J Nanostruct 8(2): 119-130, Spring 2018

RESEARCH PAPER

Fabrication of TiO_2 Hollow Spheres and its Application in Modification of Carbon Paste Electrode For Simultaneous Determination of Dopamine and Piroxicam in the Presence of Ascorbic acid

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ARTICLE INFO

Article History:
Received 08 February 2018
Accepted 21 March 2018
Published 01 April 2018

Keywords:
Dopamine
Multi-walled Carbon nanotubes
Poly-glutamic acid
Piroxicam
TiO_2 hollow spheres

ABSTRACT

In this work we report preparation TiO_2 hollow spheres and its application as an electrochemical sensor. Therefore the novel carbon paste electrode modified with TiO_2 hollow spheres (TOHS), multi-walled carbon nanotubes (MWCNTs) and poly-glutamic acid (PGA) film (PGA/TOHS/MWCNTs/CPE) was used for simultaneous determination of dopamine (DA) and piroxicam (PRX) in the presence of ascorbic acid (AA). The electro-oxidation of dopamine(DA) and piroxicam (PRX) has been investigated by application of the modified electrode using cyclic voltammetry (CV), differential pulse voltammetry (DPV) and chronoamperometry (CA) methods. The modified electrode accelerates the electron transfer reactions of DA and PRX. In addition it shows no significant interference of AA as the electroactive coexistent compounds with DA and PRX in biological systems. The fabricated sensor revealed some advantages such as excellent selectivity, good stability and high sensitivity toward DA and PRX determination. Under the optimum conditions the electrode provides a linear response versus DA and PRX concentrations in the range of 0.3–60 and 0.4–80 μM and with a detection limit of 0.2μM and 0.3μM(S/N=3) respectively using the DPV method. The modified electrode was used for determination of DA and PRX in human urine with satisfactory results.

How to cite this article
Babaei A, Moradi M , Sohrabi M, Feshki S, Marandi M. Fabrication of TiO_2 Hollow Spheres and its Application in Modification of Carbon Paste Electrode For Simultaneous Determination of Dopamine and Piroxicam in the Presence of Ascorbic acid. J Nanostruct, 2018; 8(1):119-130. DOI: 10.22052/JNS.2018.02.002

INTRODUCTION

Recently, there has been growing interest in the synthesis, characterization, properties, mechanisms and fabrication of conducting polymers [1-3]. Glutamic acid is one of the common amino acids, which can be easily electro-polymerized on electrode surface to form poly glutamic acid (PGA)[4].To prevent possible loss of the materials from the electrode surface, to improve the oxidation currents of analytes and to improve the anti-interferential ability of the sensor, PGA film was constructed on the surface of the carbon paste modified electrode.

Hollow spheres of metal oxides have indeed attracted a great deal of attention because of their wide variety of applications, including catalysis [5] photonic materials [5] drug delivery [6] and so forth. Among them, several researches have been done on the synthesis of TiO_2 Hollow Spheres.

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These sub-micrometer size structures are grown either directly or by application of some templates of soft materials. The templates could be finally sacrificed to form hollow scattering structures. TOHS could be prepared using sub-micrometer size carbon or polystyrene templates or even by template-free methods. In the template-free methods, the size distribution of TOHS is quite wide with sizes in the range of 200 nm to several micrometers. In this work, TOHS were synthesized by liquid phase deposition (LPD) of TiO_2 on the carbon spheres (CS) template. The templates can be of different composition, for example, silica [7] and carbon particles [8], have been used. Because of the facile removal of carbonaceous materials fabricated by hydrothermal carbonization, we chose carbonaceous spheres to fabricate TOHS. The TOHS photocatalyst has attracted considerable attention due to its high porosity, high surface area, low bulk density and higher photocatalytic activity for pollutants than the TiO_2 particle photocatalyst [9-11].TOHS was used for modification of this sensor because of its high surface area and electrocatalytic activity.

MWCNTs, owing to their unique structures, high stabilities, high surface to volume ratios, and low resistivities, are extremely attractive in various fields such as catalysis of redox reactions [12-14], nanoelectronics[15], electrochemical sensors [16, 17], etc. Sensors based on CNTs have received a lot of attention and have largely improved the voltammetric response (lower overvoltages and higher peak currents) of a variety of biological, clinical and environmental compounds [18].

Dopamine (DA,3,4-dihydroxyphenylethylamine) is an important neurotransmitter of the catecholamine group and is well characterized by its electrochemical activity [19]. DA has been detected with using various analytical methods such as mass spectrometric [20], fluorometric [21], radioenzymatic[22] and chromatographic [23, 24]. However, some of these methods suffer from some disadvantages such as requirement for sample pretreatment, low sensitivity or selectivity, high costs, the use of organic solvents and long analysis times. Therefore the electrochemical determination of DA has received considerable interest due to the important advantages of electrochemical instrumentation such as lower cost, good sensitivity and speed[25-27].

Piroxicam(PRX, 4-hydroxy-2-methyl-N-2-pyridinyl-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide or feldene) is a drug that possesses analgesic and antipyretic properties. PRX has been used for the treatment of inflammatory illnesses [28]. Several methods like high performance liquid chromatographic (HPLC) [29-32], double-beam UV spectrophotometry [33, 34], spectrofluorometry[35], thin layer chromatography [36], capillary electrophoresis [37] and liquid chromatography with UV detection [38-40], were reported for the determination of PRX.

It has been found that PRX produced dual effects on dopamine-related behaviors in body [41]. Normally human use ascorbic acid (AA) in food dietary or medicine and then AA could be present in human blood or urine. Therefore it would be useful to study simultaneous determination of DA and PRX in the presence of AA.

To the best of our knowledge, no study has been reported so far on the simultaneous determination of DA and PRX in the presence of AA. Herein, we report the preparation and application ofa novel carbon paste electrode modified with TOHS, MWCNTs and PGA film (PGA/TOHS/MWCNTs/CPE) for simultaneous determination of DA and PRX in the presence of AA.The modified electrode showed good sensitivity, excellent selectivityand low detection limit with wide linear dynamic range. The analytical performance of the modified electrode in quantification of DA and PRX in human urineis evaluated with satisfactory results.

