Palladium nanoparticles modified electrode for the selective detection of catecholamine neurotransmitters in presence of ascorbic acid

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ABSTRACT

Palladium (Pd) nanoparticles are directly fabricated on glassy carbon electrode (GCE) and indium tin oxide electrode (ITO) by simple electrochemical deposition process. The Pd nanoparticles modified ITO electrode surface has been studied in detail using scanning electron microscopy (SEM) and atomic force microscopy (AFM). The electrodeposited nano Pd particles are found as spherical shaped in the size range of 39–78 nm. The X-ray diffraction (XRD) analysis reveals that the electrodeposited nano Pd film possesses the face centered cubic crystalline structure. This nano Pd film modified GCE effectively exhibits the electro oxidation signals for the detection of catecholamines epinephrine (EP), norepinephrine (NEP) and dopamine (DA). Especially, the proposed nano Pd film modified GCE successfully showed two well separated anodic oxidation peaks for the detection of catecholamines and ascorbic (AA) in mixture solution. The proposed nano Pd film modified electrode also retains the advantage of easy fabrication, high sensitivity and good repeatability. Finally, this type of nano Pd film modified electrode supports the selective detection of catecholamines in injection solutions.

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1. Introduction

The design and development of nanoparticles based biosensors have been found to be interesting research in the new era. In particular, noble metal nanoparticles were found as ideal supporting materials for the electrocatalytic activities because; they have their own fascinating surface structure, good electrical and mechanical properties, strong stability and limited aggregation and high performance. These properties clearly support their use as catalysts for commercially viable applications. For example, gold and platinum nanoparticles have their own mechanical strength and electrical properties, and they could be used as catalysts. Likewise, palladium (Pd) nanoparticles are part of the platinum group of metals, which possess their own special properties for the electrode modification process. Previously, the Pd nanoparticles modified electrodes have been used for various types of electrochemical sensors. For example, there have been reports of electro deposited Pd nanoparticles on single-walled carbon nanotubes for flexible hydrogen sensors [1], Pd-modified carbon nanotubes synthesized in supercritical fluid for oxygen reduction [2], electrodeposited nano platinum-Pd alloy in nafion film-coated GCE [3], Pd nanoparticles supported multi-walled carbon nanotubes [4] for the electrocatalytic oxidation of formaldehyde, and GCE–nafion–polyaniline–Pd nanoparticles modified electrode [5] for the electrocatalytic oxidation of formic acid. Further, Pd nanoparticles modified carbon ionic liquid electrode [6], multilayer polymer films [7],

Pd nanoparticles supported boron-doped diamond and Pd plated BDD micro disc array have been utilized for the electrocatalytic oxidation process of hydrazine [8]. Its uses are not only limited to electrochemical sensors, also have been applied for biosensors applications, such as glucose, DNA and H_2O_2 sensing. For instance, Pd nanoparticles modified GCE [9], Pd nanoparticles and glucose oxidase onto nafion-solubilized carbon nanotube electrodes for glucose sensing [10], Pd nanoparticles combined carbon nanotubes modified GCE for electrochemical DNA biosensors [11], Pd nanoparticles encapsulated channels for protein biosensing and the reduction of H_2O_2 [12], conductive Pd nanoparticles nanocage for H_2O_2 sensing [13], electro spun Pd nanoparticles-loaded carbon nanofibers for H_2O_2 and NADH reduction [14] and DNA-templated preparation of Pd nanoparticles on ITO for H_2O_2 reduction and ascorbic acid (AA) oxidation [15] have been reported.

Earlier literature reports reveal that the metal nanoparticles modified electrodes have also been utilized for selective detection and determination of catecholamine neurotransmitters in the presence of AA. They are, the nano-Au self assembled GCE for selective determination of epinephrine in the presence of ascorbic acid [16], nanostructured Pt–Au hybrid film for simultaneous determination of catecholamines in the presence of ascorbic acid [17], DNA combined gold nanoparticle multi layer films for selective determination of norepinephrine in presence of AA [18], boron-doped diamond electrode modified with gold nanoparticles/polyelectrolyte-coated polystyrene colloids [19], nano-Au self-assembly on GCE [20], and ferrocene encapsulated Pd-linked ormosil based biosensor for the dopamine detection process [21], respectively.

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In this report, for the first time, we have utilized Pd nanoparticles modified electrodes for the selective detection and determination of catecholamines in presence of AA. The present work reports about the electrochemical fabrication of Pd nanoparticles on GCE and ITO by cyclic voltammetry (CV). The electrodeposited nano Pd film has been characterized by scanning electron microscopy (SEM), atomic force microscopy (AFM), X-ray diffraction (XRD) and electrochemical impedance studies (EIS). The nano Pd film modified electrodes showed significant selectivity for the detection of EP, NEP and DA in the presence of higher concentrations of AA. Also, the proposed nano Pd film modified electrode showed obvious results for the detection of catecholamines in injection solutions.

2. Experimental

2.1. Reagents

Potassium tetra chloro palladate (II) was obtained from Strem chemicals (USA). Epinephrine (EP), norepinephrine (EP), dopamine (DA), and ascorbic acid (AA) were purchased from Sigma-Aldrich (USA). All the other chemicals (Merck) used in this investigation were of analytical grade (99%). Double distilled deionized water was used to prepare all the solutions. A phosphate buffer solution (PBS) of pH 7.0 was prepared using Na_2HPO_4 (0.05 M) and NaH_2PO_4 (0.05 M). Pure nitrogen was passed through all the experimental solutions.

