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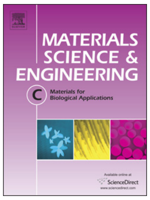

Electrocatalytic boost up of epinephrine and its simultaneous resolution in the presence of serotonin and folic acid at poly(serine)/multi-walled carbon nanotubes composite modified electrode: A voltammetric study

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ABSTRACT

The present paper describes the new strategy for the development of nanosensor based on dropcasting of multi-walled carbon nanotubes (MWCNTs) followed by electropolymerization of serine (ser) onto the glassy carbon electrode (GCE). The developed nanocomposite sensor was abbreviated as poly(ser)/MWCNTs/GCE and was characterized by using electrochemical impedance spectroscopy (EIS) technique. The EIS results confirmed the fast electron transfer rate at the surface of poly(ser)/MWCNTs/GCE. The proposed sensor exhibited good catalytic activity towards the sensing of epinephrine (EP) individually and simultaneously in the presence of serotonin (5-HT) and folic acid (FA) in 0.1 M phosphate buffer solution (PBS) at pH 7.0. The limit of detection (LOD) and limit of quantification (LOQ) of EP was found to be 6×10^{-7} M and 2×10^{-6} M respectively. The fabricated sensor showed excellent precision and accuracy with a relative standard deviation (RSD) of 4.86%. The proposed composite sensor was effectively applied towards the determination of EP in human blood serum and pharmaceutical injection sample.

1. Introduction

Epinephrine (EP) (1-(3,4-dihydroxyphenyl)-2-methyloamino-ethanol) (also known as adrenaline) is an important catecholamine neurotransmitter in the mammalian central nervous system for transporting the information among the biological cells [1], which exists in the nervous tissue and body fluids as a large organic cation. It plays significant role in the function of the central nervous, hormonal and cardiovascular systems. Many life phenomena are connected to the concentration of EP in blood. Changes of its concentration in blood show the way to many diseases [2]. Medically, EP has been used as a common emergency healthcare medicine [3]. These catecholamine drugs are used to treat hypertension, sepsis, bronchial asthma, cardiac surgery and myocardial infarction [4,5]. EP is not only healthcare medicine but also a powerful doping agent, thus, it is banned by the World Anti Doping Agency (WADA) in taking during sports competition [6]. Therefore, the determination of EP in human body fluids has attracted much attention to the researchers. Serotonin (5-hydroxy tryptamine, 5-HT) is a monoamine neurotransmitter synthesized in serotonergic neurons in the central nervous system (CNS) and plays a crucial role in the emotional system together with EP in the brain [7,8]. Folic acid (FA) (vitamin B_9) works principally in the brain and CNS. Thus, it is necessary for the production of EP and 5-HT in the nervous system. Therefore, simultaneous determination of EP, 5-HT and FA has acquired great importance.

Carbon nanotubes (CNTs) are considered as a novel material due to their unique properties and extensive applications since its discovery by Iijima [9]. Recently, CNTs are considered for the fabrication of electrochemical sensors because of their unequal properties such as anti-surface fouling, high sensitivity, a large edge plane/basal plane ratio, high surface-to-volume ratio [10], high ability to promote electron transfer [11], good electrical conductivity, and high chemical stability and tubular structure. Previously, different types of electrochemical sensors based on CNTs have been reported to detect the biological analytes such as paracetamol [12], neurotransmitters [13–15], proteins [16], cytochrome c [17], ascorbic acid [18], NADH [19] and hydrazine compounds [20,21]. Thus, CNTs are suitable materials for the development of electrochemical sensors.

Numerous analytical methods have been developed for the determination of EP. These methods consist of chemiluminescence [22], chromatographic methods [23], capillary electrophoresis [24], flow injection analysis [25] and spectroscopic methods [26]. However, these methods need more complicated procedures, costly instrumentation and over analysis time when compared to electroanalytical methods. Electroanalytical techniques have several advantages such as good sensitivity and selectivity, low cost, low background current, wide range of potential ranges, quick surface renewal, easy fabrication, and

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trouble-free operation. In electroanalytical techniques, the redox course of the analyte occurs at high over potential owing to the slow electron transfer rate at conventional electrodes (such as GCE, Au and Pt). The electrochemical determination of EP in the presence of ascorbic acid (AA), uric acid (UA), FA and 5-HT at the conventional electrodes is of poor result, to overcome this problem, the modification of the electrode surface is of great interest. The concept of electrode surface modification is one of the advanced methods in the field of electroanalytical chemistry. The modifications onto the surface of electrode lead to considerable diminish of over potentials and enhance the electron transfer rate for system of redox reactions. Various materials have been used for the modification of electrode surfaces such as polymer films [27–29], nanoparticles [30,31], surfactants [32–34], organic compounds [35,36], metals [37], and metal oxide nanoparticles [38] including MWCNTs [39,40]. Nevertheless, when compared to the above materials, polymer layer-MWCNTs composite based electrodes show high sensitivity and boost up the electro-redox reaction of analytes. A poly(serine) film onto the electrode surfaces offer extraordinary surface area, thereby, exhibits good catalytic activity towards the electrochemical determination of analyte. In this connection, in our present study, a facile and simple nanocomposite sensor (poly(ser)/MWCNTs/GCE) was synthesized for the determination of EP individually and simultaneously in the presence of FA and 5-HT.

2. Experimental

2.1. Instrumentation

CHI 660D electrochemical work station was used for the measurements of cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS). A conventional three electrode system was employed, which consists of a modified/unmodified GCE as working electrode; saturated calomel electrode (SCE) as reference electrode to measure cell potentials and glassy carbon rod as an auxiliary electrode to measure the current. Elico U 120 pH meter combined with pH CL 51 B was used to measure pH values. IR spectra were recorded on Bruker Alpha-Eco ATR–FTIR (Attenuated total reflection–Fourier transform infrared) interferometer with single reflection sampling module equipped with ZnSe crystal.

2.2. Reagents

Epinephrine was from HiMedia Laboratories Pvt. Limited, Mumbai. Serotonin and folic acid serine were from Sigma. $K_3[Fe(CN)_6]$ was from Merck Specialities Pvt. Limited, Mumbai. $K_4[Fe(CN)_6]$ and KCl were from Qualigens fine chemicals, Mumbai. Multi-walled carbon nanotubes (Dropsens, Edificio CEEI, Llanera (SPAIN)) were used as received. The stock solution of 10 mM EP was prepared and stored in a refrigerator. The working solution was prepared by diluting the stock solution with buffer solution. 0.1 M PBS was prepared from $NaH_2PO_4 \cdot 2H_2O$ and Na_2HPO_4. Adrenaline® was taken as epinephrine injection. All chemicals were of analytical grade and were used without further purification.