MATERIALS AND METHODS

DA, PRX and AA were obtained from Sigma chemical company. Glutamic acid, Glucose, Ethanol, Titanium (IV) isopropoxide (TTIP) were purchased from Merck chemical company. MWCNTs (purity more than 95%) with number of walls 3-15, and tube length 1–10 micro meter were purchased from PlasmaChem GmbH Company. The reagents were analytical grade and used without any further purification.

All solutions were freshly prepared with triply distilled water. Ammoniabuffer solutions were prepared from stock solution of 0.1M NH_3 and 0.1M NH_4Cl.The suitable pH was adjusted using concentrated HCl and ammonia solutions. Electrochemical experiments on DA and PRXwere carried out in 0.1 M Ammonia buffer at pH 7.0. Fresh human urine was purchased from Razi institute of vaccine and serum company (Tehran,

Iran). The urine sample was filtered and diluted 20 times using a 0.1 M ammonia buffer solution of pH 7.0.

Synthesis of TOHS

Preparation of CS

CS with about 200nm size were synthesized by a hydrothermal method [42]. Briefly an aqueous solution of glucose with concentration of 0.5M was prepared in the first step. Then it was transferred to a 100 ml Teflon-lined stainless steel autoclave and heated at 180 ˚C for 8 h. The dark-brown precipitate was centrifuged and washed with ethanol and water for several times. Finally the CS was dried at 70 ˚C for 5 h.

Preparation of TOHS

TOHS were prepared by LPD of TiO_2 on the CS template. 0.2g of the as-prepared CS was added to 20 ml ethanol solution and fully dispersed. Then 0.002mol of TTIP was added to the solution. The mixture was stirred at room temperature for 24 h for the LPD process. The CS/TiO_2 precipitate was centrifuged and washed with ethanol and water and dried at 40 ºC for 10 h. Finally the precipitate was heated at 450 ºC to remove the carbon templates and form the pure TiO_2 structures [43]. As illustrated schematically in Fig. 1., the process is expected to result in hollow spheres of TiO_2.

Instrumentation

All the voltammetric measurements were carried out using the proposed modified carbon paste electrode, (PGA/TOHS/MWCNTs/CPE) as the working electrode, Ag/AgCl 3MKCl as the reference electrode and platinum wire as an auxiliary electrode. DPV, CV, CA and the electrochemical impedance spectroscopy (EIS) experiments were carried out in nitrogen-saturated water by using an Autolab PGSTAT 30 Potentiostat/Galvanostat (EcoChemie, The Netherlands) coupled with a 663 VA stand (Metrohm, Switzerland). pH measurements were performed with a Metrohm 744 pH meter using a combination glass electrode. The morphology of the TOHS was investigated by scanning electron microscopy (SEM, Leica Cambridge, model S 360). Infrared spectra were recorded as pressed KBr discs, using a Unicom Galaxy Series FT-IR 5000 spectrophotometer.

Preparation of PGA /TOHS/MWCNTs/CPE

The proportions of TOHS and MWCNTs affect the sensitivity of the modified sensor. It was found when the proportions by mass of TOHS and MWCNTs increased from 3 to 20 % and 1 to 6 % respectively, the response of the electrode improved and when the proportions were more than 20% and 6% for TOHS and MWCNTs respectively, the response decreased with larger background current (Fig. S1). Therefore TOHS/MWCNTs/CPE was prepared by mixing appropriate amount of graphite powder, MWCNTs and TOHS (%w/w, 74:6:20) thoroughly in a mortar and then proper amount of paraffin oil was added to form a carbon paste. A portion of the carbon paste was firmly filled into one end of a glass tube (ca. 2 mm i.d. and 10 cm long) and a copper wire was inserted through the opposite end to establish an electrical contact. Prior to use, the TOHS/MWCNTs/CPE was polished on a piece of weighing paper and rinsed with double distilled water. Afterwards, the PGA/TOHS/MWCNTs/CPE was prepared by electro-polymerization of TOHS/MWCNTs/CPE in 0.1M ammonia buffer at pH 7.0 containing 0.05 M glutamic acid in the potential range of −0.2 to 2.0 V at 100 $mV \cdot s^{-1}$ for 15 cycles (Fig. S2).

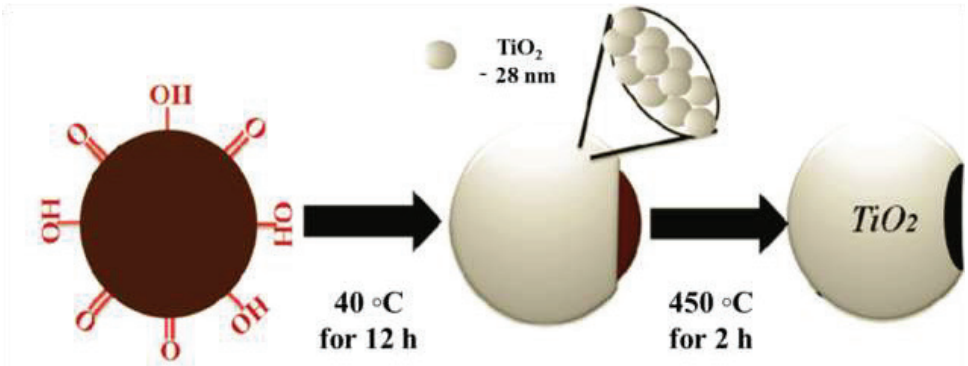

Fig. 1. Scheme of three phases of TOHS, illustrating the growth of nanostructured file over a CS and the removal of template.

Sample preparation for FTIR spectroscopy analysis

Before FTIR spectroscopy analysis, a graphite rod as working electrode was used. The PGA was obtained by electro-polymerization on the graphite rod working electrode in 0.1M Ammonia buffer at pH 7.0 containing 0.05M glutamic acid in the potential range of −0.2 to 2.0 V at 100mV·s^{-1} for 15 cycles. After electro-polymerization, the PGA was separated from the surface of working electrode and dispersed in ethanol solution by sonication of graphite rod in 2ml ethanol solution. After this step, 30 µL of ethanol solution containing the PGA was poured on the pressed KBr disc to obtain FTIR spectrum of PGA.

