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Pristine multi-walled carbon nanotubes/SDS modified carbon paste electrode as an amperometric sensor for epinephrine

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ABSTRACT

An amperometric sensor for the determination of epinephrine (EP) was fabricated by modifying the carbon paste electrode (CPE) with pristine multi-walled carbon nanotubes (pMWCNTs) using bulk modification followed by drop casting of sodium dodecyl sulfate (SDS) onto the surface for its optimal potential application. The modified electrode showed an excellent electrocatalytic activity towards EP by decreasing the overpotential and greatly enhancing the current sensitivity. FE-SEM images confirmed the dispersion of pMWCNTs in the CPE matrix. EDX analysis ensured the surface coverage of SDS. A comparative study of pMWCNTs with those of oxidized MWCNTs ($MWCNTs_{OX}$) modified electrodes reveals that the former is the best base material for the construction of the sensor with advantages of lower oxidation overpotential and the least background current. The performance of the modified electrode was impressive in terms of the least charge transfer resistance (R_{ct}), highest values for diffusion coefficient (D_{EP}) and standard heterogeneous electron transfer rate constant (k°). Analytical characterization of the modified electrode exhibited two linear dynamic ranges from 1.0×10^{-7} to 1.0×10^{-6} M and 1.0×10^{-6} to 1.0×10^{-4} M with a detection limit of $(4.5\pm0.18)\times10^{-8}$ M. A 100-fold excess of serotonin, acetaminophen, folic acid, uric acid, tryptophan, tyrosine and cysteine, 10-fold excess of ascorbic acid and twofold excess of dopamine do not interfere in the quantification of EP at this electrode. The analytical applications of the modified electrode were demonstrated by determining EP in spiked blood serum and adrenaline tartrate injection. The modified electrode involves a simple fabrication procedure, minimum usage of the modifier, quick response, excellent stability, reproducibility and anti-fouling effects.

1. Introduction

Epinephrine (EP, adrenaline) is one of the largest catecholamine neurotransmitters in the mammalian central nervous system and a hormone possessing various physiological and pharmacological functions [1]. It serves as a chemical mediator for transmitting nerve impulses to efferent organs [2]. EP is secreted from the chromaffin cell of the suprarenal gland which exists as a large organic cation in nerve cells and body fluids [3,4]. It is synthesized naturally in human system from L-phenylalanine and L-tyrosine [5]. Clinically, EP is used as a common emergency health care medicine in the treatment of severe allergic reactions, anaphylaxis, sepsis, emphysema and myocardial infarction [6,7]. It is also used in the treatment of glaucoma [8]. Low levels of EP are found in patients of Parkinson's disease and orthostatic hypotension [9]. High levels of EP are associated with stress and thyroid hormone deficiency [10]. EP prepares the body for actions during perceived emergency situations by boosting the supply of oxygen and glucose to the brain and muscles [11]. It simultaneously elevates the blood sugar level by inducing the conversion of glycogen to glucose in liver and lipolysis in adipose tissue. Hence, it can be used as a potent doping agent and its use is banned by World Anti Doping Agency in competitive games [12]. Therefore, it is necessary to develop a simple, fast and accurate method for the determination of EP which would play an important role in neurophysiology, clinical diagnosis and quality control.

Many analytical methods have been developed for the determination of EP in various samples. These methods include spectrophotometry, fluorimetry, liquid chromatography, capillary electrophoresis, thermal lens microscopy (TLM), chemiluminescence, electrochemiluminescence and flow injection analysis [13,14]. However, these techniques involve cumbersome procedures, expensive instrumentation, lack of selectivity or specificity and prolonged analysis time. Therefore, these techniques are less

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desirable in the routine analysis of EP. Being an electroactive compound, electroanalytical techniques are commonly employed for the quantification of EP. These techniques make possible a simple, fast-and-inexpensive path to estimate EP with high sensitivity and selectivity [15]. Nevertheless, the electroanalysis of EP faces certain challenges. The redox process of EP occurs at high overpotential due to the sluggish electrode kinetics at traditional bare working electrodes like Au, Ag, Pt, glassy carbon (GC) and graphite. Voltammetric response of EP at these electrodes is very close to the other electroactive biomolecules such as ascorbic acid (AA), dopamine (DA) and uric acid (UA), and their signals usually overlap [16]. Adsorption of EP causes the passivation of the electrode [17]. These facts limit the accuracy and sensitivity of the determination of EP. Hence, it is necessary to resolve these problems by modifying the electrodes with suitable materials. CPE has been widely used in the development of electrochemical sensors for biomolecules because of its simple method of preparation, renewable surface, low background current, acquiescence with various types of modification procedures and above all, its biocompatibility [18].

Since the discovery of carbon nanotubes (CNTs) in 1991 by Iijima [19], these materials have turned out to hold great promise in various fields in science and technology. In the 21st century, the applications of CNTs include lab-on-chip sensors, drug delivery vehicles, conducting nanowires, energy storage devices and electrochemical sensors [20,21]. Electrochemical sensors based on CNTs are reported to have certain advantages such as increased heterogeneous electron transfer rates, prevention of surface fouling, high sensitivity, quick response time and electrocatalytic activity towards wide varieties of compounds [22,23].Various types of CNT modified electrodes such as CNT–polymer nanocomposite electrodes, CNT–paste electrodes, CNT/sol–gel nanocomposite electrodes and layer-by-layer assembly of CNT film electrodes, have been used as electrochemical sensors for catecholamines [24].

Surfactants have been widely used in electroanalysis because of their ability to improve the properties of the electrode/solution interface [25]. Surfactant modified electrodes have been used as an electrochemical sensor for catecholamines [26–28]. The drop-cast method of modification is endowed with a dual advantage: entire coverage of the electrode surface with the modifier and minimum utilization of the modifier [29]. Hence, it is but natural that this method gets a preference over other methods of modification. There is no report of sodium dodecyl sulfate (SDS) being used in combination with pMWCNTs as an amperometric sensor for the quantification of EP.