2.2. Apparatus

Electrochemical measurements like cyclic voltammetry (CV), and differential pulse voltammetry (DPV) were performed using CHI 410a potentiostats (CH Instruments, Austin, TX, USA). A conventional three-electrode cell, consisting of BAS glassy carbon electrode (GCE) (φ = 0.3 cm in diameter) were in the form of disks sealed in a Teflon jacket having an exposed geometric surface area of 0.07 cm^2 as the working electrode, Ag/AgCl (saturated KCl) electrode as the reference electrode and a platinum wire as the counter electrode, were used at room temperature. All the potentials mentioned in this paper were referred to Ag/AgCl (saturated KCl) reference electrode. The surface morphological characterization of the film was studied by SEM (Hitachi S-3000H, Japan) and AFM (Being Nano-Instruments, CSPM-4000, China). For our convenience, indium tin oxide (ITO) thin film coated glass electrodes have been used for SEM and AFM analysis. Electrochemical impedance studies (EIS) were performed by using ZAHNER impedance analyzer (ZAHNER Elektrik Gmbh & Co KG, Germany).

2.3. Fabrication of Pd particles modified GCE

Prior to the electrode modification process, the bare GCE was polished with the help of BAS polishing kit using aqueous slurries of alumina powder (0.05 μm), rinsed and ultrasonicated in double distilled deionized water. The Pd particles are electrochemically deposited on GCE from 0.5 M H_2SO_4 solution containing 1×10^{-3} M potassium tetra chloro palladate (II), with a repetitive potential scan between 1.2 and −0.25 V (at the scan rate of 0.1 V s^{-1}) for seven cycles. Thus, the Pd particles modified GCE has been fabricated. Then the Pd particles modified GCE was rinsed with deionized water and applied for the further electrochemical studies.

3. Results and discussion

3.1. Characterization of Pd film modified GCE

Fig. 1 shows the cyclic voltammogram (CV) of Pd nanoparticles deposition process. Here the CV show the characteristic current features of Pd reduction (0.38 V), Pd oxide formation (0.67 V)
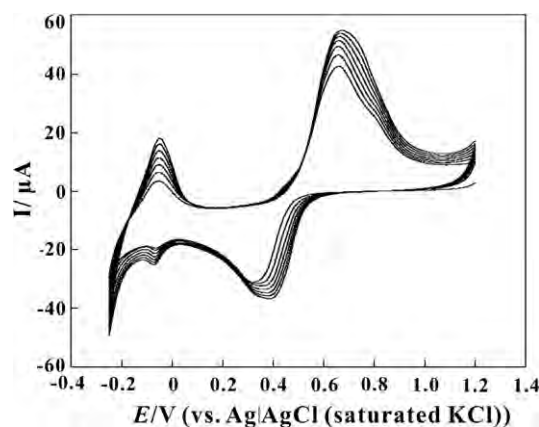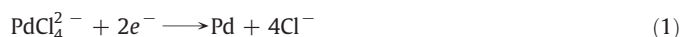

Fig. 1. (A) Repeated cyclic voltammogram of nano Pd film electro deposited on GCE from 0.5 M H_2SO_4 containing K_2PdCl_4 (1×10^{-3} M) and potential scan between 1.2 and −0.25 V for seven cycles (Scan rate of 0.1 V s^{-1}).

hydrogen adsorption and desorption (0.1 to −0.1 V) process. On scanning the potential in negative direction, Pd particles are deposited on the electrode surface and the peak at −0.08 V shows the reduction process of protons to hydrogen, which are adsorbed on the Pd surface. This reduction peak confirms the hydrogen adsorption process on the Pd surface. During the positive potential scanning process, the peak at −0.05 V is appears due to the oxidation of hydrogen atoms.

In the next step the deposited Pd particles are further oxidized to Pd^{2+} to form a Pd oxide layer (0.67 V) on the electrode surface [22]. The formed Pd oxides are further reduced on the negative-going scans, leading back to Pd particles with hydrogen adsorption process. During this repetitive cycling process, all the peaks are found growing which confirms the Pd nano particles deposition process on GCE. These observations clearly suggest the successive formation of nano Pd film on the GCE surface. Thus, nano Pd film modified GCE has been fabricated. The expected reduction process of the $PdCl_4^{2-}$ complex to Pd is as follows [22–24];

$$PdCl_4^{2-} + 2e^- \longrightarrow Pd + 4Cl^- \qquad (1)$$

The electrodeposited nano Pd film modified GCE was further transferred in pH 7.0 PBS for different scan rate studies (0.01–1 V s^{-1}) (Fig not shown). Here the cathodic reduction peak currents of nano Pd film are linearly proportional to the scan rate (Fig not shown) in the range of 0.01 to 1 V s^{-1}, and expected as a surface confined process with a correlation coefficient of 0.998. Also, the peak to peak potential separations of the nano Pd film is directly proportional to the scan rate. Further the surface coverage of nano Pd film has been calculated using the following equation:

$$\Gamma = Q / nFA \qquad (2)$$

The surface coverage (Γ) of nano Pd film is estimated to be about 5.70×10^{-10} mol cm^{-2} by integrating the charge (Q) of cathodic peak current of nano Pd film at the scan rate of 0.02 V/s. (where: n = no of electrons involved in the electron transfer process (n = 2); F — Faraday constant; Q = 7.78×10^{-6} C; A — geometric area of the GCE (0.0707 cm^2)) [25].