2.3. Preparation of MWCNTs/GCE

The GCE was polished to a mirror finished on polish pads with 0.3 and 0.05 μm alumina slurry and cleaned thoroughly with distilled water successively. MWCNTs were solubilized in ethanol (1 mg/ml) and then ultrasonicated for 5 min to get MWCNTs dispersion. Immobilization of MWCNTs was achieved by drop casting of 5 μL onto the surface of GCE and dried at room temperature in the air. Hereafter the resultant electrode was abbreviated as MWCNTs/GCE and was used as working electrode.

2.4. Preparation of poly(ser)/GCE and poly(ser)/MWCNTs/GCE

Fig. 1 shows the uninterrupted 5 CV cycles at MWCNTs/GCE and GCE in 1 mM serine aqueous solution (pH 7.0). Initially, the scan was run from −0.5 to 1.0 V, it was observed that no polymer film was formed on the MWCNTs/GCE and GCE surface. However, when the window was extended from −0.5 to 2.0 V the effective polymerization was achieved. On the other hand, when the potential window was extended in negative direction, the polymerization was not observed. Therefore, the window −0.5 to 2.0 V was considered for the successful polymerization process. The film coated electrode was washed with distilled water to remove the physically adsorbed material. Hereafter the resulted electrodes were abbreviated as poly(ser)/GCE and poly(ser)/MWCNTs/GCE respectively and were used as working electrodes. The steps involved towards the fabrication of poly(ser)/MWCNTs/GCE was illustrated in Fig. 2.

3. Result and discussion

3.1. Characterization of poly(ser)/MWCNTs composite

FT-IR spectrum (Fig. S1) of the poly(ser)/MWCNTs composite gives a confirmative evidence for the presence of poly(ser) molecule and the MWCNTs. The asymmetric stretching of the CH group of the poly(ser) molecule was found as a prominent band at 2926 cm^{-1}. A strong and characteristic band at 1744 cm^{-1} is due to stretching vibration of carbonyl group. Appearance of a band at 3438 cm^{-1} confirms the stretching vibrations of the NH and OH functions. Strong stretching bands are evident in the region 1600–1400 cm^{-1} particularly at 1403, 1465, 1580 cm^{-1} for MWCNTs [41].

3.2. Electrochemical impedance study of poly(ser)/MWCNTs/GCE

Electrochemical impedance spectroscopy (EIS) technique is a powerful and emerging tool for the study of interfacial electron transfer properties and is used to identify the impedance changes of the electrode surface during the modification process. By using EIS, the impedance spectral data was recorded for bare GCE, poly(ser)/GCE, MWCNTs/GCE and poly(ser)/MWCNTs/GCE (in the form of Nyquist plots) in 1 M KCl containing 2.5 mM $[Fe(CN)_6]^{3-/4-}$ as a redox probe at the initial potential of 0.209 V with the range from 1 to 10^5 Hz (Fig. 3). Primarily, the semicircle portion at high frequencies in the Nyquist plots corresponds to the magnitude of the charge transfer resistance (R_{ct}) in the equivalent circuit. This value extensively varies based on the modification of the electrode surfaces [42]. In order to work out the R_{ct} values, the impedance values were fitted to Randles equivalent circuit (Fig. 3

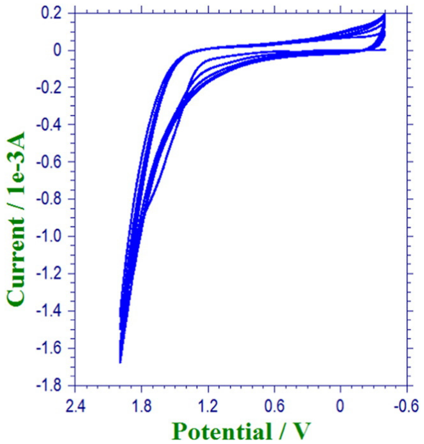

Fig. 1. Five continuous cyclic voltammograms for the electrochemical polymerization of serine onto the surface of MWCNTs/GCE.

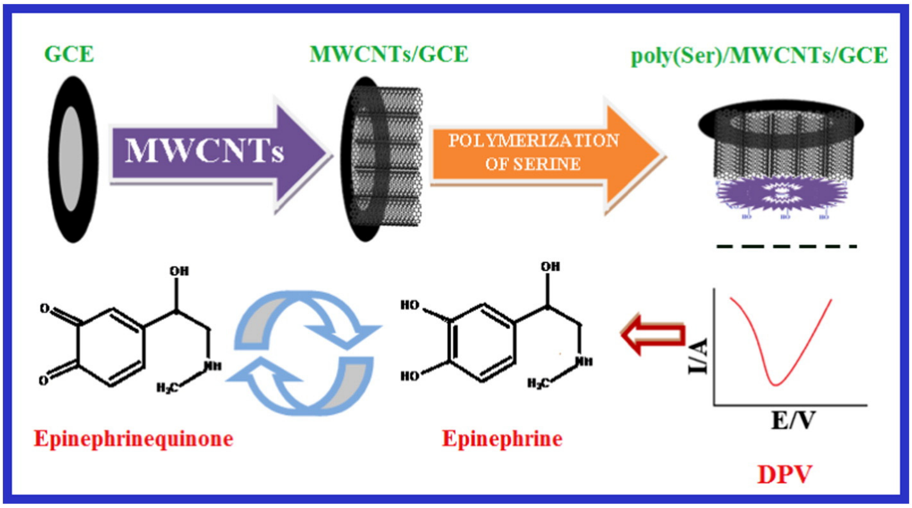

Fig. 2. A schematic diagram showing different steps involved in developing poly(ser)/MWCNTs/GCE towards the determination of EP.

inset), which was considered based on the features of the obtained impedance spectrum. As can be seen from Fig. 3, a semicircle with large diameter was observed with R_{ct} of 114.7 Ω at bare GCE. On the one hand, the diameter of semi-circle diminished to R_{ct} 107.9 Ω at poly(ser)/GCE. The decreased R_{ct} indicates that the presence of better conductive polymer layer of serine onto the GCE. In contrast, on drop casting the MWCNTs onto the GCE, responded with low diameter of semicircle (R_{ct} 10.55 Ω) because of surface anti-fouling activity and high enhanced conductivity. On the other hand, by the electropolymerization of serine onto the MWCNTs/GCE, the diameter of semicircle was drastically lowered to 7.145 Ω. This was mainly observed due to the synergistic effect of MWCNTs and polymer of serine. This observed effect showed the extraordinary catalytic activity. This is an evidence that the support of charge transfer kinetics was a huge extent at poly(ser)/MWCNTs/GCE on account of a poor charge transfer resistance at this nanocomposite sensor.