In keeping with our endeavor to modify CPE using different materials and also to use them as sensor for different applications [30–36], our objective in the present work was to develop a simple amperometric sensor for the determination of EP by combining properties of pMWCNTs as well as that of SDS. Here, in order to optimize the potential application of the sensor, we have exploited the advantages of MWCNTs such as electrocatalytic activity, favorable charge transfer, good conductivity, good adsorptive properties in combination with the properties of SDS such as increased surface area, high columbic forces and amenability to adsorption on the electrode surface through the hydrophobic end of the chain. The modified electrode was intended to be free from the interference of other molecules so that it would provide a better detection limit and linear dynamic range. To minimize the usage of MWCNTs, we bulk modified the CPE with MWCNTs. In order to investigate a better support to drop cast SDS onto the electrode surface, a parallel study was carried out using CPE bulk modified with oxidized MWCNTs ($MWCNTs_{OX}$). Our study demonstrates that the CPE bulk modified with pMWCNTs is a better supporting material for this purpose. This inspired us to bulk modify the CPE with pMWCNTs and use this surface for further modification with SDS by the drop cast method. Electrochemical behavior of EP was thoroughly investigated at the modified electrode. The interference studies were carried out in the presence of other biomolecules. Analytical performance of the modified electrode was examined by estimating the EP in pharmaceuticals and the blood serum. The modification of the electrode in the present study results in a reduction in oxidation overpotential and a better linear dynamic range for EP as compared to our previous report [37].

2. Experimental

2.1. Chemicals and reagents

Epinephrine hydrochloride, dopamine hydrochloride, serotonin hydrochloride (Sigma-Aldrich), L-tryptophan (Trp), L-tyrosine (Tyr), L-cysteine (Cys), UA, and folic acid (FA) were obtained from SRL, acetaminophen (AAP) was procured from Micro Labs Ltd., and AA, KH_2PO_4, H_3PO_4, NaOH pellets, $HClO_4$, silicone oil, and SDS were from Merck; all the chemicals were of analytical grade and were used as received. All aqueous solutions were prepared with ultra pure water (>18.2 MΩ cm) from Milli-Q Plus system (Millipore). The stock solutions of 25.0×10^{-3} M EP, DA and serotonin (5-HT) were prepared in 0.1 M $HClO_4$, UA, Cys, FA and Tyr in 0.1 M NaOH and Trp, AAP and AA were prepared in water. Phosphate buffer solutions were prepared from KH_2PO_4 and NaOH and pH was adjusted using H_3PO_4 or NaOH. The graphite powder was obtained from Graphite India Ltd. The thin pristine MWCNTs (NC7000 series), obtained from Nanocyl S.A. (Belgium), are produced via the catalytic carbon vapor deposition (CCVD) process. The MWCNTs were used as received. The MWCNTs have an average diameter of 9.5 nm and length of 1.5 μm.

2.2. Apparatus

All electrochemical experiments were performed using a ChemiLink model EA-201 Electro Analyzer. A conventional three electrode system was used for all electrochemical experiments, which comprises of a bare or modified CPE as working electrode, a platinum wire as auxiliary electrode and all potentials were measured and applied using saturated calomel electrode (SCE) as a reference electrode. The tip of the Lugin capillary was set at a fixed distance from the working electrode surface throughout the experimental studies. The cyclic voltammetric (CV) studies were performed in quiescent solution and the amperometric experiments were carried out under hydrodynamic conditions. The electrochemical experiments and voltammetric curves were recorded at room temperature (~300.0 K). The surface morphology of the electrodes was studied using field-emission scanning electron microscopy (FE-SEM) and the energy dispersive X-ray analysis (EDX) using Quanta 200, FEI, Germany; SUPRA 40 VP, Gemini, Zeiss, Germany. A Philips Tecnai 100 transmission electron microscope (TEM) was used to observe the morphology of MWCNTs. Electrochemical impedance spectroscopy (EIS) was performed using VersaSTAT 3. A digital pH/mV meter (ELICO LI 614) was employed to measure the pH of the prepared buffer solutions.

2.3. Generation of oxygen functionalities on pMWCNTs

Since the oxygen functionalities on the surface of the MWCNTs improve their electrochemical properties, the same were generated by treating 2.2 g of NC7000 with 170.0 mL of a mixture of nitric acid (38.0 mL at 70 wt%) and sulfuric acid (132.0 mL at 98 wt%). This mixture was then heated at 50 °C for 4 h under constant stirring. It was allowed to cool down to room temperature and then filtered. The resulting mass was washed several times with deionized water until neutral pH was attained [38,39]. The residue ($MWCNTs_{OX}$) was dried and used for electrode modification.

2.4. Preparation of bare and pMWCNTs modified carbon paste electrodes

After having optimized the ratio of graphite powder to binder, CPE was prepared by thoroughly hand mixing the graphite powder and silicone oil in a ratio 70:30 (w/w) and was homogenized in an agate mortar using a pestle to obtain a paste. A portion of the resulting homogeneous paste was packed into the cave of the Teflon tube. A copper wire fixed to a graphite rod and inserted into the Teflon tube served to establish electrical contact with the external circuit. Similarly, pMWCNTs and $MWCNTs_{OX}$ modified CPEs were prepared separately by mixing the graphite powder, silicone oil and pMWCNTs or $MWCNTs_{OX}$ in a ratio 60:30:10 (w/w) after optimization. The graphite powder and pMWCNTs or $MWCNTs_{OX}$ were initially mixed in a mortar to ensure a uniform dispersion of pMWCNTs or $MWCNTs_{OX}$. Silicone oil was added later to the above mixtures and mixed together to form a homogeneous paste. These electrodes were designated as MCPE/pMWCNTs and MCPE/$MWCNTs_{OX}$.

2.5. Preparation of SDS modified electrodes

SDS modified CPE, MCPE/pMWCNTs and MCPE/$MWCNTs_{OX}$ were prepared by the drop cast method. A 10.0 μL of 3.0×10^{-3} M SDS solution was cast onto the surface of CPE, MCPE/pMWCNTs and MCPE/$MWCNTs_{OX}$. After 8.0 min, the electrodes were thoroughly rinsed with Milli-Q water to remove unadsorbed SDS and these electrodes were used for electroanalysis. The SDS modified electrodes were abbreviated as MCPE/SDS, MCPE/pMWCNTs/SDS and MCPE/$MWCNTs_{OX}$/SDS respectively.