3.2. SEM and AFM analysis

Fig. 2(A) shows the typical SEM image obtained for the electrodeposited nano Pd film on ITO. Herein the SEM image has been obtained for 5 μm surface areas (view angle 90 degree). The SEM image shows that the Pd nanoparticles are uniformly dispersed on the electrode surface. During the electrochemical deposition process, few Pd nanoparticles are gathered to form as a group of nanoparticles
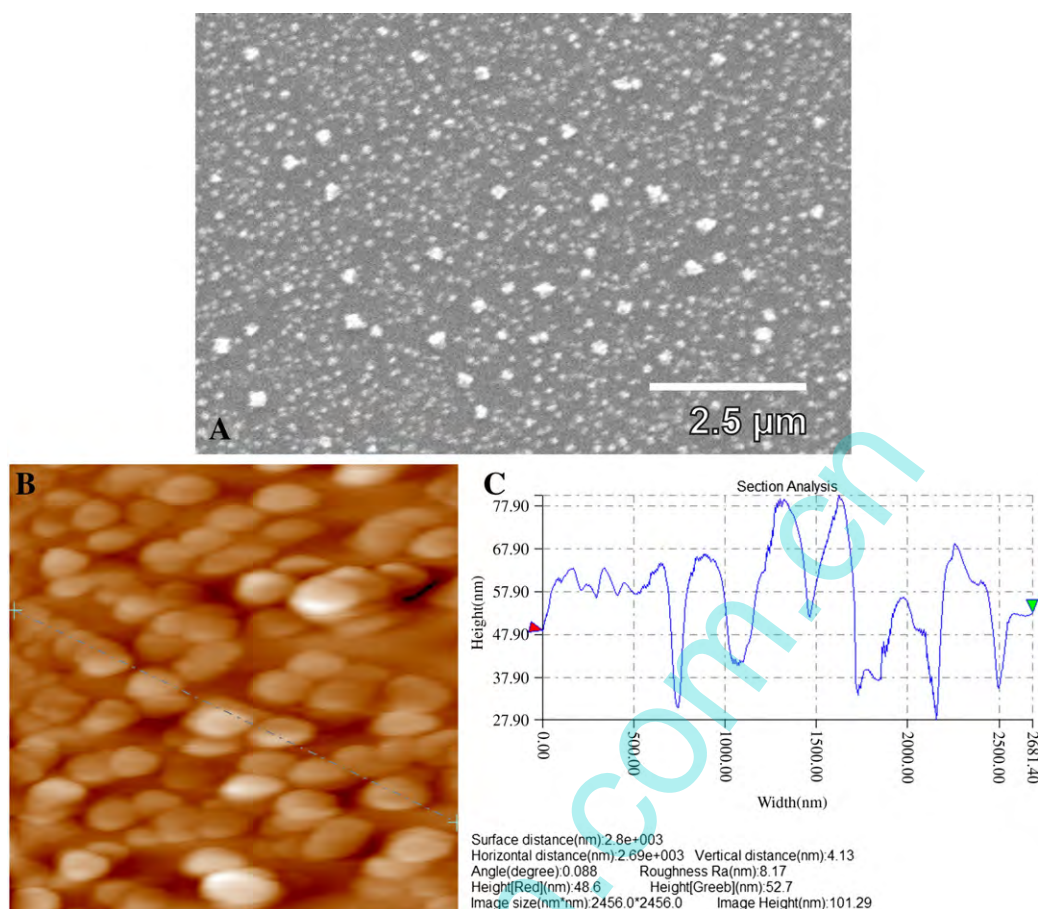
Fig. 2. (A) SEM image of nano Pd film on ITO (magnification 20 K, 90°). (B) AFM Topographic 2D image of nano Pd film on ITO. (C) Cross-sectional graph of the nano Pd film.

which has been noticed in the SEM. However, this type of formation has been found as few on the electrode surface and remaining the area are uniformly electro deposited with Pd nanoparticles. Based on this SEM analysis, the electrodeposited nano Pd particles have been found to be in the size range of 39–78 nm, respectively. Finally, the SEM analysis result authenticates the deposition of nano Pd particles on the electrode surface.

The electrodeposited nano Pd particles were also examined by using AFM technique. The AFM imaging provides more detailed information about the surface morphology and homogeneity of the nano Pd film. Fig. 2B and C shows a typical AFM 2D image and cross section analysis graph of the electrodeposited nano Pd film. The AFM tapping mode has been employed for the surface analysis of nano Pd film on ITO. Particularly, Fig. 2B represents the magnified close view of Pd nano particle film. From the Fig. 2B, it can be seen that the electrodeposited nano Pd film has been found as uniform one, which indicates that the Pd nanoparticles are found to be in the well dispersed stage. Further the

electro deposition process of the nano Pd film has been optimized by varying the concentration, no of cycles and scan rates. All these variation studies have been examined using AFM (Table 1).