3.3. Electrochemical investigation and electrochemistry of EP

The electrochemical behavior of 1 mM EP in 0.1 M PBS (pH 7.0) was investigated using the CV technique. Fig. 4 shows the response of EP at (a) GCE, (b) MWCNTs/GCE and (c) poly(Ser)/MWCNTs/GCE. At the bare GCE, the electrochemical oxidation of EP occurs at about 0.332 V and the peak was broad with poor response signifying slow electron transfer kinetics at GCE. While, the peak potential was reduced to 0.127 V with considerable enlargement in the peak current during the oxidation of EP at MWCNTs/GCE suggesting the MWCNT composite sensor as a well-organized electron promoter in electrochemical reaction of EP. However, a large improvement in the anodic peak current was observed for the electrochemical oxidation of EP at poly(ser)/MWCNTs/GCE surface, which was about 2.7 and 5 times higher in comparison with MWCNTs/GCE and GCE, respectively. The obtained results clearly demonstrates that the increase in the peak current and lowering of over oxidation potential at poly(ser)/MWCNTs/GCE were due to electrocatalysis [43]. Therefore, the MWCNTs were responsible in the improvement of surface area onto the GCE and the layer of serine polymer on MWCNTs/GCE could improve the reversibility of the electron transfer processes [44]. The electrochemical response of EP shows enormous variation at poly(ser)/MWCNTs/GCE when compared to GCE and MWCNTs/GCE. As can be seen in Fig. 4, there were no observable peaks at GCE (system 'a') i.e., the anodic peak 'A_2' was not resolved well since the electron diffusion at this electrode was very slow. While, high catalytic activity was observed at MWCNTs/GCE due to large surface area, but noticeable peak current was not appeared (system 'b'). However, at poly(ser)/MWCNTs/GCE (system 'c'), well resolved peaks were observed with highly enhanced peak currents, indicating that the polymer of serine has picked up the electron transfer rate between EP and nanocomposite sensor. This behavior might be due to the fact that secondary amine group of serine on MWCNTs attracts the electroactive OH groups

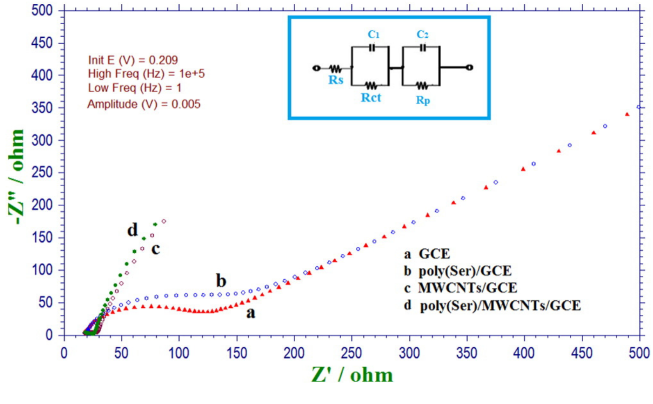

Fig. 3. EIS spectrum of 1 M KCl solution containing 2.5 mM $[Fe(CN)_6]^{-3/-4}$ at different electrodes; inset — Randles equivalent circuit to poly(ser)/MWCNTs/GCE.

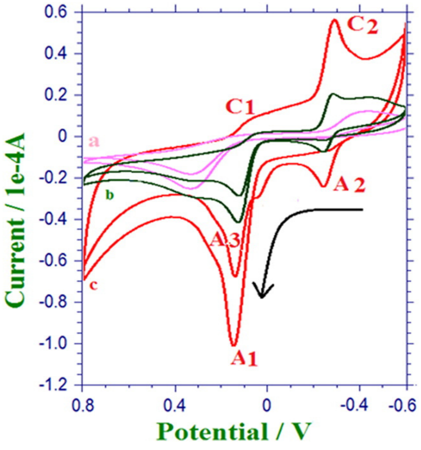

Fig. 4. Cyclic voltammograms for the electrochemical response of 1 mM EP at (a) bare GCE, (b) MWCNTs/GCE and (c) poly(ser)/MWCNTs/GCE in 0.1 M PBS of pH 7.0 at a scan rate of 0.05 $V \cdot s^{-1}$.

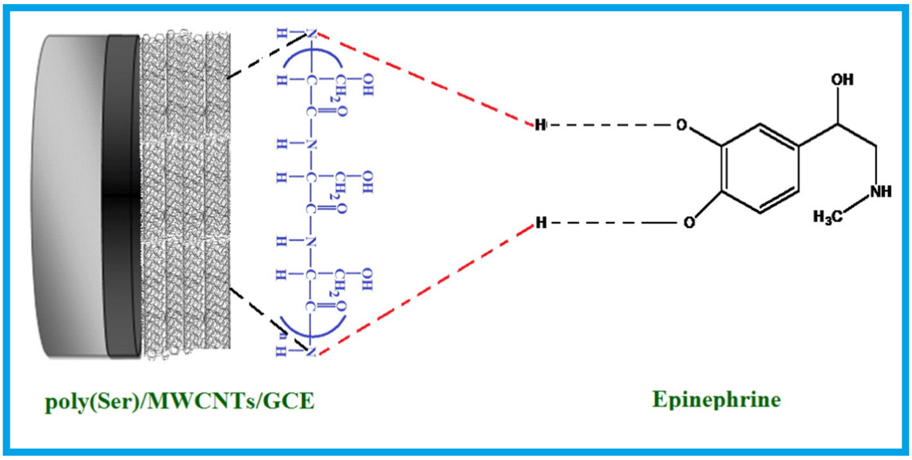

Fig. 5. A schematic diagram showing the probable catalytic mechanism of poly(ser)/MWCNTs/GCE towards EP.

of EP, thereby, leading to boost up the EP concentration on the electrode surface (Fig. 5). The probable catalytic mechanism of EP was shown in Fig. 6 [45]. From Fig. 4 (system 'c') electrochemical reaction mechanism of EP at poly(ser)/MWCNTs/GCE can be expressed as follows: in the positive scan direction i.e., from −0.6 to 0.8 V, only one peak 'A_1' appeared at 0.332 V corresponding to the electro-oxidation of EP to open-chain quinone by the deprotonation process. In the reverse scan direction i.e., from 0.8 to −0.6 V, epinephrine quinone has undergone reduction at 0.090 V (peak 'C_1'), but it was less favorable. However, epinephrine quinone Cyclises to epinephrinechrome via 1,4-Micheal addition reaction. Peak 'C_2' at −0.291 V in the potential direction 0.8 to −0.6 V corresponds to the reduction of epinephrinechrome to leucoepinephrinechrome. However, by extending the scan direction again in the positive direction i.e., from −0.6 to 0.8 V, two new anodic peaks 'A_2'and 'A_3' were observed at −0.243 and 0.033 V, respectively. A new peak 'A_2' corresponds to the reversible oxidation of leucoepinephrinechrome to epinephrinechrome. The leucoepinephrinechrome is a spontaneous process and is converted to 5,6-dihydroxy-N-methylindole. This 5,6-dihydroxy-N-methylindole is electroactive in nature, thereby, it undergoes further oxidation to give 5,6-diquinone-N-methylindole (A_3 peak). Therefore, the developed nanocomposite sensor is reliable for the study of electrochemical behavior of EP.