General Procedure

The general procedure used to obtain voltammograms was as follows. Each sample solution (10 mL) containing 0.1M Ammonia buffer at pH 7.0 and appropriate amount of analysts were pipetted into a voltammetric cell. The modified electrode was regenerated by successive washing with triply distilled water and then 0.5% sodium hydroxide solution. The electrode was finally rinsed carefully with distilled water to remove all adsorbate from the electrode surface and to provide a fresh surface before running subsequent experiments. Before running electrochemical experiment, the solution underwent 200 seconds stirring.

EIS was performed in a solution containing 5mM of each of Fe $(CN)_6^{3-}$ and$Fe(CN)_6^{4-}$ and 0.1 M KCl at 0.23 V with the frequency swept from 10^5 to 0.01 Hz.

RESULTS AND DISCUSSION

Characterization of TOHS and TOHS/MWCNTs/Carbon composite

The scale of TOHS was characterized by means of SEM. Fig. 2a shows the hierarchical submicrometer TOHS that synthesis by using CS as template. CS is synthesized by a facile and environmentally friendly route. The produced CS shows a rough surface with high capacity for metal ion adsorption. A 3D network of hierarchical submicrometer TOHS were formed after removing the CS template at high temperature, exhibiting high specific surface area as well as good electrocatalytic property. In addition, it can be observed from Fig. 2a that TOHS have a spherical shapes and hollow structures with a diameter of about 200 nm. The shell thickness of TOHS is aggregation of several TiO_2 nanoparticles with about 50 nm size. Fig. 2b shows SEM image of TOHS/MWCNTs/Carbon composite. The results shows the TOHS and MWCNTs have a good dispersion in to carbon powder.

Characterization of PGA

Fig. S3 displays the FTIR spectra of glutamic acid (a) and PGA film (b). As can be seen, some of the peaks in the FT-IR spectrum of (curve a), vanishes in the FT-IR spectrum of (curve b) suggesting the successful formation of PGA.The FT-IR absorption spectrum of solid glutamic acid is constituted of the peaks of C—O stretching vibration at 1643 cm^{-1},C—H, N—H and O—H stretching vibration of in the range of 2500 to 3500 cm^{-1}(curve a). Only after polymerization, a new band between 1550 cm^{-1} and 1620 cm^{-1} is registered. The new large band of higher intensity corresponds to the δNH vibrations [44]. Also, at 3400 cm^{-1} absorption bands for N–H stretch corresponding to secondary amides is registered [44].

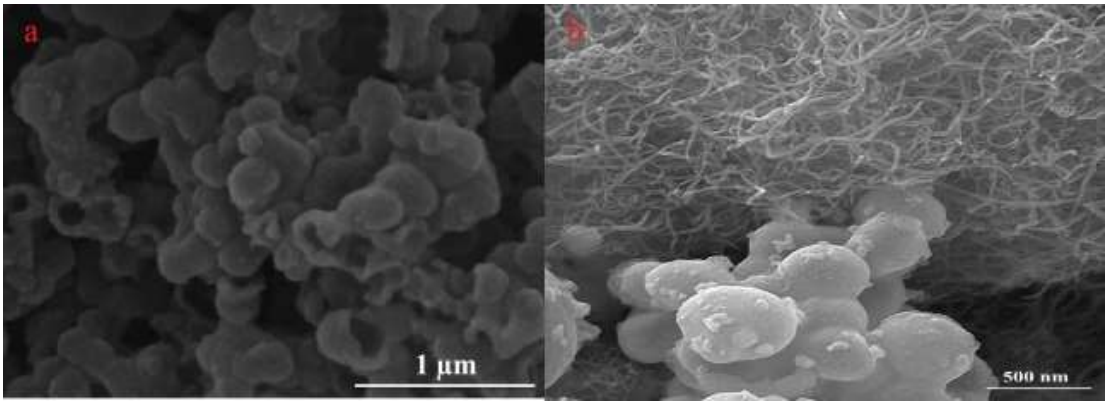

Fig. 2. SEM image of the TOHS (a) and TOHS/MWCNTs/Carbon composite (b).

Optimization of Operational Parameters

The electrochemical oxidation peak currents of modified electrode toward DA and PRX were measured in different media as phosphate, Britton-Robinson, ammonia and acetate buffer solutions at pH 7.0. The best sensitivity toward DA and PRX was obtained in ammonia buffer solution.

The influence of solution pH on the voltammetric response of the PGA/TOHS/MWCNTs/CPE towards DA and PRX in the simultaneous determination of 80µM DA and 100µM PRX was investigated by using CV method (Fig. 3). As can be seen from Fig. 3B, the highest oxidation peaks for simultaneously determination of DA and PRX could be obtained at pH=7. Therefore the pH value of 7.0 was chosen as an optimum solution pH for further experiments.

Relationship between oxidation peak potential of DA and PRX with pH was showed in Fig. 3C. It was found that by the variation of pH, the corresponding oxidation peak potentials for DA and PRX vary linearly with pH as follows:

E_{pa} versus ·Ag/AgCl (V) = 0.61–0.0574 pH (R^2 = 0.995) **DA**

Ipa(µA) = 0.546ν (mV s^{-1}) + 12.519 (R^2= 0.991) **PRX**

The slope of the equation 1 is close to the Nernstian amounts which suggest an equal number of electron and proton transfers are involved in the electrochemical oxidation. However the slope of the equation 2 is close to the system which expected for a two electron and one proton electrode reaction, which is 0.0296 V/pH at 25 °C [45]. In our previous report, we conclude that mechanism of the electrochemical oxidation of PRX is two electron and one proton electrode reaction [46].