3. Results and discussion

3.1. Surface morphology of electrodes

The morphological characteristics of all the electrodes were compared by recording the FE-SEM images. Fig. 1a and b shows the FE-SEM images of CPE and MCPE/pMWCNTs, respectively. It is understood from Fig. 1 that a smoother surface was obtained for CPE compared to MCPE/pMWCNTs. Fig. 1b reveals the uniform dispersion of pMWCNTs in the CPE matrix. Modification with SDS does not drastically alter the surface morphologies of the above electrodes. Fig. S1a–d, (Supplementary information) shows the EDX analysis of CPE, MCPE/pMWCNTs, MCPE/SDS and MCPE/pMWCNTs/SDS, respectively. EDX analysis of the electrodes indicates the presence of C, Si and O. The presence of S both at MCPE/SDS and MCPE/pMWCNTs/SDS (Fig. S1c and d) convincingly confirms the adsorption of SDS at CPE and MCPE/pMWCNTs. Fig. S2 (Supplementary information) shows the TEM image of MWCNTs which are used for electrode modification.

3.2. Characterization of electrodes by electrochemical impedance spectroscopy

Electrochemical impedance spectroscopy (EIS) is an effective and powerful technique to probe the charge transfer properties of electrode/solution interface [40]. EIS spectra of CPE, MCPE/pMWCNTs, MCPE/SDS and MCPE/pMWCNTs/SDS are shown in the form of the Nyquist plot in Fig. 2. EIS data of electrodes were recorded in ac frequency range varying from 0.1 Hz to 100.0 kHz at an oxidation peak potential (Epa, 215.0 mV) of 5.0×10^{-4} M EP in 0.1 M phosphate buffer solution of pH 7.0. The charge transfer resistance (R_{ct}) value reflects the ease of electron transfer kinetics of EP at electrode/electrolyte interface. R_{ct} values at different electrodes were obtained by fitting the obtained impedance data to an appropriate circuit (Fig. 2, inset). The components of equivalent circuit are correlated to the resistance offered by supporting electrolyte (Rs), the double layer capacitance (Cdl), the charge transfer resistance (R_{ct}), the Warburg impedance resulting from the diffusion of ions from bulk solution to the electrode surface (W), the constant phase element (Cf) aroused due to the rough and inhomogeneous microscopic structure of electrode surfaces and the resistance offered by the electrode surface along with adsorbed SDS film (Rf) [41,42]. The R_{ct} values of 5.0×10^{-4} M EP at CPE, MCPE/pMWCNTs, MCPE/SDS and MCPE/pMWCNTs/SDS are displayed in Table 1. The semicircle part of the Nyquist plot at higher frequency region corresponds to the electron transfer limited process which is being magnified for each of the electrodes and displayed in Fig. S3 (Supplementary information). The presence of pMWCNTs in carbon paste matrix

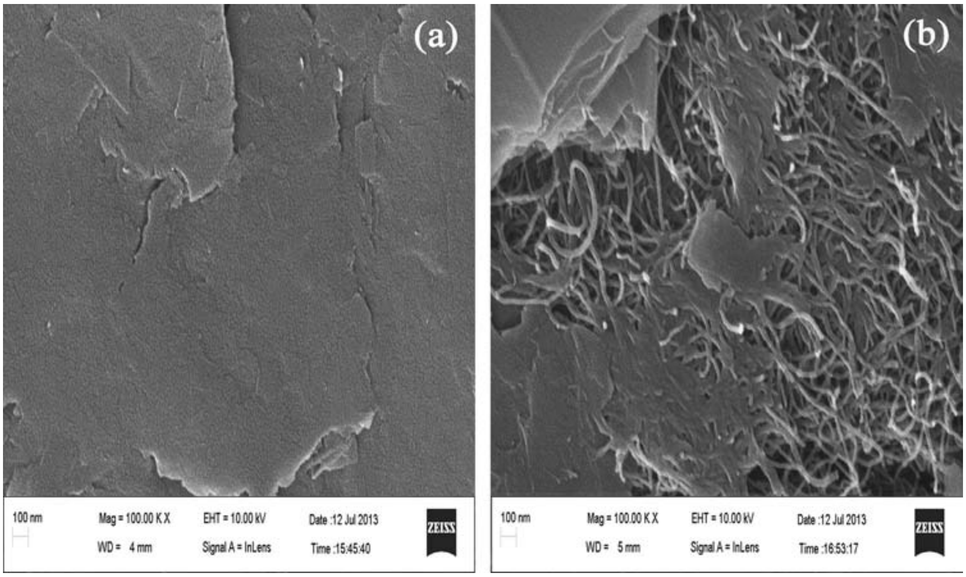


Fig. 1. FE-SEM images of (a) CPE and (b) MCPE/pMWCNTs.

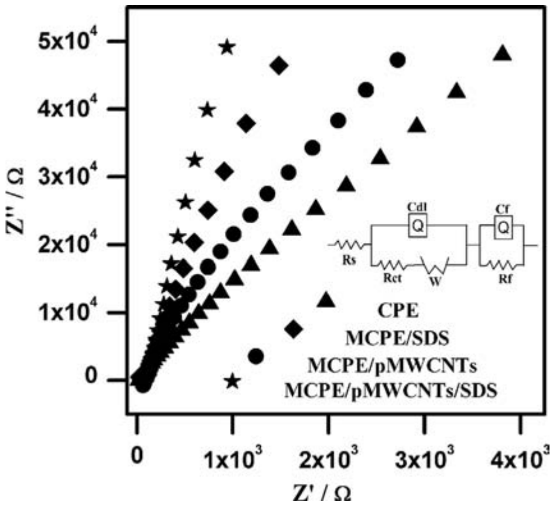


Fig. 2. Nyquist plots of 5.0×10^{-4} M EP in 0.1 M phosphate buffer of pH 7.0 at different electrodes. Inset shows the equivalent circuit used for the analysis.