3.4. Effect of solution pH

In order to optimize the solution pH and to obtain the best anodic peak resolution and high sensitivity, 0.1 M PBS containing 1 mM EP was studied at poly(ser)/MWCNTs/GCE by employing CV technique. As illustrated in Fig. 7, the experiment was performed in the pH range from 5.0 to 8.0. As the pH of the solution was increased, the anodic peak current density gradually increases from 5.0 to 7.0. When the pH of the solution is above 7.0, the anodic peak current diminished. However, the maximum peak resolution and sensitivity was observed at pH 7.0. Hence, this pH was chosen as an optimal pH for the entire work. Furthermore, the anodic peak potential shifted linearly towards more negative side as the pH of the solution was increased from 5.0 to 8.0. This indicates the participation of protons in the electrochemical oxidation of EP at poly(ser)/MWCNTs/GCE. A plot of pH of the solution vs anodic peak potential (Fig. 7 — inset) was constructed and was linearly connected with a linear regression equation of

$$E_{pa}(V) = 0.69154 - 0.0765\ \ pH \quad (R = 0.99288).$$

The plot has linearity with a slope of 76 mV pH^{-1}, this value was nearly closer to the theoretical value (59 mV pH^{-1}) of the Nernstian equation for equal number of electrons and protons transfer reaction [46]. This indicates the involvement of equal number of protons and electrons in the oxidation of EP at poly(ser)/MWCNTs/GCE.

3.5. Effect of potential scan rate

The effect of potential scan rate on the peak currents was investigated in 1 mM EP at poly(ser)/MWCNTs/GCE. As illustrated in Fig. 8A, the peak currents were increasing with the increase in the scan rate. The plot of square of scan rate ($\nu^{1/2}$) vs redox peak currents was linearly associated with the correlation coefficients of R = 0.99842, 0.99958, respectively, with a corresponding linear regression equations of

$$I_{pa}\left(10^{-5}A\right) = -0.53769 - 37.71943\ \ \left(V \cdot s^{-1}\right)^{1/2}$$

$$I_{pc}\left(10^{-5}A\right) = -0.62304 + 17.3842\ \ \left(V \cdot s^{-1}\right)^{1/2}.$$

This indicates that the electrochemical reaction of EP at nanocomposite modified electrode was a diffusion controlled process.

To support the electrochemical reaction mechanism of EP, the variation of the peak potentials as a function of the scan rate was analyzed. The formal potential (E_0) of EP was deduced from the intercept of ν vs E_{pa} plot (Fig. 8B). From the plot (E_{pa}–E_0) vs ln I_{pa} (Fig. 8C), it was possible to calculate the value of the energy transfer coefficient (α) and was found to be as 0.384. The number of electrons involved during the oxidation of EP step can be calculated by using the following Eq. (2) [47].

$$m = RT/[(1-\alpha)nf] \tag{2}$$

where'α' is the energy transfer coefficient and 'n' is the number of electrons transferred during the reaction. R, F and T are the universal gas constant, Faraday constant and absolute temperature, respectively. The 'α' value and the slope obtained from the plot ln ν vs E_{pa} (Fig. 8D) was substituted in Eq. (2). From the calculations, the number of electrons involved during oxidation of EP step was found to be as 1.77 (≈2). This result was experimentally evident that the electro-oxidation of EP was a two electron process.

3.6. Effect of EP concentration

The effect of EP concentration was examined in 0.1 M PBS (pH 7.0) at the surface of the developed nanocomposite sensor using DPV technique. Fig. 9 illustrates the increase in anodic peak current with the increase in the concentration of EP. From the plot between the

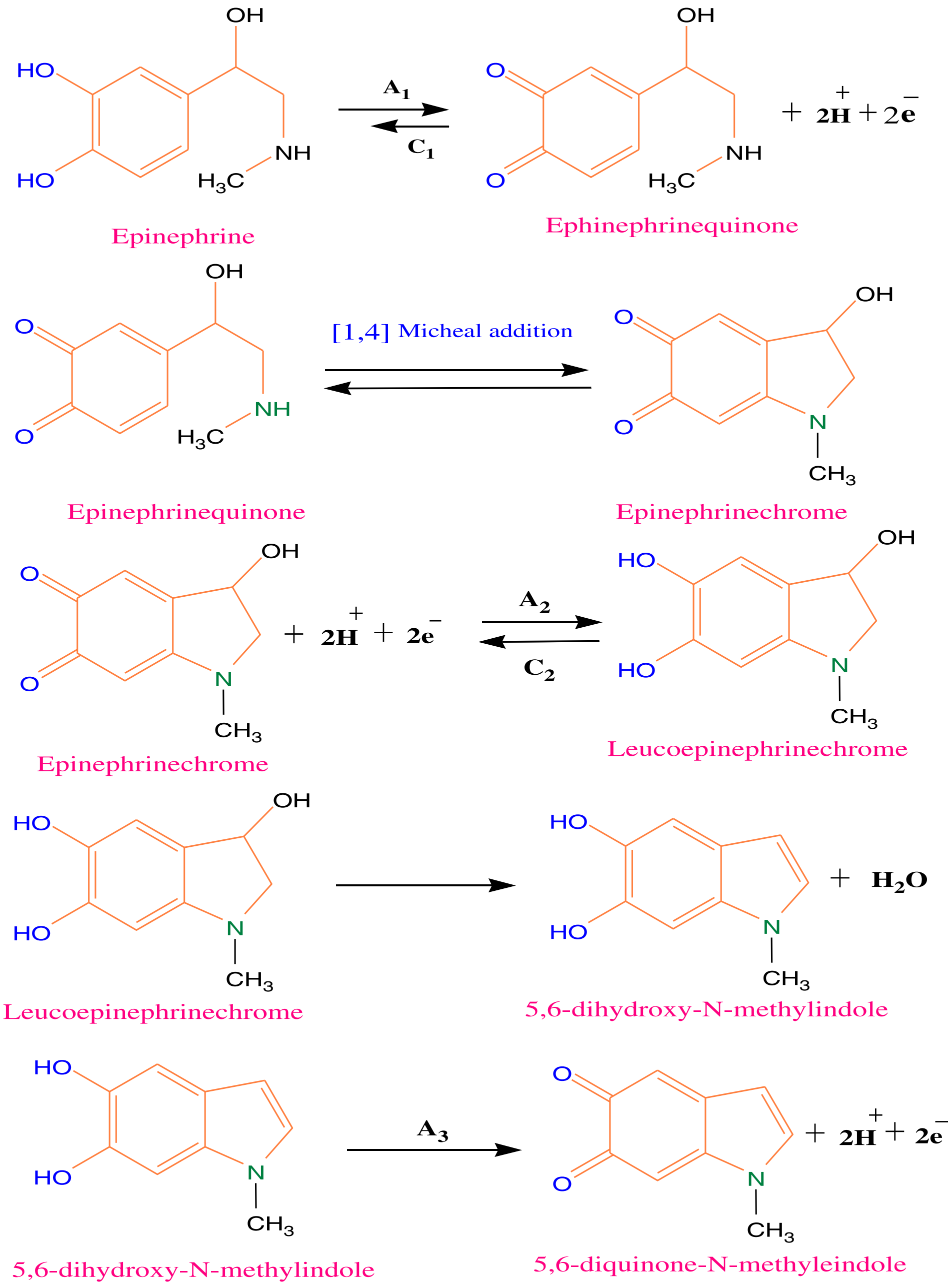

Fig. 6. The proposed electrochemical mechanism of EP at poly(ser)/MWCNTs/GCE.