The effect of accumulation time ranged from 40 to 400 s on the oxidation peak currents of DA and PRX at the PGA/TOHS/MWCNTs/CPE for the simultaneous determination of 80µM DA and 50 µM PRX was investigated using CV method in ammonia buffer at pH 7.0 (not shown). The anodic peak currents of DA and PRX improve with accumulation time, but after 200 s for DA and after 150 s for PRX remained almost stable then it level off. This may be due to saturation of the amount of DA and PRX adsorbed on the modified electrode surface. Thus, the accumulation time of 200 s was selected as an optimum time for subsequent experiments.

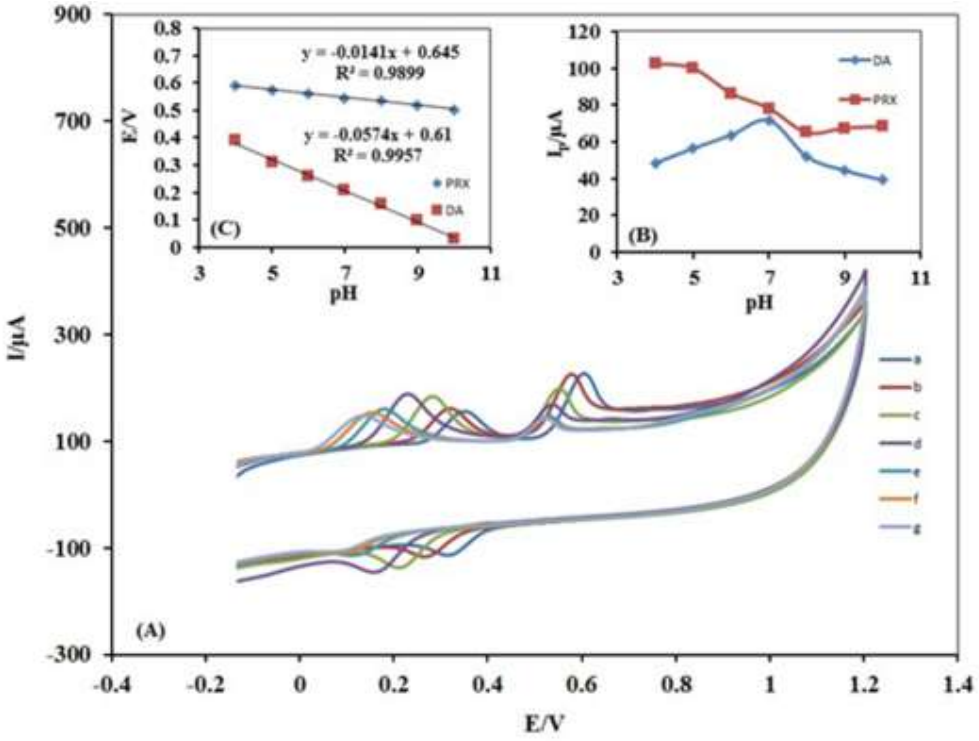

Fig. 3. (A) Cyclic voltammograms of 80 µM DA and 100 µM PRX compounds at PGA/TOHS/MWCNTs/CPE in 0.1 M ammonia buffer solution at different pHs: 4(a), 5(b), 6(c), 7(d), 8(e), 9(f), 10(g). Insets: (B) Plot of anodic peak currents (Ipa) of DA and PRX as a function of pH values. (C) Plot of potential values as a function of pH values.

Electrochemical Impedance Spectroscopy Studies

Through the modification process, the impedance on the surface of the electrode noticeably changes which can then usefully employed in EIS. Fig. 4 shows the Nyquist plots (-z'' vs. z') for (a) CPE, (b) CPE modified with TOHS/MWCNTs/PGA. Nyquist plots were obtained when the electrodes were immersed in a 0.1 M KCl solution containing 5mM in both $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$. In the Nyquist diagram, the semicircle diameter of EIS is equal to R_{ct}.

The results showed the diameter of the semicircle for the modified electrode with TOHS/MWCNTs/PGA is smaller than that of the unmodified CPE. As a result, the modified electrode provides lower electron transfer resistance in comparison with CPE. These results demonstrate that PGA/TOHS/MWCNTs/CPE have an excellent potential to be an electric conductive material which are also able to accelerate the electron. Therefore this composite was used for electrochemical determination of DA and PRX.

Electrochemical Studies of DA and PRX on Modified Electrode

In the pH range of 4–10 PGA (pK_a = 4.45) with a carboxylic acid group which did not participate in the polymerization reaction and AA (pKa = 4.10) exist in anionic form. At neutral pH, PRX exist in different prototropic forms, which include neutral form, zwitterions and anionic form [47]. In addition DA is in cationic form. Therefore under physiological conditions a PGA film strongly repulses anionic AA. Therefore the modified electrode can apply for simultaneous determination of DA and PRX in presence of AA.

Differential pulse voltammograms recorded for a solution of 100mM of DA and PRX in presence of 0.4mM of AA in ammonia buffer at pH 7.0 at (a) CPE, (b) MWCNTs/CPE,(c)TOHS/MWCNTs/CPE and (d) PGA/TOHS/MWCNTs/CPE are shown in Fig.5. As can beseen in Fig. 5, the PGA/TOHS/MWCNTs/CPE provides higher peak currents for the oxidation of DA and PRX. In addition we can see the oxidation peak current due to presence of AA in Fig 5b and c disappears in Fig. 5d. Therefore, PGA film on the surface of electrode can strongly repulses anionic AA and highly attracts cationic DA and PRX.Therefore, it was concluded that PGA/TOHS/MWCNTs/CPE can be used as the excellent sensor for selective simultaneous electrochemical determination of DA and PRX in presence of a