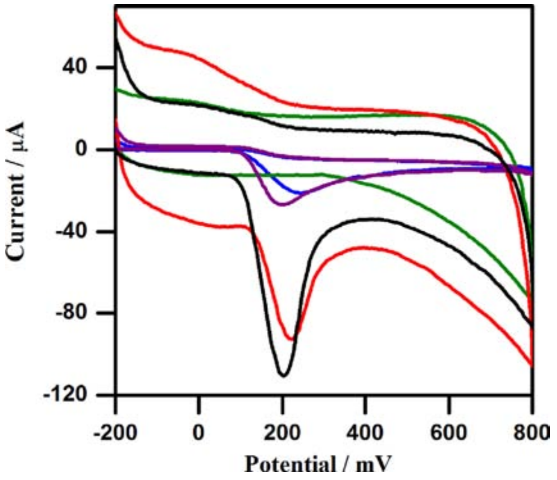


Fig. 3. Cyclic voltammograms of 5.0×10^{-4} M EP in 0.1 M phosphate buffer of pH.7.0 at CPE (blue), MCPE/pMWCNTs (violet), MCPE/SDS (red), MCPE/pMWCNTs/SDS (black) and blank (green). Scan rate: 50 mV s^{-1}. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Electrochemical and kinetic parameters for the electrochemical oxidation of EP at different electrodes.

Electrodes	R_{ct} (Ω)	D (cm^2 s^{-1})	k° (cm s^{-1})
CPE	1000.7	3.13×10^{-6}	1.47×10^{-3}
MCPE/pMWCNTs	752.4	4.11×10^{-6}	2.13×10^{-3}
MCPE/SDS	234.2	1.89×10^{-5}	4.51×10^{-3}
MCPE/pMWCNTs/SDS	198.9	4.10×10^{-5}	6.38×10^{-3}

and adsorption of SDS at surface result in a least charge transfer resistance and improve the charge transfer kinetics at MCPE/pMWCNTs/SDS. Hence, MCPE/pMWCNTs/SDS was employed for the development of the amperometric sensor for EP.

3.3. Electrochemical behavior of EP

The electrochemical behavior of EP was investigated using the CV technique. The resulting cyclic voltammograms for 5.0×10^{-4} M EP in 0.1 M potassium phosphate buffer solutions (KPBS) of pH 7.0 at CPE (blue), MCPE/pMWCNTs (violet), MCPE/SDS (red) and MCPE/pMWCNTs/SDS (black) are shown in Fig. 3. The absence of voltammetric peaks for the blank (Fig. 3, green) at MCPE/pMWCNTs/SDS indicates its electrochemical inertness in the potential window of interest. EP shows an irreversible electrochemical behavior at all the electrodes. Epa of EP at CPE, MCPE/pMWCNTs, MCPE/SDS and MCPE/pMWCNTs/SDS is 248.0 ± 2.4, 200.0 ± 4.8, 223.0 ± 4.1 and 199.0 ± 3.9 mV respectively. The corresponding anodic peak current (Ipa) of EP at the respective electrodes are -20.8 ± 1.8, -23.7 ± 3.2, -53.3 ± 4.1 and -88.4 ± 4.3 µA, respectively. A negative shift 49.0 mV in Epa and 4.4 times enhancement in Ipa clearly demonstrates the electrocatatalytic activity of MCPE/pMWCNTs/SDS towards the oxidation of EP. The negative shift is quite evident at MCPE/pMWCNTs and MCPE/pMWCNTs/SDS compared to CPE and MCPE/SDS. Hence, it can be concluded that the presence of pMWCNTs in the CPE matrix is responsible for the shift in potential on account of the favorable electronic properties of pMWCNTs. The edge plane or edge plane like defects on the surface of pMWCNTs also facilitates the electron transfer [43]. SDS is an anionic surfactant. The adsorption of SDS at the surface of the electrodes takes place in such a way that the hydrophobic alkyl chain will get adsorbed spontaneously at the surface of hydrophobic CPE and MCPE/pMWCNTs through Van der Waals interactions, keeping the sulfate group orienting outwards [44]. This creates high negative charge density at the surface of these electrodes and promotes the electrostatic attraction of EP, which is a cation at pH 7.0 (pKa=8.8) [45]. The hydroxyl group of EP can form hydrogen bond with the electronegative oxygen atoms of the sulfate group in SDS [46]. This weakens the bond energy and makes the oxidation more viable. Also, modification with pMWCNTs provides more surface area for SDS molecules to get adsorbed. This causes maximum current sensitivity at MCPE/pMWCNTs/SDS. In order to ascertain that the pMWCNTs are the best base electrode material for the study of EP, the electrochemical behavior of 5.0×10^{-4} M EP was investigated at MCPE/$MWCNTs_{OX}$ (orange) and MCPE/$MWCNTs_{OX}$/SDS (magenta) in 0.1 M KPBS of pH 7.0 under the same conditions which are displayed in Fig. 4. Epa of EP at MCPE/$MWCNTs_{OX}$ and MCPE/$MWCNTs_{OX}$/SDS is 215.0 ± 6.8 and 248.0 ± 7.9 mV, respectively. The electrochemical oxidation of EP at MCPE/$MWCNTs_{OX}$/SDS involves higher energy. The corresponding Ipa for EP at the respective electrodes is -48.5 ± 6.3 and -62.8 ± 5.4 µA. It is evident from Fig. 4, that the electrodes modified with $MWCNTs_{OX}$ offer a higher background current as compared to the ones modified with pMWCNTs (black and violet). The $MWCNTs_{OX}$ carries oxygen functionalities which are possibly responsible for high background current. The adsorption of SDS molecules at the negatively charged surface of MCPE/$MWCNTs_{OX}$ may not be uniform due to the repulsion. Moreover, it was also observed that the hydrophilicity of MCPE/$MWCNTs_{OX}$ surface due to the presence of $MWCNTs_{OX}$ caused surface rupture during the 8 min adsorption time of the surfactant and this was subsequently reflected in the measurement. A 49.0 mV positive shift in Epa and 25.6 µA decrease in Ipa of EP were observed at MCPE/$MWCNTs_{OX}$/SDS as compared to MCPE/pMWCNTs/SDS which proves the poor redox kinetics at MCPE/$MWCNTs_{OX}$/SDS. The pMWCNTs modified electrode suppresses the background current to a greater extent, provides better redox kinetics and a maximum current sensitivity for EP. These being the desired qualities for an electrochemical sensor, MCPE/pMWCNTs/SDS is more suitable for this purpose.