At the 1×10^{-3} M concentration range, for 14 cycles, the film exhibited with the deposition of linear size range of nanoparticles and possesses the higher roughness value. For 28 cycles, in the same concentration range (1×10^{-3} M), it shows a non linear size range of nanoparticles deposition along with decrease in the average surface roughness. For 56 cycles, in the same concentration range, the film exhibits as a distorted one and failed to produce the nanoparticles. At the 5×10^{-3} M concentration range, for seven cycles (at the scan rate of 0.02 V s^{-1}), big size nanoparticles are deposited on the electrode surface. In the same concentration range (5×10^{-3} M), for 0.05 and 0.1 V s^{-1} scan rate studies big size Pd particles are deposited. Therefore, the utilization of higher concentrations (5×10^{-3} M) resulted in the formation of big size Pd particles and failed to produce the Pd particles in the nano scale range, respectively. Finally, at 1×10^{-2} M concentration

Table 1
AFM analysis table for the Pd deposition process by varying the concentration, scan rate and number of cycles.

Number	Concentration of K_2PdCl_4 in 0.5 M H_2SO_4	Scan rate (V)	No of cycles	AFM parameters measured (nm)		Results observed in AFM analysis
				Roughness Average (AR) nm	Root mean square roughness (RMS) (nm)	
1	1×10^{-3} M	0.1	7	8.17	10.9	Linear size range of nanoparticles deposited
2	1×10^{-3} M	0.1	14	8.52	10.9	Linear size range of nanoparticles deposited
			28	7.53	9.77	Non linear size range of nanoparticles deposited
			56	–	–	Distorted thin film
3	5×10^{-3} M	0.02	7	–	–	Big size particles (absence of nano size)
		0.05	7	–	–	Big size particles (absence of nano size)
		0.1	7	–	–	Big size particles (absence of nano size)
4	1×10^{-2} M	0.1	7	–	–	Distorted thin film

range, for seven cycles (at 0.1 V s⁻¹) it exhibited as distorted Pd thin film. Based on these out coming experimental results, we conclude that the utilization of increasing concentration (5×10^{-3} and 1×10^{-2} M) doesn't favorable for the fabrication of nanoparticles and only distorted thin film with big size particles are obtained. For this reason, we have selected the seven cycles (at the scan rate of 0.1 V s⁻¹) as the optimized electro deposition condition for the Pd nanoparticles deposition process. A detailed surface morphological study has been done for the above mentioned film and various surface parameters have been analyzed. First, the average roughness (AR) value (Table 1) is obtained from the several image topographies of corresponding nano Pd film. These values are average calculated from several images acquired in different regions of the respective sample. In most of the regions, the Pd nanoparticle film exhibited as most regular uniform particle dimensions with low ARs. Generally, the bare ITO surface exhibits a very smooth morphology, with the RMS roughness being smaller than 0.25 nm. However, the increase in the RMS roughness value has linear relationship with the density and the size of Pd nanoparticles on the electrode surface. Based on this AFM analysis, the maximum diameter of the nano Pd particles are found in the size range of 76.37 nm, and the minimum size was 39.02 nm, respectively. Finally, the morphological characteristic natures of nano Pd film observed in AFM analysis are consistent with the SEM results.

3.3. XRD and electrochemical impedance studies of nano Pd film

X-ray diffraction spectrum of nano Pd film on ITO was shown in Fig. 3(A). Here the XRD spectrum of nano Pd film shows the reflection due to (111), (200) and (220) planes at $2\theta = 38.38$, 45.15 and 64.98, respectively. The XRD spectrum displays like broad peaks because of the presence of nano size Pd particles. In particular, the XRD spectrum shows one main characteristic peak of Pd (200) at 45.15, which validates the successful reduction of Pd metal complex to Pd. Based on these observed planes, the electrodeposited nano Pd particles possess the face centered cubic crystalline structure which is similar to Pd in the bulk phase.

Impedance spectroscopy is an effective method to probe the features of surface modified electrodes [26]. This study has been employed to analyze the detailed electrochemical activities of modified electrode with individual or mixed components. Here the complex impedance can be presented as a sum of the real, Z' (ω), and imaginary Z'' (ω), components that originate mainly from the resistance and capacitance of the cell. From the shape of an impedance spectrum, the electron-transfer kinetics and diffusion characteristics can be extracted. The respective semicircle parameters correspond to the electron transfer resistance (R_{et}) and the double layer capacity (C_{dl}) nature of the modified electrode. Fig. 3(B) shows the Faradaic impedance spectra, presented as Nyquist plots (Z'' vs. Z') for the nano Pd film modified GCE and bare GCE, respectively. The bare GC electrode exhibits an almost straight line (□) with a very small depressed semi circle arc ($R_{et} = 0.1$ ($Z'/K\Omega$)) which represents the characteristics of diffusion limited electron-transfer process on the electrode surface. At the same time, the nano Pd film shows like a depressed semi circle arc with an interfacial resistance due to the electrostatic repulsion between the charged surface and probe molecule Fe(CN)₆⁻⁴⁻ (o). This depressed semi circle arc ($R_{et} = 1.7(Z'/K\Omega)$) clearly indicates the capacitance behavior of the nano Pd film modified GCE. Thus, the electron transfer process will become as a slow process on the nano Pd film modified GCE. Finally, based on these illustrations, the slow electron transfer kinetics nature of nano Pd film has been authenticated.