concentration of EP (10^{-7} M) and anodic peak current (10^{-7} M), a linearity was observed with a corresponding linear regression equation of

$$I_{pa}\left(10^{-7}A\right) = -0.98686 + 0.09168\left(10^{-7}A\right) \qquad R = 0.99949.$$

The limit of detection (LOD) and limit of quantification (LOQ) were found to be as 6×10^{-7} M and 2×10^{-6} M, respectively. The LOD and LOQ values were calculated by using the following equations [48,49]

$$LOD = 3S/M$$

$$LOQ = 10S/M$$

where 'S' is the standard deviation of peak currents and 'M' is the slope of the working curve. The LOD and linearity range of EP at various modified electrodes were compared with our present method and are shown in Table 1.

3.7. Stability, repeatability and reproducibility of poly(ser)/MWCNTs/GCE

To investigate the stability of the poly(ser)/MWCNTs/GCE, the CV of 1 mM EP in PBS (pH 7.0) solution was recorded for every 3 min intervals. A total of 20 parallel CV runs were recorded without any observable decline in the composite response and was found that both anodic and cathodic peaks remained comparatively stable after the first cycle (Fig. 10). The relative standard deviation (RSD) of these 20 cycles was 4.86%. The repeated measurements specify that the developed nanocomposite sensor had a good reproducibility and does not suffer from surface fouling during the experimental conditions. In addition, the reproducibility of the poly(ser)/MWCNTs/GCE was studied by

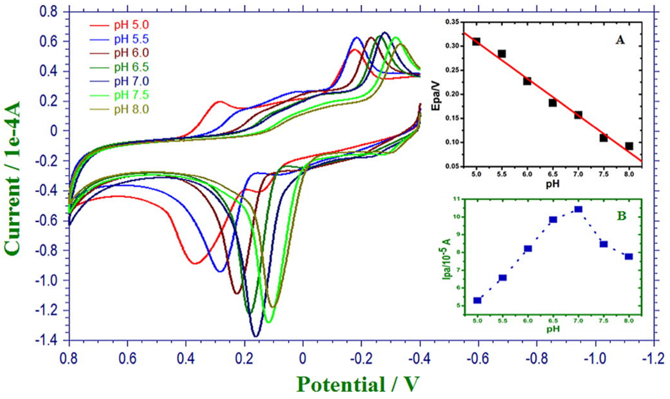

Fig. 7. Cyclic voltammograms obtained at poly(ser)/MWCNTs/GCE in 0.1 M PBS solution containing 1 mM EP at different pH values. Inset — (A): A plot between EP oxidation peak potential vs pH of PBS; inset — (B): A plot of EP oxidation peak current vs pH.

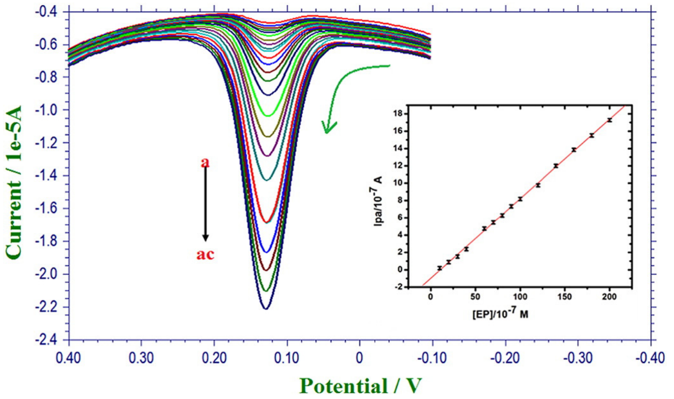

Fig. 9. Differential pulse voltammograms of EP with the different concentrations in PBS at pH 7.0: (a) 1×10^{-6} M (b) 2×10^{-6} M (c) 3×10^{-6} M (d) 4×10^{-6} M (e) 6×10^{-6} M (f) 7×10^{-6} M (g) 8×10^{-6} M (h) 9×10^{-6} M (i) 1×10^{-5} M (j) 1.2×10^{-5} M (k) 1.4×10^{-5} M (l) 1.6×10^{-5} M (m) 1.8×10^{-5} M (n) 2×10^{-5} M (o) 2.5×10^{-5} M (p) 3×10^{-5} M (q) 3.5×10^{-5} M (r) 4×10^{-5} M (s) 5×10^{-5} M (t) 6×10^{-5} M (u) 7×10^{-5} M (v) 8×10^{-5} M (w) 1×10^{-4} M (x) 1.2×10^{-4} M (y) 1.4×10^{-4} M (z) 1.6×10^{-4} M (aa) 1.8×10^{-4} M (ab) 2×10^{-4} M (ac) 2.2×10^{-4} M; inset — calibration plot of EP concentration.

constructing four nanocomposite modified electrodes of the same kind and these were used to test in 1 mM of EP and found that there was no much change in the results.

3.8. Simultaneous resolution of EP, 5-HT and FA

The simultaneous resolution of EP, 5-HT and FA at a conventional GCE suffers from interference due to their closely related oxidation potentials. However, the achievability of the simultaneous resolution of EP, 5-HT and FA at a nanocomposite sensor was made easier to vary because, they were well resolved with their oxidation potentials by reducing their over potentials. The investigation was carried out by varying the concentration of each individual and by keeping the concentration of remaining two species constant.