The influence of potential scan rate on the electrochemical oxidation of 5.0×10^{-4} M EP at MCPE/pMWCNTs/SDS was investigated using CV. Ipa was observed to vary linearly with the square root of scan rate ($\sqrt{\nu}$) in the range 10–150 mV s^{-1} as depicted in Fig. S4 (Supplementary information). Such a behavior suggests that the electrochemical oxidation of EP at MCPE/pMWCNTs/SDS is

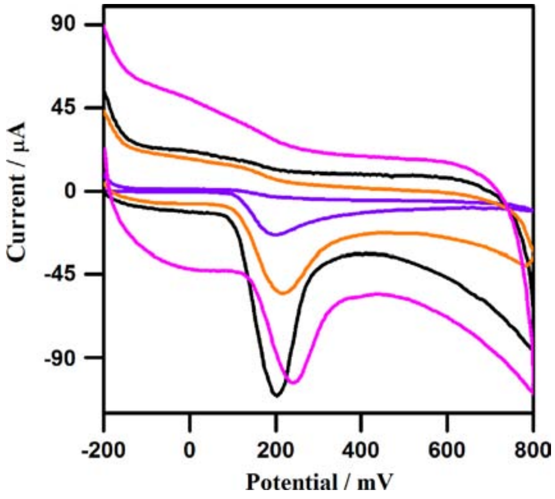


Fig. 4. Cyclic voltammograms of 5.0×10^{-4} M EP in 0.1 M phosphate buffer of pH.7.0 at MCPE/pMWCNTs (violet), MCPE/pMWCNTs/SDS (black), MCPE/MWCNTs$_{OX}$ (orange) and MCPE/MWCNTs$_{OX}$/SDS (magenta). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

a diffusion-controlled process [47]. The linear regression equation for the potential range studied is Ipa (µA)=2.23–11.9$\sqrt{\nu}$ (mV s^{-1})$^{1/2}$ with R^2=0.9906. Epa of EP shifts in the positive direction with an increase in scan rate.

3.4. Effect of solution pH

The pH of the supporting electrolytes plays a significant role in determining the mechanism of electrochemical oxidation of EP at MCPE/pMWCNTs/SDS. Hence, the effect of pH on Epa and Ipa of 5.0×10^{-4} M EP was investigated in 0.1 M KPBS of different pH ranging from 3.0 to 8.0 using the CV technique. As represented in Fig. S5a (Supplementary information) the Ipa attains maximum value at pH 7.0. It was observed that the Ipa decreased with increase in pH between 3.0 and 6.0. Hence, it could be concluded that the protons could catalyze the electrochemical oxidation of EP. Moreover, the sulfate groups of SDS will remain protonated in acidic pH and the electrostatic attraction of EP cation will not prevail. At neural pH, the electrostatic attraction of EP cation and SDS reaches a maximum value and a sharp increase in the value of Ipa is observed. As expected, there was a large decline in current in basic pH. Since the maximum current sensitivity was at pH 7.0 which was closer to the physiological pH, we maintained the same pH in all our experiments. Furthermore, it could be observed that Epa shifts linearly in negative direction with increase in pH as depicted in Fig. S5b (Supplementary information), which indicates the involvement of protons in the electrochemical oxidation of EP at MCPE/pMWCNTs/SDS. The linear regression equation of Epa versus pH plot is Epa (mV)=672.5–65.3 pH with R^2=0.9875. Eq. (1) represents the Nernst equation which describes the relationship between Epa and pH, where n and m represent the number of electrons and protons involved in the reaction, and a and b are coefficients of oxidant and reductant in the reaction [48].

$$\mathrm{Epa} = E^\circ + \left(\frac{0.0591}{n}\right)\log\left[\frac{(\mathrm{OX})^a}{(R)^b}\right] - \left(0.0591\frac{m}{n}\right)\mathrm{pH} \tag{1}$$

A slope of −65.9 pH/mV obtained closely matches with the Nernstian slope for the electrode reaction which involves an equal number of electrons and protons (m=n). Two protons and two electrons are involved in electrochemical oxidation of EP at MCPE/pMWCNTs/SDS, which is in accordance with mechanism of EP already reported elsewhere [49,50]. Scheme 1 represents the electrochemical oxidation mechanism of EP at MCPE/pMWCNTs/SDS.

3.5. Determination of diffusion coefficient and heterogeneous electron transfer rate constant

Diffusion coefficients of EP (D_{EP}) in 0.1 M KPBS of pH 7.0 at CPE, MCPE/pMWCNTs, MCPE/SDS and MCPE/pMWCNTs/SDS were determined using the chronoamperometry technique as reported elsewhere [51]. The chronoamperograms of various concentrations of EP (1.0×10^{-4}–1.0×10^{-3}) were recorded at different electrodes (Fig. S6a, Supplementary information). The current (I) generated during the electrochemical oxidation of EP with D_{EP} under mass transport limited conditions is described by the Cottrell equation

$$I = \frac{nFAD_{EP}^{1/2}C_{EP}}{\sqrt{\pi t}} \tag{2}$$

where n, F, A, C_{EP} and t are the number of electrons transferred, the Faraday constant (96,485 C/mol), the geometric area of the electrode (cm^2), the concentration of EP (mol/cm^3) and the time (s) respectively. The experimental plots of I versus $t^{-1/2}$ were constructed for different concentrations of EP for all the four electrodes (Fig. S6b, Supplementary information). The slope of the resulting straight lines was plotted against C_{EP} (Fig. S6c, Supplementary information). D_{EP} calculated from these slopes and the Cottrell equation are displayed in Table 1. It is clear from Table 1 that the values of D_{EP} were enhanced upon modification with pMWCNTs and SDS. The maximum value for D_{EP} was observed at MCPE/pMWCNTs/SDS. The electrostatic attraction of EP molecules and SDS film at the electrode surface causes the D_{EP} to increase.