3.4. EP, NEP and DA detection by CV and DPV

The electro catalytic oxidations of EP, NEP and DA have been examined on nano Pd modified GCE and bare GCE. Fig. 4A, B and C show the individual catalytic oxidation CVs of EP, NEP and DA on nano Pd modified and bare GCE. Here, the EP oxidation, at the bare electrode takes place at 0.375 V, but for the nano Pd film modified GCE, the oxidation potential shifts, and appears with a much enhanced anodic peak current at 0.267 V.
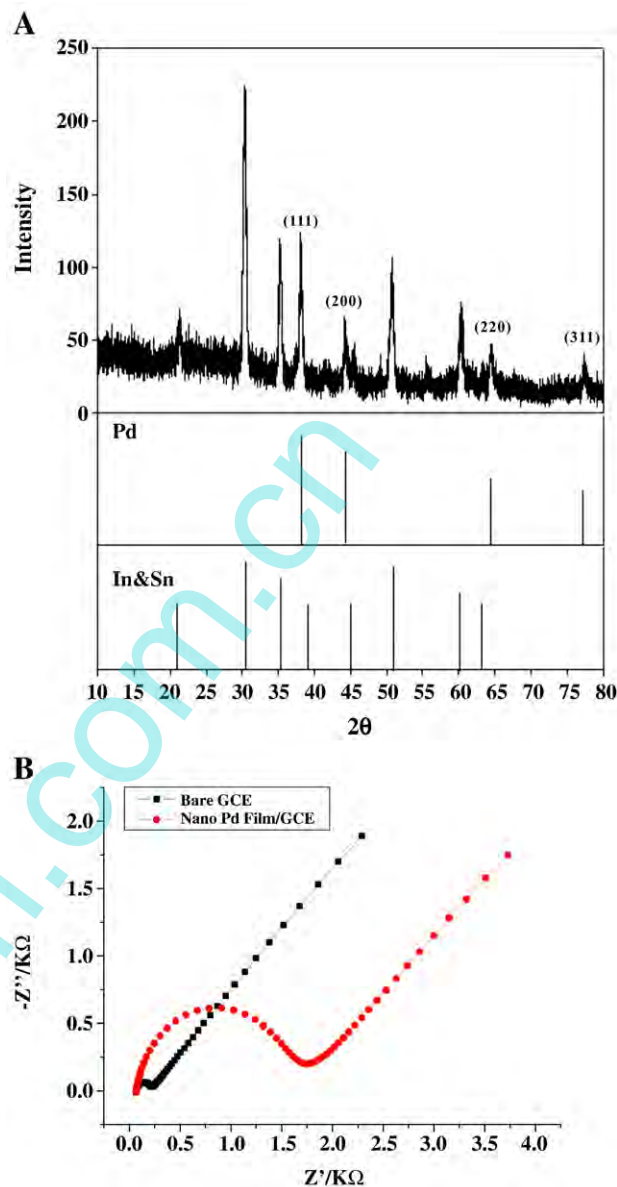

Fig. 3. (A) XRD pattern of nano Pd film on ITO. (B) Electrochemical impedance spectra curves of (□) bare GCE (o) nano Pd film modified GCE in pH 7.0 PBS containing 5×10^{-3} M [Fe (CN)₆]⁻³/⁻⁴. (Amplitude: 5 mV).

For NEP, the oxidation potential shifts to 0.239 V (for the bare GCE, it is at 0.320 V)). In the case of DA, the oxidation peak current appears at 0.239 V for bare GCE, and for nano Pd film modified GCE, it appears at 0.214 V. Thus, the potential shifts and current increases show that the nano Pd film modified GCE possesses good electrocatalytic activity for detecting EP, NEP and DA comparing with bare GCE. Finally, these results clearly explicate the advantage of using nano Pd film modified GCE.

Since the differential pulse voltammetry (DPV) has a better sensitivity and resolution than cyclic voltammetry (CV), it is used for the determination of EP, NEP and DA on nano Pd film modified GCE. Fig. 5A shows the DPVs of the nano Pd film with various concentrations of EP in pH 7.0 PBS. Here the oxidation peak current increases linearly with the increasing concentrations of EP. This shows that the nano Pd film successfully catalyzes the EP. The oxidation peak currents of EP have been measured and plotted against the concentration.

