilities, collection of blood samples, and preparation of blood plasma.

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