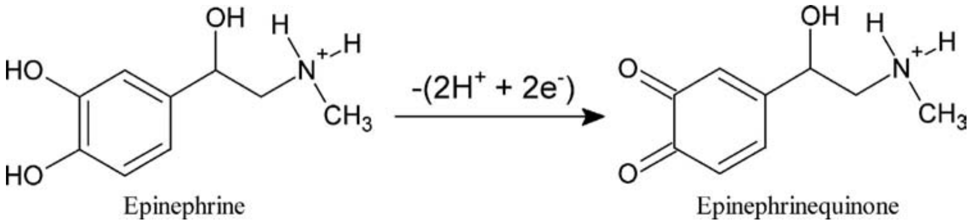
The standard heterogeneous electron transfer rate constant (k°) for the irreversible electrochemical oxidation of EP at CPE, MCPE/pMWCNTs, MCPE/SDS and MCPE/pMWCNTs/SDS was calculated using the following equation, as reported elsewhere [52].

$$k^\circ = 1.11 D_{EP}^{1/2}(E_p - E_{p/2})^{-1/2}\nu^{1/2} \tag{3}$$

where E_p, $E_{p/2}$ (mV) and ν (mV s^{-1}) are the peak potential, the half wave potential and the scan rate respectively. The k° values at different electrodes are displayed in Table 1. The increase in k° on modification indicates a better charge transfer kinetics at the modified electrodes. The highest value for k° was observed at MCPE/pMWCNTs/SDS which confirms the electrocatalytic activity of the electrode towards EP. Therefore, it can be concluded from Table 1 that the R_{ct} values decrease on modification while D_{EP} and k° values increase. The observations made during the CV investigation of EP were consistent with these results.

3.6. Effect of loading pMWCNTs in the carbon paste matrix

The amount of pMWCNTs in CPE matrix plays an important role in the performance of MCPE/pMWCNTs/SDS. Moreover, the presence of pMWCNTs affects the adsorption of SDS at MCPE/pMWCNTs. Hence, MCPE/pMWCNTs/SDS was prepared by mixing different quantities of pMWCNTs (1.0–5.0 mg). Ipa attains the highest value at an electrode containing 2.0 mg of pMWCNTs as shown in Fig. S7 (Supplementary information). The higher loading of pMWCNTs induced higher background current and resulted in the reduction of Ipa. Furthermore, the adsorption of SDS at electrodes diminishes as the amount of pMWCNTs in CPE matrix increases because of the decrease in hydrophobicity of electrode surface. These two factors largely determine the sensitivity of MCPE/pMWCNTs/SDS. It was further supported by the observation that the adsorption of SDS at CPE surface (in the absence of



Scheme 1. Electrochemical oxidation mechanism of EP at MCPE/pMWCNTs/SDS.

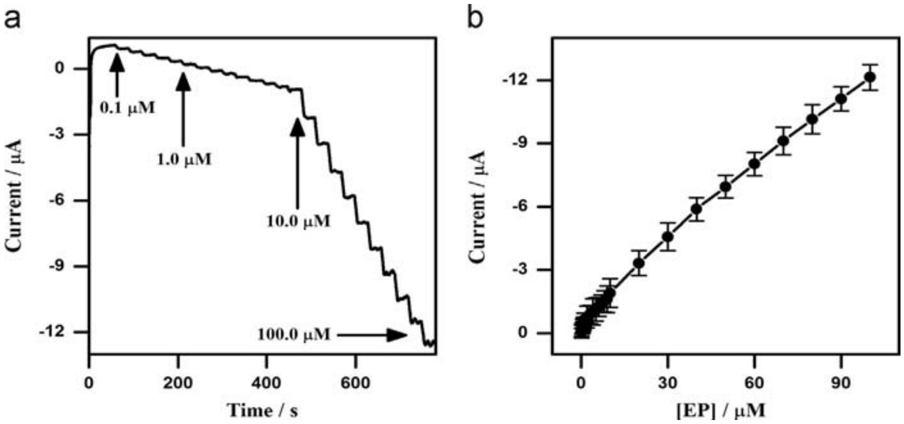


Fig. 5. (a) Amperometric current response of different concentrations of EP at MCPE/pMWCNTs/SDS in 0.1 M phosphate buffer solution of pH 7.0 under hydrodynamic conditions. Applied potential: 215.0 mV. (b) Calibration plot of Ipa versus concentration of EP at MCPE/pMWCNTs/SDS.

pMWCNTs) under identical conditions presented high background currents (Fig. 3). Thus, MCPE/pMWCNTs/SDS was prepared using 2.0 mg of pMWCNTs and the same was maintained throughout our studies.

3.7. *Effect of concentration of SDS*

The effect of concentration of SDS on the voltammetric response of EP and hence on the performance of the sensor was investigated by varying the concentrations of SDS between 1.0 and 9.0 mM. The variation of Ipa with the concentration of SDS is depicted in Fig. S8 (Supplementary information). It was found that the Ipa attained its highest value at 3.0 mM, after which it decreased and then remained almost a constant till 7.0 mM and again decreased thereafter. Hence, it could be concluded that the degree of SDS adsorption reaches a maximum value at 3.0 mM and a further increase in concentration does not improve the adsorption of SDS. Therefore, Ipa remained constant in the concentration range 3.0–7.0 mM. When concentration of SDS approaches its critical micelle concentration (CMC, 8.0 mM), there is a corresponding decrease in the number of free SDS molecules available for adsorption [53]. Consequently, the Ipa of EP started to decrease beyond the concentration of 7.0 mM. Therefore, 3.0 mM SDS was used in the preparation of MCPE/pMWCNTs/SDS.