As shown in the inset of Fig. 5A, the dependence of peak current vs. concentration of EP is in the linear range of 34 to 349 μM. From this calibration plot, the linear regression equation of EP electro oxidation has been expressed as I_{pa} (μA) = 0.1508 C (μM) + 0.6339 with a
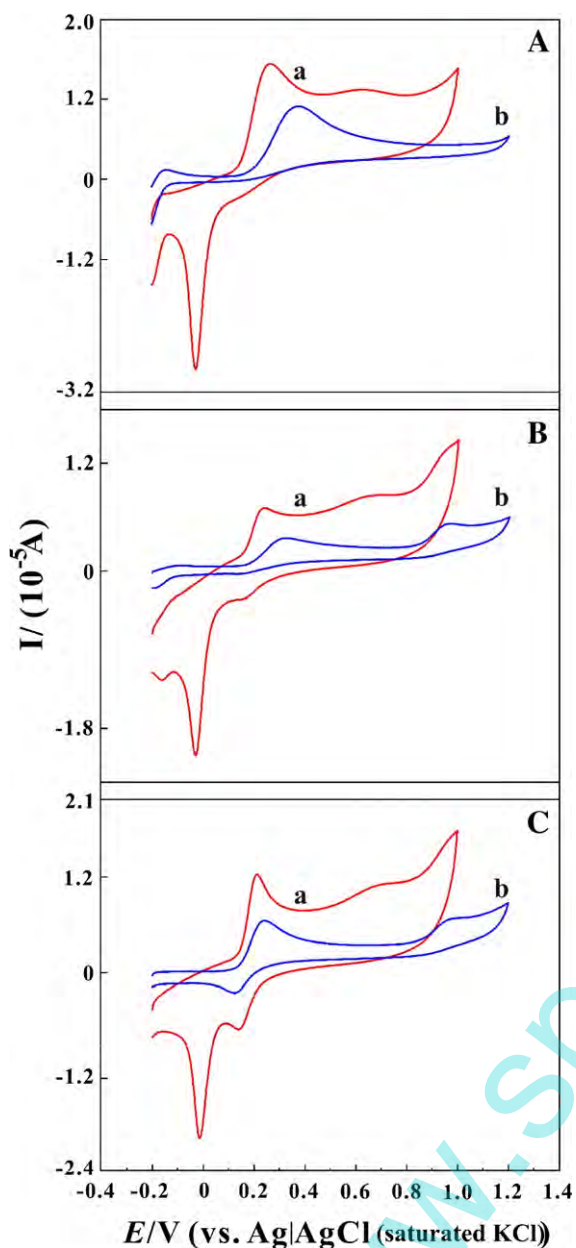
Fig. 4. (A) Cyclic voltammetric response of epinephrine at (a) nano Pd film modified and (b) bare GCE, epinephrine concentration = 3.5×10^{-4} M. (B) Cyclic voltammetric response of norepinephrine at (a) nano Pd film modified and (b) bare GCE, norepinephrine concentration = 1.8×10^{-4} M. (C) Cyclic voltammetric response of epinephrine at (a) nano Pd film modified and (b) bare GCE, dopamine concentration = 1.8×10^{-4} M.

3.5. Selective detection of EP, NEP and DA in higher concentrations of AA

The selective detection and determination of EP and NEP in the presence of higher concentrations of AA is difficult at bare unmodified solid electrodes. At the same time, it is possible at nano Pd film modified GCE. Based on the effect of pH and considering the physiological pH condition, we have selected pH 7.0 PBS buffer solution for the selective determination of EP, NEP and DA at nano Pd film modified GCE. In this, DPV technique has been employed because; it could provide a better peak resolution and current sensitivity, which is most suitable for selective determination of these species in mixture. Fig. 6A shows the DPV curves of EP for various concentrations (in the presence of 1.3 mM AA) at nano Pd film modified GCE. Here the sufficient enough peak separation occurred between the AA-EP oxidation peak potentials at the nano Pd film modified GCE. Further the oxidation peak currents of EP increase linearly in conjunction with increasing concentrations in the linear range of 34 to 279 µM. This result shows that the nano Pd film
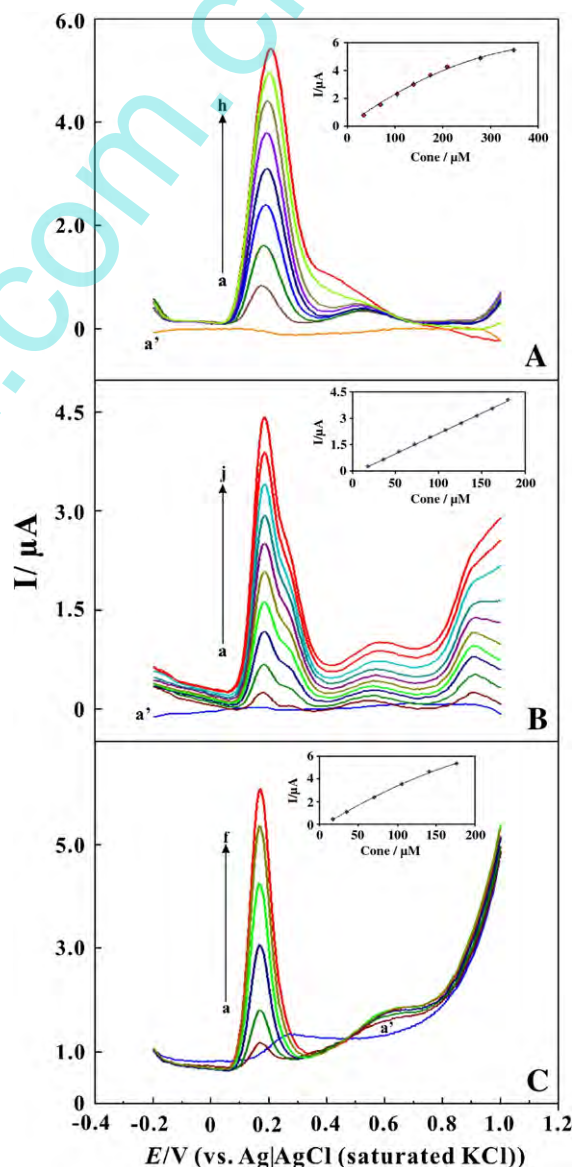