The determination of EP in the presence of 10 μM 5-HT and 5 μM FA was performed at poly(ser)/MWCNTs/GCE with the constant increase in the concentration of EP from 29 μM to 69 μM (Fig. 11A). As illustrated in the Fig. 11A, the shape of the anodic peaks was well resolved

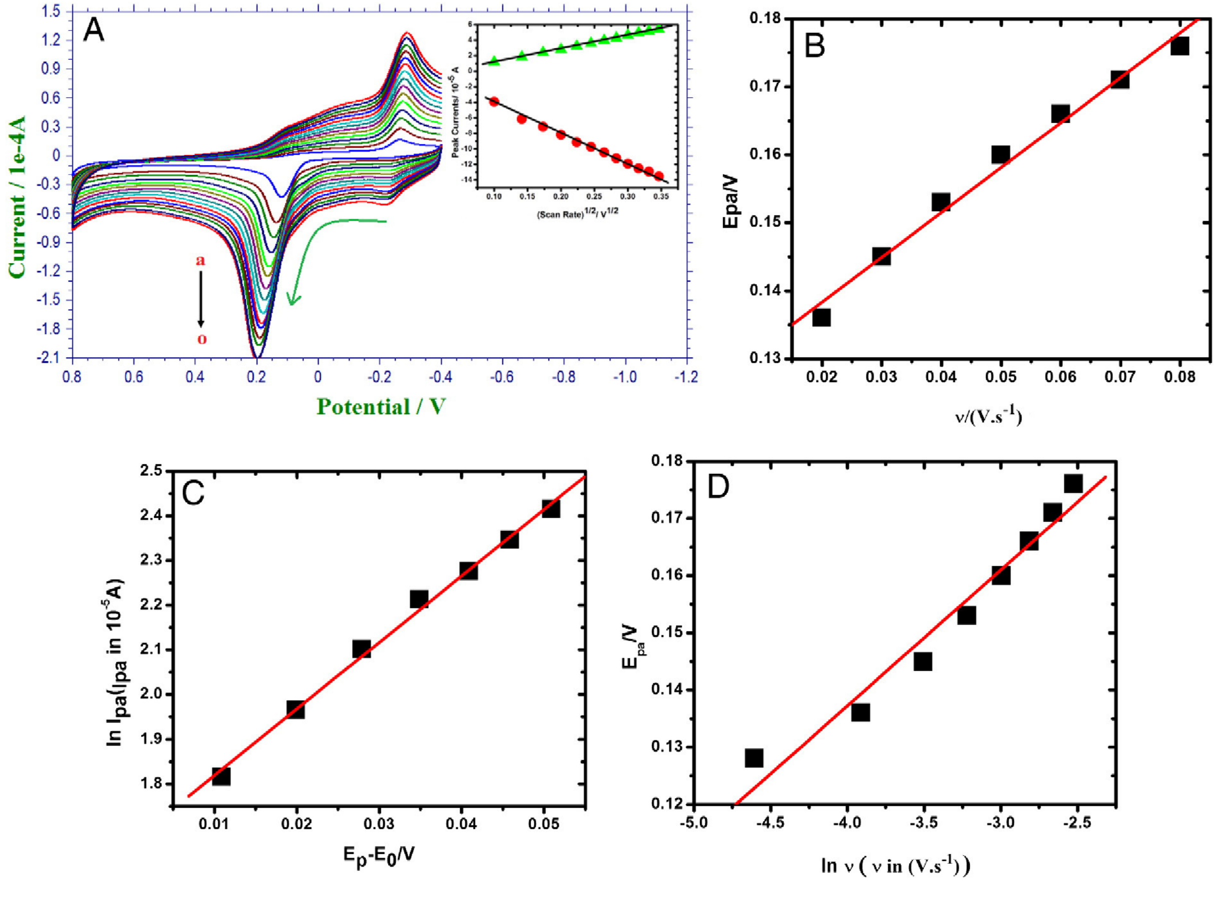

Fig. 8. (A). Cyclic voltammograms of EP at poly(ser)/MWCNTs/GCE in 0.1 M PBS solution of pH 7.0 at different scan rates (a to o, 0.01–0.15 V.s^{-1}). Inset — calibration plots for the redox peak currents vs the square root of scan rate; (B) relation between the scan rate (ν) and peak potential (E_{pa}); (C) experimental variation of peak current (ln Ipa) as a function of the difference of $E_{pa} - E_0$; (D) experimental variation of peak current (ln ν) against a function of E_{pa}.

Table 1
Comparison of the efficiency of some modified electrodes used in the electro-oxidation of EP.

Electrode	Method	pH	LOD (M)	Ref.
Ru[a]/WGE[b]	DPV	4.0	8.0×10^{-7}	[50]
GNPs[c]/GCE	SWV	7.0	5.0×10^{-6}	[51]
GN[d]-SPEs[e]	CV	5.4	1.0×10^{-4}	[52]
5-Amino-3′,4′-dimethylbiphenyl-2-ol/CPE	CV	–	6.2×10^{-7}	[53]
Au–Cys[f]–SWCNT[g]–CoTAPc[h]	SWV	7.0	6×10^{-6}	[54]
Poly(ser)/MWCNTs/GCE	DPV	7.0	6×10^{-7}	Present work

[a] Rutin.
[b] Paraffin-impregnated graphite electrode.
[c] Gold nanoparticle.
[d] Graphene.
[e] Screen printed electrodes.
[f] Cysteamine.
[g] Single-walled carbon nanotubes.
[h] Cobalt(II)tetra-aminophthalocyanine.

corresponding to 5-HT and FA. From Fig. 11A (inset), it was observed that the anodic peak response was linearly connected with the concentration of EP with a linear regression equation of

$$I_{pa}\left(10^{-7}\mathrm{A}\right) = -1.94232 + 0.42148\ [\mathrm{EP}]\ (\mu\mathrm{M}) \quad R = 0.99751.$$

Likewise, the determination of 5-HT was carried out in the presence of 10 μM EP and 12 μM FA. The concentration of 5-HT was increased linearly from 5 μM to 30 μM (Fig. 11B). From the plot between the concentration of 5-HT and peak currents (Fig. 11B(inset)), a linear relation was observed with a linear regression equation of

$$I_{pa}\left(10^{-7}\mathrm{A}\right) = 3.83067 + 1.21823\ [\text{5-HT}]\ (\mu\mathrm{M}) \quad R = 0.99512.$$

FA was also determined in the presence of 5 μM 5-HT and 10 μM EP. The concentration of FA was increased from 5 μM to 110 μM (Fig. 11C) in the same way as discussed above. From the plot between the concentration and peak currents (Fig. 11C (inset)), a linearity was observed with a linear regression equation of

$$I_{pa}\left(10^{-7}\mathrm{A}\right) = -5.0302 + 0.44836\ [\mathrm{FA}]\ (\mu\mathrm{M}) \quad R = 0.99831.$$

These results proved that the nanocomposite sensor was efficient in simultaneous sensing of EP, 5-HT and FA.