3.8. *Analytical characterization of MCPE/pMWCNTs/SDS*

Trace level detection of EP at MCPE/pMWCNTs/SDS was carried out under hydrodynamic conditions using amperometry. Fig. 5a shows the amperometric response of EP in 0.1 M KPBS of pH 7.0 at MCPE/pMWCNTs/SDS whose potential was set at 215.0 mV versus SCE. MCPE/pMWCNTs/SDS provided quick response on the addition of aliquots of EP with an interval of 30.0 s and response attained stability in 5.0 s. Fig. 5b displays a linear increase in current response with an increase in the concentration of EP. The calibration plot presents two linear dynamic ranges, from 1.0×10^{-7} to 1.0×10^{-6} M and 1.0×10^{16} to 1.0×10^{-4} M respectively for EP. The linear regression equations corresponding to these ranges are Ipa (μA)=0.02–0.61C_{EP} (μM), with R^2=0.9991 and Ipa (μA)=−0.69–0.12C_{EP} (μM), with R^2=0.9958 respectively. The detection limit of EP at MCPE/pMWCNTs/SDS is $(4.5\pm0.18)\times10^{-8}$ M. The analytical performance of the MCPE/pMWCNTs/SDS was compared with other modified electrodes and the results are displayed in Table 2.

The preparation of Pt/*Phanerochaete chyrsosporium* [54] involves the pretreatment of Pt electrode and the immobilization of *P. chyrsosporium* which is a complex procedure. Also, it requires the presence of a mediator such as $K_3[Fe(CN)_6]$ for the proper functioning of Pt/*P. chyrsosporium*, with an optimum response at pH 4.5, which restricts its applications for biological samples. The preparation of MCPE/pMWCNTs/SDS is relatively simpler and eliminates the need for exhaustive pretreatment and processing steps. Analytical characterization of Ru/WGE [55] was carried out at pH 4.0 and the real sample analysis has not been carried out. Au–Cys–SWCNT–CoTAPc [56] involves a tedious preparation procedure and the use of Au electrode and the modifier, SWCNT makes it an expensive sensor, apart from it requiring a chemical modification of SWCNT in its preparation. The *D* and *k* values for electrochemical oxidation of EP are much higher at MCPE/pMWCNTs/SDS as compared to MDWCNTPE [57]. GCE has to undergo a rigorous pretreatment prior to the preparation in the case of GCE/poly(taurine) [58] and OMC/Nafion/GCE [59]. The utility of the sensor for the estimation of EP in biological samples has not been demonstrated for GCE/poly(taurine). Preparation of Au/4α-Ni^{II}TAPc SAMF [60] and DMSA/Au [61] take a long time of 48.0 and 30.0 h respectively and they are expensive too. Moreover, the electrochemical determination of EP at Au/4α-Ni^{II}TAPc SAMF has been carried out in an acidic medium. The Au electrode employed for modification requires pretreatment prior to its modification. A higher oxidation overpotential has been reported at MZ-CPE [62] for the determination of EP as compared to MCPE/pMWCNTs/SDS with an additional advantage of a twofold higher

Table 2
Comparison of the present work at MCPE/pMWCNTs/SDS with the performance of other electrodes reported in the literature.

Electrode	pH	Linear dynamic range (M)	Detection limit (M)	Technique used	Ref.
Pt/*P. chyrsosporium*	4.5	5.0×10^{-6}–1.0×10^{-4}	1.04×10^{-6}	CV	[54]
Ru/WGE	4.0	3.0×10^{-6}–9.0×10^{-5}	8.0×10^{-7}	DPV	[55]
Au–Cys–SWCNT–CoTAPc	7.0	1.22×10^{-5}–1.3×10^{-4}	6.0×10^{-6}	SWV	[56]
MDWCNTPE	7.0	7.0×10^{-7}–1.2×10^{-3}	2.16×10^{-7}	DPV	[57]
GCE/poly(taurine)	7.4	2.0×10^{-6}–6.0×10^{-4}	3.0×10^{-7}	DPV	[58]
OMC/Nafion/GCE	7.0	5.0×10^{-7}–2.0×10^{-4}	2.0×10^{-7}	DPV	[59]
Au/4α-Ni^{II}TAPc SAMF	5.0	5.0×10^{-8}–5.0×10^{-7}	5.0×10^{-8}	Amperometry	[60]
DMSA/Au	7.7	4.0×10^{-4}–4.0×10^{-3}	5.8×10^{-8}	DPV	[61]
MZ-CPE	7.0	1.0×10^{-6}–2.5×10^{-3}	5.0×10^{-7}	DPV	[62]
MIP/PIL-MWNT/ITO	7.4	2.0×10^{-7}–6.7×10^{-4}	6.0×10^{-8}	Amperometry	[63]
MCPE/pMWCNTs/SDS	7.0	1.0×10^{-7}–1.0×10^{-6} and 1.0×10^{-6}–1.0×10^{-4}	$(4.5\pm0.18)\times10^{-8}$	Amperometry	Present work

value of D for MCPE/pMWCNTs/SDS. A higher energy is required for the oxidation of EP at MIP/PIL-MWNT/ITO [63] as compared to MCPE/pMWCNTs/SDS apart from a cumbersome two stages involved in its preparation. The sensor suffers from a set back as it has not been tried for any analytical applications. On being looked at from various angles, MCPE/pMWCNTs/SDS has the much desired properties for a modified electrode to be used as a sensor. The properties – a relatively easy method of modification, inexpensiveness, freedom from pretreatment, good reproducibility, sensitivity, stability, better linear dynamic range, amenability to be used at physiological pH and better detection limits – make it superior and more convenient for use as a sensor as compared to many modified electrodes reported in the literature for the detection of EP.

3.9. *Interference studies*

EP co-exists with many biomolecules in the biological matrix. Many such molecules are electroactive and have redox potential similar to EP. In order to rule out the possibility of interference, we have investigated the effect of these molecules on the amperometric determination of EP. Amperometric response of interfering molecules has been investigated at MCPE/pMWCNTs/SDS after fixing its potential at 215.0 mV versus SCE. It is evident from Fig. 6 that 1.0×10^{-5} M 5-HT, AAP, FA, UA, Trp, Tyr and Cys have not given any amperometric signal at MCPE/pMWCNTs/SDS. It was also observed that concentration of DA less than 3.0×10^{-7} M and AA at concentrations lower than 1.0×10^{-6} M have not been shown to be detected at MCPE/pMWCNTs/SDS. The individual amperometric analysis of DA and AA at MCPE/pMWCNTs/SDS reveals that the lowest concentration of DA and AA that can be detected at this electrode at step potential of 215.0 mV is 3.0×10^{-7} M and 1.0×10^{-6} M, respectively. The DA and AA interfere with amperometric detection of EP above these concentrations at MCPE/pMWCNTs/SDS. The CV studies of DA and AA at MCPE/pMWCNTs/SDS indicate that Epa of these molecules are lower than step potential of EP. Hence, it co-oxidizes with EP at higher concentrations. AA exists as anion while DA exists as a cation at physiological pH. AA was repelled at MCPE/pMWCNTs/SDS while DA got attracted. This leads to an increased sensitivity for DA while the sensitivity for AA is decreased at MCPE/pMWCNTs/SDS. Since the MCPE/pMWCNTs/SDS is completely free from the interference of all the above mentioned molecules at their significantly high concentrations, it can be used as potential electrochemical sensor for EP.