Fig. 5. (A) Anodic DPVs of nano Pd film for the different concentrations of EP in pH 7.0 PBS ($a' = 0$, a–h; 34, 69, 104, 139, 174, 209, 279 and 349 µM). Inset shows a current vs. concentration plot of EP. (B) DPVs of nano Pd film for the different concentrations of NEP in pH 7.0 PBS ($a' = 0$, a–j; 18, 36, 54, 72, 90, 108, 126, 144, 162 and 180 µM). Inset shows a current vs. concentration plot of NEP. (C) DPVs of nano Pd film for the different concentrations of DA in pH 7.0 PBS ($a' = 0$, a–f; 17, 35, 70, 106, 141 and 177 µM). Inset shows a current vs. concentration plot of DA.

correlation coefficient of $R^2 = 0.958$. The next attempt is employed for the NEP electro oxidation process. Fig. 5B shows the DPVs of NEP oxidation on nano Pd film modified GCE. In this, the oxidation peak currents of NEP increase linearly with the increasing concentrations in the range of 18 to 180 µM. The inset of Fig. 5B shows the oxidation current vs. concentration plot of NEP and based on this calibration plot the linear regression equation has been expressed as I_{pa} (µA) = 0.23 C (µM)−0.158, $R^2 = 0.999$. On the other hand, Fig. 5C shows the DPVs of DA oxidation on nano Pd film modified GCE. Here the oxidation peak currents of DA increase linearly along with increasing concentrations in the linear range of range of 17 to 177 µM. From the inset of Fig. 5C, the linear regression equation for DA oxidation has been found as I_{pa} (µA) = 0.3147 C (µM) + 0.005, $R^2 = 0.994$. Finally, all these DPV results validate that the proposed nano Pd film is a remarkable film for the individual electrocatalytic oxidation of catecholamines, respectively.

modified GCE possess the special electrocatalytic activity which could be considered as the main reason for the successful anodic peak separation between the catecholamines and AA. Here the sloping background current occurred at initial itself because; the electro catalytic current response of the higher concentration of AA (1.3 mM). The inset of Fig. 6A shows the current vs. concentration plot for EP in the presence of 1.3 mM AA. Based on this calibration plot, the linear regression equation for the selective determination of EP has been expressed as I_{pa} (μA) = 0.160 C (μM) + 0.5658, R^2 = 0.9858. Finally, the above result validates the capability of the nano Pd film modified GCE for the selective detection of EP in the presence of AA (1.3 mM).

Fig. 6B shows the DPV curves for the selective determination of NEP in the higher concentrations of AA (1.3 mM) at nano Pd film modified GCE. In this the oxidation peak current of NEP increases linearly along with increasing concentrations in the range of 9 to 126 μM. Here also the nano
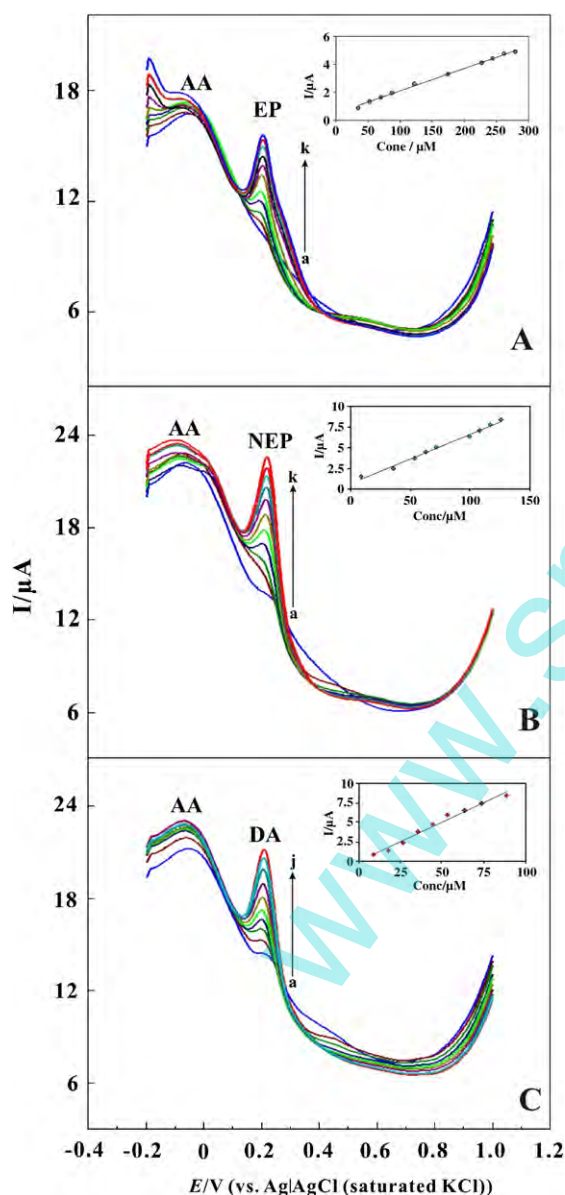

Fig. 6. (A) Anodic DPVs recorded for EP in pH 7.0 PBS containing 1.3 mM of AA. EP was in the concentration range of (a–k; 0, 34, 52, 69, 87, 122, 174, 227, 244, 261 and 279 μM). Inset shows a current vs. concentration plot of EP. (B) Anodic DPVs recorded for NEP in pH 7.0 PBS containing 1.3 mM of AA. NEP was in the concentration range of (a–k; 0, 9, 36, 54, 63, 72, 82, 99, 108, 117 and 126 μM). Inset shows a current vs. concentration plot of NEP. (C) Anodic DPVs recorded for DA in pH 7.0 PBS containing 1.3 mM of AA. DA was in the concentration range of (a–j; 0, 8, 17, 26, 35, 44, 53, 63, 73 and 88 μM) Inset shows a current vs. concentration plot of DA.