3.9. Determination of EP in real samples

In order to validate the reliability of the present method, the analysis of EP in the injection sample (adrenaline specified content: 1.00 mg $\mathrm{mL^{-1}}$) at poly(ser)/MWCNTs/GCE was carried out. The specified concentration of injection sample was analyzed in 0.1 M PBS (pH 7.0) and blood serum using DPV technique with a standard addition of working analyte. The results of the analysis were given in Table 2. The result shows that the content values evaluated by the proposed method were closer to the labeled content. Thus, the sensing performance of poly(ser)/MWCNTs/GCE towards EP was reliable in the pharmaceutical as well as blood serum samples.

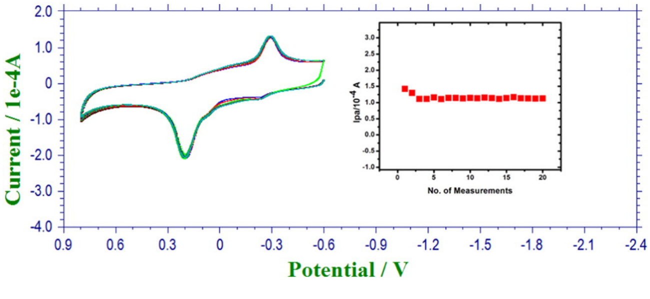

Fig. 10. Cyclic voltammograms for 20 parallel cycles of 1 mM EP in 0.1 M PBS solution of pH 7.0 at a scan rate of 0.05 $\mathrm{V \cdot s^{-1}}$; inset — a plot of 20 parallel cycles of CVs vs I_{pa}.

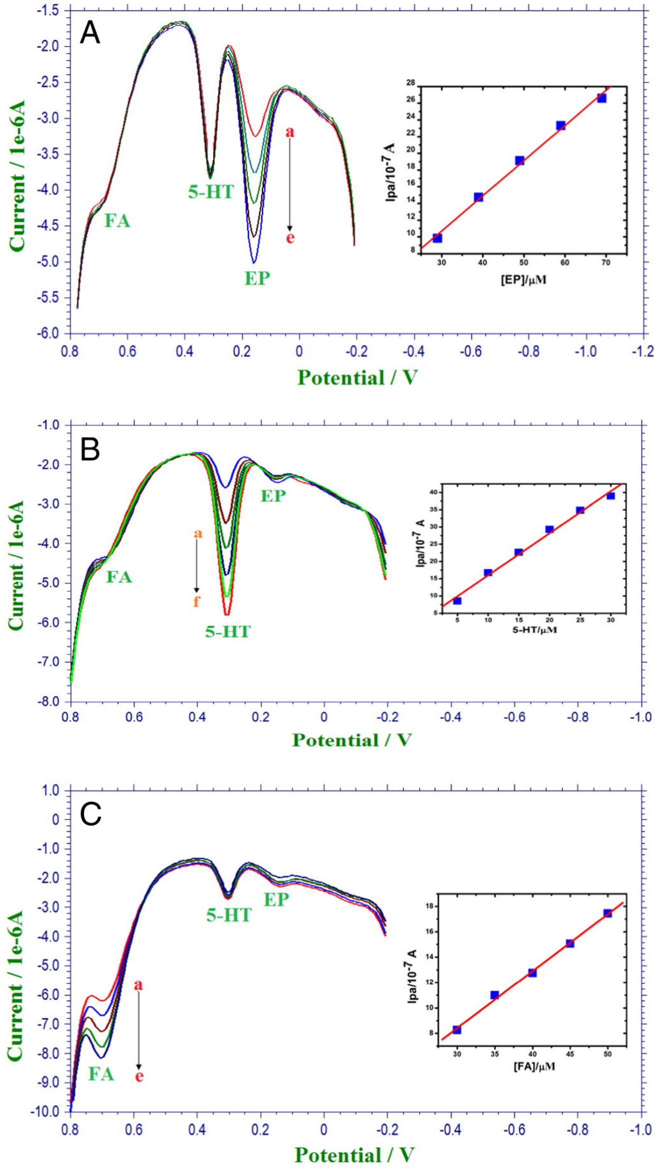

Fig. 11. (A) Differential pulse voltammograms obtained for EP, in the presence of 1×10^{-5} M 5-HT and 5×10^{-6} M FA at poly(ser)/MWCNTs/GCE. Concentration of EP: (a) 2.9×10^{-5} M (b) 3.9×10^{-5} M (c) 4.9×10^{-5} M (d) 5.9×10^{-5} M (e) 6.9×10^{-5} M; inset — calibration plot of EP determination. (B) Differential pulse voltammograms obtained for 5-HT, in the presence of 1×10^{-5} M EP and 1.2×10^{-5} M FA at poly(ser)/MWCNTs/GCE. Concentration of 5-HT: (a) 5×10^{-6} M (b) 1×10^{-5} M (c) 1.5×10^{-5} M (d) 2×10^{-5} M (e) 2.5×10^{-5} M (f) 3×10^{-5} M; inset — calibration plot of 5-HT determination. (C) Differential pulse voltammograms obtained for FA, in the presence of 5×10^{-6} M 5-HT and 1×10^{-5} M EP at poly(ser)/MWCNTs/GCE. Concentration of FA: (a) 3×10^{-5} M (b) 3.5×10^{-5} M (c) 4×10^{-5} M (d) 4.5×10^{-5} M (e) 5×10^{-5} M; inset — calibration plot of FA determination.

Table 2
Determination of EP in real samples.

Real sample	Added (μM)	Found (μM)	Recovery (%)	Bias
Adrineline® injection	100	98.20	98.2	−1.8
Blood serum	100	97.95	97.95	−2.05

4. Conclusions

Present paper demonstrates that the fabricated poly(ser)/MWCNTs/GCE showed satisfactory sensitivity, selectivity and stability in sensing the EP in 0.1 M PBS at pH 7.0. The present nanocomposite sensor holds great promise for simultaneous determination of EP, 5-HT and FA. The sensor was also effectively employed for the determination of EP in the commercial injection and human blood serum samples. Due to its high stability and repeatability of poly(ser)/MWCNTs/GCE, it has high potential for the future developments of nanosensors in clinical and pharmaceutical research.