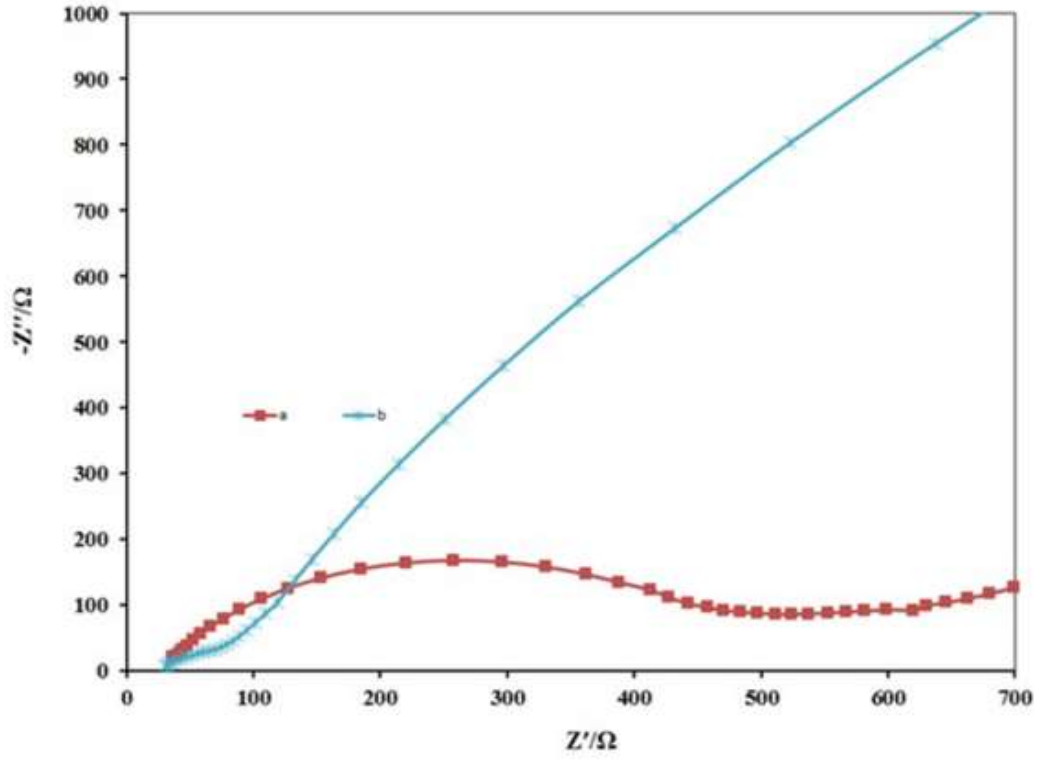

Fig. 4. Nyquist plots for CPE before (a) and after modification with (b) PGA/ TOHS/MWCNTs obtained when the electrodes immersed into solutions of 5mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ and 0.1M KCl solution.

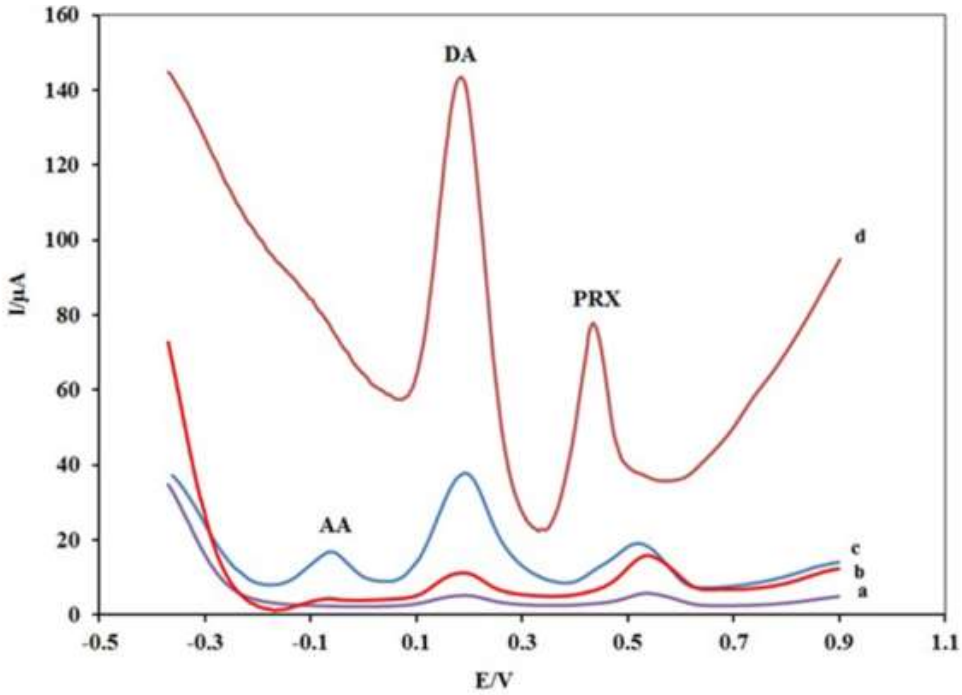

Fig. 5. The DPV voltammograms of CPE (a), MWCNTs/CPE (b), TOHS/MWCNTs/CPE (c) and PGA/TOHS/MWCNTs/CPE (d) for 100 μM DA PRX in presence of 0.4mM AA in 0.1M ammonia buffer solution (pH 7.0) at scan rate of 100 mV s^{-1}.

large excess of AA.

The influence of variation of scan rate on the oxidation responses of DA and PRX over the 10-650 mVs^{-1} range of scan rate at the PGA/TOHS/MWCNTs/CPEin 0.1M ammonia buffer solution (pH 7.0) were investigated by CV (Fig. 6). The linear relationships between the oxidation peak currents and scan rates were observed for DA in the range of 10-200 mVs^{-1} and PRX in the range of 10-160 mVs^{-1} as follow:

I_{pa}/μA = 28.01$v^{1/2}$–52.21 (v /mV s^{-1})$^{1/2}$ (R^2 = 0.990) **DA**

I_{pa}/μA = 0.101$v^{1/2}$+ 23.62 (v /mV s^{-1})$^{1/2}$ (R^2 = 0.995) **PRX**

These phenomena suggest that the redox reactions of two compounds at PGA/TOHS/MWCNTs/CPE are adsorption-controlled processes at such scan rates. At sweep rates from 200.0 to 650.0 mV.$s^{-1}$and 160.0 to 650.0 mV.s^{-1} for DA and PRX respectively, the plot of currents versus scan rate deviate from linearity. However the corresponding peak currents relate linearly with the square root of scan rate ($v^{1/2}$) as follow:

E_{pa} versus ·Ag/AgCl (V) = 0.645–0.0141 pH (R^2 = 0.989) **PRX**

Ipa(μA) = 1.652ν (mV s^{-1}) + 1.076 (R^2 = 0.997) **DA**

The results indicate the oxidation of DA and PRX are diffusion control at such scan rates. Peak separations (DE_p) for DA begin to increase, at scan rates higher than 200/n mV s^{-1}, demonstrating the limitation due to charge transfer kinetics (Fig. 6a). Based on Laviron theory [48] the charge transfer coefficient (α) and electron transfer rate constant (k_s) can be determined by measuring the variation of ΔE_p vs. log scan rate. The slopes of E_{pc} vs. log (n) are -0.05 and 0.07 for reduction and oxidation of DA, respectively. Using the equations of:

$$E_{pc} = K - 2.303\ (RT/\alpha_c nF)\ \log\ (\nu) \quad (7)$$

$$E_{pa} = K + 2.303\ (RT/\alpha_a nF)\ \log\ (\nu) \quad (8)$$

By considering two electrons transferred for DA, cathodic (α_c) and anodic (α_a) charge transfer coefficients of 0.422 and 0.465 were obtained. Substituting the α value into the following equation, an apparent surface electron transfer rate constant, k_s= 2.55 s^{-1}, was obtained.

$$\text{Log } k_s = \alpha \log (1-\alpha) + (1-\alpha) \log\alpha - \log (RT/nF\upsilon) - \alpha(1-\alpha)\ NFE/2.3RT$$

For an irreversible anodic reaction, the

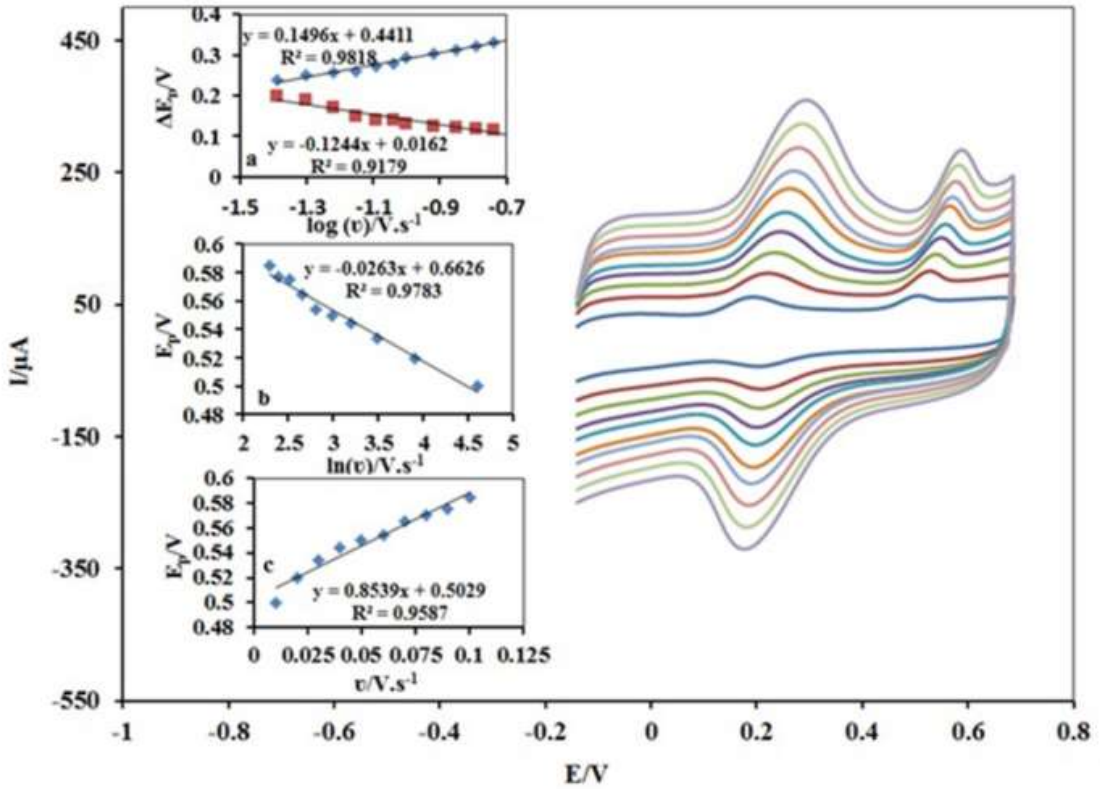

Fig. 6.Cyclic voltammograms100 μM DA and PRXat different scan rates (from inner to outer) 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.1Vs. Insets: (a) peak separations (DEp) for DA as a function of log (υ), (b) peak potential for PRX as a function of ln (υ) and (c) peak potential for PRX as a function of (υ).

relationship between E_p and v describes by Laviron's theory as follows:

$$E_p = E^0 - \left(\frac{RT}{\alpha_a nF}\right)\ln\left(\frac{RTk_s}{\alpha_a nF}\right) + \left(\frac{RT}{\alpha_a nF}\right)\ln \nu \quad (10)$$

According to the slope of the straight line of E_p against lnv (Fig. 6b), the value of α_a of the PRX was calculated to be 0.49. The value of E^0 in Eq.(10) can be obtained from the intercept of Ep vs. v (Fig. 6c) curve by extrapolating to the vertical axis at v=0[49]. From E^0, 0.5021 for DA and α_a value, the value of k_s for the DA was calculated equal to 1.03 s^{-1}.

The large value of the electron transfer rate constants shows the high ability of the modified electrode for promoting electron transfer between the DA, PRX and the electrode surface.

Linear dynamic range and detection limit of the method

The electrochemical behaviors of simultaneous additions of solutions of DA and PRX in the presence of the 0.4mM of AA at the PGA/TOHS/MWCNTs/CPE in 0.1M ammonia buffer solution (pH 7.0) are depicted in Fig.7. Fig. 7a and b shows the corresponding calibration curves of mixture of DA and PRX in presence of 0.4mM AA respectively. Oxidation peak currents of DA and PRX were proportional to the concentration in the range of 0.3-60 μM and 0.4-80 μM, respectively.A detection limit of 0.2 μM and 0.3 μM (S/N = 3) was obtained for DA and PRX, respectively.The investigations revealed that these linear ranges were kept in mixture solutions of DA and PRX, showing high efficiency of the fabricated modified electrode for determinations of these compounds.