Table 2

Comparison chart for the selective determination of catecholamines in various metal nanoparticles based literature reports.

Number	Type of film modified electrode	AA (mM)	EP (μM)	NEP (μM)	DA (μM)	References
1	Nano Au self assembly	1	20–100	–	–	[16]
2	DNA and nano Au	1	–	0.005–800	–	[18]
3	Nano Au array	0.1	–	–	0.22–5.92	[27]
4	Polymer–nano Au	1	–	–	0.002–0.022	[28]
5	Sulfhydryl–nano Au	0.15	–	–	20–145	[29]
6	Nano Au–polyelectrolyte	1	–	–	5–100	[19]
7	Nano Au self assembly	0.2	–	–	2–7	[20]
8	Nano Pd	1.3	34–279	9–126	8–88	This work

Pd film modified GCE successfully exhibits two well separated electro oxidation peaks for the detection of NEP and AA. This peak separation is sufficient enough for the selective determination of NEP. Further the inset of Fig. 6B shows the calibration plot for the selective determination of NEP in the presence of AA and the linear regression equation has been found as I_{pa}(μA) = 0.6006 C (μM) + 0.5534, R^2 = 0.9901.

Fig. 6C shows the DPV response of nano Pd film modified GCE for the selective detection of DA in the presence of 1.3 mM AA. The nano Pd film modified GCE successfully exhibits two well separated anodic oxidation peaks for the selective detection and determination of AA and DA. The obtained peak separation at nano Pd film modified GCE is sufficient enough for the selective detection of DA in the presence of higher concentrations of AA (1.3 mM). Further the nano Pd film shows the current increase for the increasing concentrations of DA in the linear range of 8 to 88 μM. The inset of Fig. 6C shows the current vs. concentration plot for the selective detection and determination of DA at nano Pd film modified GCE. From this calibration plot, the linear regression equation for the selective detection of DA was expressed as I_{pa} (μA) = 1.014 C (μM) + 0.0625, R^2 = 0.9833. Based on these result, we conclude that the proposed nano Pd film modified GCE possess the capability for the selective detection and determination of AA-EP and AA-NEP, AA-DA successfully without any fouling effect. To validate the repeatability results, three GC electrodes are electrochemically deposited with nano Pd film and their responses towards the selective determination of EP, NEP and DA (in the presence of AA(1.3 mM)) have been investigated. Herein the peak to peak separation between the voltammetric signals of EP, NEP and DA with AA remained almost same for all the three electrodes, which authenticates the repeatable nature of the nano Pd film modified GCE. Finally, Table 2 shows the comparison chart for the selective determination of catecholamines at nano Pd film modified GCE with various metal nanoparticles based literature reports.

3.6. Selective determination of EP and DA in injection samples

The practical analytical performance of the nano Pd film modified GCE has been examined by evaluating the selective detection of EP and DA in injection sample mixtures. The adrenalin injection solution (epinephrine 1 mg ml^{-1}), dopamine injection solution (40 mg ml^{-1}), and ascorbic acid (500 mg) tablets are utilized for the real sample analysis. The Tables 3 and 4 show the real sample analysis results for the selective detection of EP and DA using the injection solution and tablet in mixtures. The real sample analysis results (Tables 3 and 4) have been obtained by standard addition method. The recoveries obtained here validates the pertinent nature of the nano Pd film modified GCE for the sensor applications, respectively.

Table 3

Determination of epinephrine in the presence of 1.3 mM vitamin C tablet solution.

Number	EP added (μM)	EP found (μM)[a]	Recovery (%)
1	34.9	34.8	99.7
2	52.3	54.0	103
3	69.0	70.0	100.2

[a] (n = 5).

Table 4
Determination of dopamine in the presence of 1.3 mM vitamin C tablet solution.

Number	DA Added (µM)	DA Found (µM) [a]	Recovery (%)
1	17.7	18.0	101.6
2	26.5	26.4	99.6
3	35.4	35.0	98.7

[a] ($n=5$).

3.7. Stability of nano Pd film

The stability of the nano Pd film modified GCEs have been investigated by storing them in pH 7.0 PBS (at room temperature). At room temperature, for continuous 5 h examination, it showed only 3% current decrease from the original one. Further at 4 °C, it has been stable for one week and after that a gradual decrease (10%) occurred from the current initial value. These results show that the proposed nano Pd film modified GCEs possess the sufficient stability.

4. Conclusion

In this report, we have employed a simple and general methodology for the electrochemical fabrication of Pd nanoparticles on GCE and ITO. The surface morphology of the nano Pd film modified ITO have been examined by using SEM, AFM and XRD analysis. The nano Pd film modified GCE possess the high electro active surface area and showed interesting electrocatalytic activity towards the selective detection and determination of catecholamine neurotransmitters in the presence of higher concentrations of AA (1.3 mM). The proposed nano Pd film modified GCE remarkably suppressed the interference effect and showed two well defined oxidation peaks for the selective detection of catecholamines and AA. In addition, the high surface area of the nano Pd film modified GCE electrode well suited for the selective determination of catecholamines. Finally, the nano Pd film modified GCEs also applied for the detection of catecholamines in injection sample mixtures.

Acknowledgment

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