3.10. *Reproducibility and stability of the electrode*

A series of six modified electrodes were prepared in the same manner and amperometric response of 5.0×10^{-6} M EP was recorded consecutively. A RSD of 2.7% observed for these measurements indicates an excellent reproducibility of MCPE/pMWCNTs/SDS.

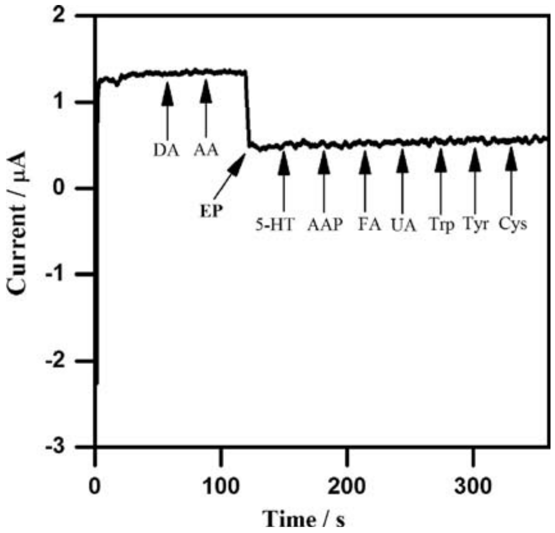


Fig. 6. Amperometric response of 3.0×10^{-7} M DA, 1.0×10^{-6} M AA, 1.0×10^{-5} M 5-HT, AAP, FA, UA, Trp, Tyr, Cys and 5.0×10^{-6} M EP at MCPE/pMWCNTs/SDS. Applied potential: 215.0 mV.

It was also found that properly stored MCPE/pMWCNTs/SDS could retain 93.6% of initial amperometric response for 5.0×10^{-6} M EP even after two weeks. This confirms the long term stability of MCPE/pMWCNTs/SDS. The amperometric response of 1.0×10^{-4} M EP at MCPE/pMWCNTs/SDS over a period of half an hour is displayed in Fig. S9 (Supplementary information). The amperometric response remains constant throughout the experiment. Hence, it can be concluded that MCPE/pMWCNTs/SDS is stable and does not undergo any surface fouling.

3.11. *Analytical applications of MCPE/pMWCNTs/SDS*

The ultimate use of any sensor depends on how effectively it could be applied to the detection of an analyte in real samples. In this respect, we wanted to put this electrode to test for its analytical utility. The MCPE/pMWCNTs/SDS was employed to determine the EP content in adrenaline tartrate injection and human blood serum by using a procedure reported elsewhere [64]. The standard addition method was used in order to rule out the matrix effect [65]. The estimation of EP was done by recovery studies in both the samples. An appropriate quantity of adrenaline tartrate injection (Harson Laboratories, specified content of adrenaline is 1.8 mg mL^{-1}) whose concentration should fall within working concentration range, was directly added to 0.1 M KPBS of pH 7.0 under stirring and the amperometric response was recorded at MCPE/pMWCNTs/SDS. The blood serum was diluted

Table 3
Results of real sample analysis at MCPE/pMWCNTs/SDS.

Real samples	EP added (μM)	EP found (μM)	Recovery (%)	RSD (%)
Blood serum	1.0	1.03 ± 0.0014	103.0	2.7
	3.0	2.93 ± 0.0018	97.7	2.9
	5.0	4.95 ± 0.0012	99.0	2.2
Adrenaline injection	0.2	0.19 ± 0.0011	95.0	2.6
	0.4	0.41 ± 0.0008	102.5	2.4
	0.6	0.58 ± 0.0012	97.2	2.6

twice with 0.1 M KPBS of pH 7.0 and this was followed by it being spiked with standard solutions of EP. The corresponding amperometric responses were recorded. Good quantitative recoveries were obtained for EP; both in adrenaline tartrate injection and the blood serum at MCPE/pMWCNTs/SDS. The results are listed in Table 3. This procedure was repeated five times ($n=5$) for each sample. The values of relative standard deviation (RSD) for all analysis were less than 3.0% which is an indication of good precision of MCPE/pMWCNTs/SDS in EP analysis. These observations ascertain the utility of MCPE/pMWCNTs/SDS as a reliable probe for the analysis of EP, both in pharmaceuticals and biological fluids.

4. Conclusions

The present work demonstrates that MCPE/pMWCNTs/SDS with adequate sensitivity, selectivity and stability can be used successfully as an amperometric sensor for the determination of EP. The MCPE/pMWCNTs had significant influence as a supporting material for the modification using SDS in comparison with MCPE/MWCNTs$_{OX}$ in the performance of the sensor in terms of better current sensitivity, oxidation overpotential and background current. The modified electrode exhibited excellent electrocatalytic activity towards the oxidation of EP. 100-fold excess of 5-HT, AAP, FA, UA, Trp, Tyr and Cys did not interfere in the determination of EP at MCPE/pMWCNTs/SDS. 10-fold excess of AA and twofold excess of DA do not alter the amperometric signal of EP. Small amounts of the modifiers used were sufficient to bring about an impressive electrocatalytic response. The pMWCNTs used for the modification can be used directly without subjecting them to any kind of processing. Practical applications of MCPE/pMWCNTs/SDS were demonstrated by estimating EP in pharmaceuticals and in body fluids. A wide linear dynamic range, low detection limit, simple fabrication procedure and quick response proved that MCPE/pMWCNTs/SDS is an efficient electrochemical sensor for the determination of EP in various matrices.

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Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.03.027.

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