CA measurements

The diffusion coefficient (D) of DA and PRX at PGA/TOHS/MWCNTs/CPE can also be determined by CA method (Fig. 8). Chronoamperometric determinations were carried out at the modified electrode by setting the working electrode potential at 320 mV and 600 mV for DA and PRX respectively. In accordance to Cottrell equation [50], for an electroactive material (DA and PRX in

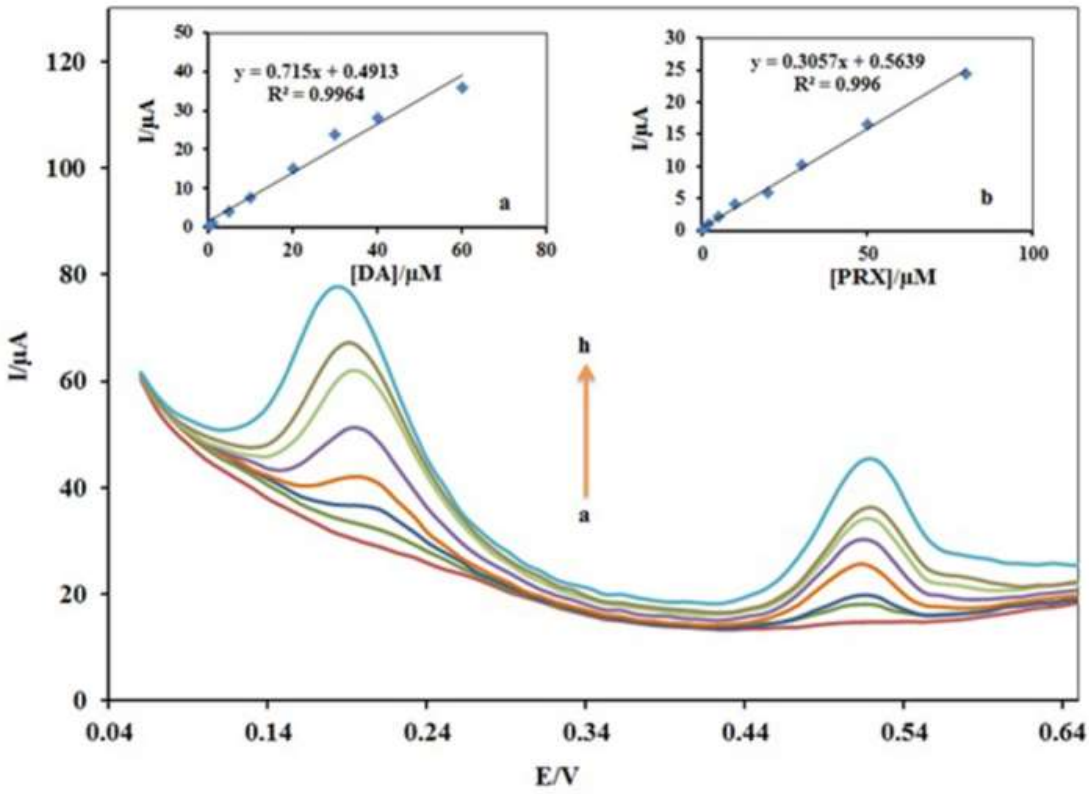

Fig. 7. Differential pulse voltammograms for different concentrations of DA and PRX mixture in presence of 0.4mM AA as (a) 0.3 + 0.4, (b) 1 + 2, (c) 5 + 5, (d) 10 + 10, (e) 20 + 20, (f) 30 + 30, (g) 40 + 50 and (h) 60 + 80 respectively, in which the first value is the concentration of DA in µM and the second value is the concentration of PRX in µM. Insets: (a) Plot of peak currents as a function of DA concentration. (b) Plot of the peak currents as a function of PRX concentration.

this case) with a diffusion coefficient of D, currents due to electrochemical reactions, are described by the following equation:

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2}$$

where D and C_b are regarded as diffusion coefficient ($cm^2\ s^{-1}$) and bulk concentration (mol cm^{-3}), respectively. When an electrochemical reaction is diffusion control (mass transport), a plot of I vs. $t^{-1/2}$ is linear, and the value of D can be obtained from the slope. Fig. 8c and d shows the plots of I vs. $t^{-1/2}$ for DA and PRX respectively. The mean values of D were found to be 20.35 $\mu cm^2\ s^{-1}$ and 28.610 $\mu cm^2\ s^{-1}$ for DA and PRX respectively.

Stability and Repeatability of the PGA/TOHS / MWCNTs/CPE

To evaluate the repeatability of the PGA/TOHS / MWCNTs/CPE, the peak currents of 10 successive measurements by DPV in a mixture solution of 100µM DA and 100µM PRX were determined. The relative standard deviations (R.S.D.) of 1.14% and 1.61% were obtained for DA and PRX, respectively. The results showed that the modified sensor is not subject to surface fouling by the oxidation products.

The proposed modified electrode showed good stability. This was tested by measuring the decrease in voltammetric current during repetitive DPV measurements of DA and PRX solutions with PGA/TOHS/MWCNTs/CPE stored in air or solution for certain period of time. For example, in the determination of 100 µM DA and 100 µM PRX in 0.1M ammonia buffer solution (pH 7.0), when the sensor was subjected to an experiment every 30 min, after 10h gave less than 9.32 and 8.27 % decrease in the oxidation currents of DA and PRX, respectively. When the electrode was stored in the atmosphere for 10 days, the oxidation peak current of DA and PRX in the solution were reduced less than 6.79 and 11.91 %, respectively. The results showed that the proposed modified PGA/TOHS/ MWCNTs/CPE has very good stability to use for

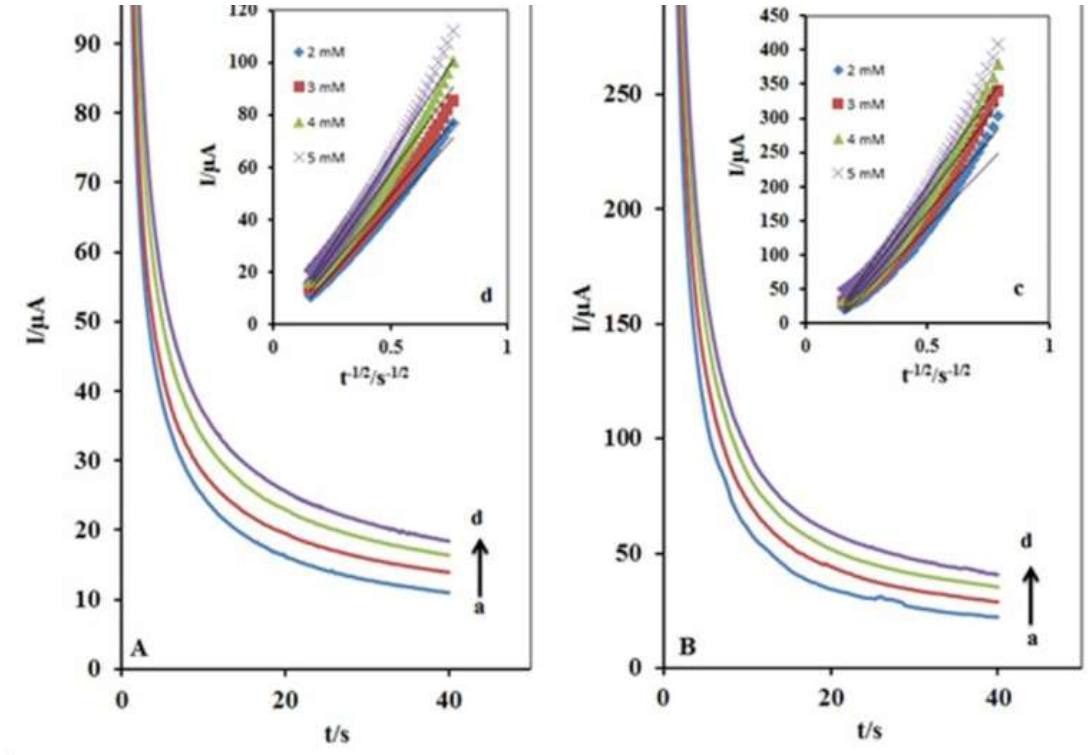

Fig. 8. (A) Chronoamperograms obtained in 0.1 M ammonia buffer solution (pH 7.0) in the presence of (a) 2, (b) 3, (c) 4 and (d) 5 mM of DA at PGA/TOHS/MWCNTs/CPE. (B) Chronoamperograms obtained in 0.1 M ammonia buffer solution (pH 7.0) in the presence of (a) 2, (b) 3, (c) 4 and (d) 5 mM of PRX at PGA/TOHS /MWCNTs/CPE. Insets: (d) Plots of I vs. $t^{-1/2}$ obtained from chronoamperograms in (A) for DA and (d) Plots of I vs. $t^{-1/2}$ obtained from chronoamperograms in (B) for PRX.

detection of these compounds in routine analysis.

Effect of Interferences and Analytical Applications

At pH 7.0, AA exists in anionic form. The free carboxylic acid group at anionic form in the structure of PGA on the CPE surface selectively attracts cationic species and allows them to pass through to the electrode surface. In contrast, anionic species were prevented from reaching the electrode surface. Therefore AA did not exchange electrons with the electrode. The effects of common interfering species in solutions of 50 μM DA and 50 μM PRX were investigated in the optimum measurement conditions. Table 1 lists the tolerance limit for each potential interferent, which is defined as the concentration of the interferent that gives an error of ≤ 10% in the determination of DA or PRX. The data show that interferences are only significant at relatively high concentrations, confirming that the proposed method is likely to be free from interferences from common components of biological samples.

The proposed method was applied to the simultaneous determination of DA and PRX in presence of AA in human urine at optimum conditions by DPV method with satisfactory results. The results for the determination of DA and PRX in human urine are summarized in Table 2. The samples were diluted 20 times before analysis and spiked with appropriate amounts of DA and PRX. The concentration of DA and PRX was

Table 1. Maximum tolerable concentration of interfering species.

Interfering species	DA C_{int}/ (μM)	PRX C_{int}/ (μM)
Tyrosin	1200	850
L-cysteine	500	600
Tramadol hydrochloride	500	400
Uric acid	400	550
Codeine	300	450

C_{int} refers to interfering compound concentration

Table 2. Estimationof DA and PRX in diluted (20-fold) urine.

Spiked (μM)		Found (μM)		[a]R.S.D. (%)		Recovery	
DA	PRX	DA	PRX	DA	PRX	DA	PRX
5	20	4.8	19.2	1.6	1.9	96.0	96.0
15	30	15.3	29.5	1.3	1.5	102	98.3

[a] Average of five determinations at optimum conditions

calculated using standard additions method in order to reduce any matrix effect. Good recoveries were obtained for spiked analysts revealing further evidence the proposed method is a reliable method for the direct simultaneous determination of DA and PRX in biological fluids.

CONCLUSION

This work demonstrates that PGA /TOHS/ MWCNTs modified carbon paste electrode is suitable for a selective simultaneous determination of trace amounts of DA and PRX. The electrode was coated with PGA film, a conductive polymer which is practically impermeable to ascorbic acid and the combination of TOHS, MWCNTs and carbon powder leads to excellent electrocatalytic performance for the simultaneous determination of DA and PRX. The electrode was used for simultaneous determination of DA and PRX in real biological sample with satisfactory results. The high selectivity, excellent sensitivity, wide linear range, low detection limit, high stability and good reproducibility for repeated determination suggest that the proposed electrode will be a good and attractive candidate for practical applications.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the research council of Arak University for providing financial support for this work.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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