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References

[1] Q.M. Xue, Physiological and Pathological Chemistry of Nervous System, Science Press, Beijing, 1978. 102.
[2] C.-W. Hsu, M.-C. Yang, Electrochemical epinephrine sensor using artificial receptor synthesized by sol–gel process, Sensors Actuators B 134 (2008) 680–686.
[3] T.N. Deftereos, A.C. Calokerinos, C.E. Efstathiou, Flow injection chemiluminometric determination of epinephrine, norepinephrine, dopamine and l-dopa, Analyst 118 (1993) 627–632.
[4] P. Hernandez, I. Sanchez, F. Paton, L. Hernandez, Cyclic voltammetry determination of epinephrine with a carbon fiber ultramicroelectrode, Talanta 46 (1998) 985–991.
[5] J. Bergquist, A. Sciubis, A. Kaczor, J. Silberring, Catecholamines and methods for their identification and quantitation in biological tissues and fluids, J. Neurosci. Methods 113 (2002) 1–13.
[6] T. Tavana, M.A. Khalilzadeh, H. Karimi-Maleh, A.A. Ensafi, H. Beitollahi, D. Zareyee, Sensitive voltammetric determination of epinephrine in the presence of acetaminophen at a novel ionic liquid modified carbon nanotubes paste electrode, J. Mol. Liq. 168 (2012) 69–74.
[7] S.X.M. Li, K.W. Perry, D.T. Wong, Influence of fluoxetine on the ability of bupropion to modulate extracellular dopamine and norepinephrine concentrations in three mesocorticolimbic areas of rats, Neuropharmacology 42 (2002) 181–190.
[8] H. Maharaj, D.S. Maharaj, K.S. Saravanan, K.P. Mohanakumar, S. Daya, Aspirin curtails the acetaminophen-induced rise in brain norepinephrine levels, Metab. Brain Dis. 19 (2004) 71–77.
[9] S. Iijima, Helical microtubules of graphitic carbon, Nature 354 (1991) 56–58.
[10] A. Merkoc, Carbon nanotubes in analytical sciences, Microchim. Acta 152 (2006) 157–174.
[11] Z.Y. Liu, R. Yuan, Y.Q. Chai, Y. Zhuo, C.L. Hong, X. Yang, H.L. Su, X.Q. Qian, Highly sensitive, reusable electrochemical aptasensor for adenosine, Electrochim. Acta 54 (2009) 6207–6211.
[12] T.-L. Lu, Y.-C. Tsai, Sensitive electrochemical determination of acetaminophen in pharmaceutical formulations at multi-walled carbon nanotube-alumina-coated silica nanocomposite modified electrode, Sensors Actuators B 153 (2011) 439–444.
[13] T. Thomas, R.J. Mascarenhas, B.E. Kumara Swamy, P. Martis, Z. Mekhalif, B.S. Sherigara, Multi-walled carbon nanotube/poly(glycine) modified carbon paste electrode for the determination of dopamine in biological fluids and pharmaceuticals, Colloids Surf. B 110 (2013) 458–465.
[14] P.-Y. Chen, R. Vittal, P.-C. Nien, K.-C. Ho, Enhancing dopamine detection using a glassy carbon electrode modified with MWCNTs, quercetin, and Nafion®, Biosens. Bioelectron. 24 (2009) 3504–3509.
[15] Y.F. Zhao, Y.Q. Gao, D.P. Zhan, H. Liu, Q. Zhao, Y. Kou, Y.H. Shao, M.X. Li, Q.K. Zhuang, Z.W. Zhu, Selective detection of dopamine in the presence of ascorbic acid and uric acid by a carbon nanotubes-ionic liquid gel modified electrode, Talanta 66 (2005) 51–57.
[16] J. Wang, T. Tangkuaram, S. Loyprasert, T. Vazquez-Alvarez, W. Veerasai, P. Kanatharana, P. Thavarungkul, Electrocatalytic detection of insulin at Ru_{Ox}/carbon nanotube-modified carbon electrodes, Anal. Chim. Acta 581 (2007) 1–6.
[17] Y. Zhang, J.B. Zheng, Direct electrochemistry and electrocatalysis of cytochrome c based on chitosan–room temperature ionic liquid-carbon nanotubes composite, Electrochim. Acta 54 (2008) 749–754.
[18] U. Yogeswaran, S.M. Chen, Multi-walled carbon nanotubes with poly(methylene blue) composite film for the enhancement and separation of electroanalytical responses of catecholamine and ascorbic acid, Sensors Actuators B 130 (2008) 739–749.
[19] B. Ge, Y.M. Tan, Q.J. Xie, M. Ma, S.Z. Yao, Preparation of chitosan–dopamine-multiwalled carbon nanotubes nanocomposite for electrocatalytic oxidation and sensitive electroanalysis of NADH, Sensors Actuators B 137 (2009) 547–554.
[20] L. Zheng, J.F. Song, Curcumin multi-wall carbon nanotubes modified glassy carbon electrode and its electrocatalytic activity towards oxidation of hydrazine, Sensors Actuators B 135 (2009) 650–655.
[21] D.A. Geraldo, C.A. Togo, J. Limson, T. Nyokong, Electrooxidation of hydrazine catalyzed by noncovalently functionalized single-walled carbon nanotubes with CoPc, Electrochim. Acta 53 (2008) 8051–8057.
[22] T. Li, Z. Wang, H. Xie, Z. Fu, Highly sensitive trivalent copper chelate-luminol chemiluminescence system for capillary electrophoresis detection of epinephrine in the urine of smoker, J. Chromatogr. B 911 (2012) 1–5.
[23] G. Zhang, Y. Zhang, C. Ji, T. McDonald, J. Walton, E.A. Groeber, R.C. Steenwyk, Z. Lin, Ultra sensitive measurement of endogenous epinephrine and norepinephrine in human plasma by semi-automated SPE-LC–MS/MS, J. Chromatogr. B 895–896 (2012) 186–190.
[24] Y. Zhao, S. Zhao, J. Huang, F. Ye, Quantum dot-enhanced chemiluminescence detection for simultaneous determination of dopamine and epinephrine by capillary electrophoresis, Talanta 85 (2011) 2650–2654.
[25] A.V. Bulatov, A.V. Petrova, A.B. Vishnikin, A.L. Moskvin, L.N. Moskvin, Stepwise injection spectrophotometric determination of epinephrine, Talanta 96 (2012) 62–67.
[26] E.V. Efremov, F. Ariese, C. Gooijer, Achievements in resonance Raman spectroscopy review of a technique with a distinct analytical chemistry potential, Anal. Chim. Acta 606 (2008) 119–134.
[27] O. Gilbert, B.E. Kumara Swamy, U. Chandra, B.S. Sherigara, Simultaneous detection of dopamine and ascorbic acid using polyglycine modified carbon paste electrode: a cyclic voltammetric study, J. Electroanal. Chem. 636 (2009) 80–85.
[28] P.V. Narayana, T. Madhusudana Reddy, P. Gopal, K. Reddaiah, P. Raghu, Development of electrochemical sensor based on poly(xylenol orange) film towards the determination of L-dopa and its simultaneous resolution in the presence of uric acid: a cyclic voltammetric study, Res. J. Chem. Sci. 4 (2014) 37–43.
[29] U. Chandra, B.E. Kumara Swamy, O. Gilbert, M. Pandurangachar, Sathish Reddy, S.S. Shankar, B.S. Sherigara, Poly(amaranth) film based sensor for resolution of dopamine in the presence of uric acid: a voltammetric study, Chin. Chem. Lett. 21 (2010) 1490–1492.
[30] Y. Liu, J.S. Huang, H.Q. Hou, T.Y. You, Simultaneous determination of dopamine, ascorbic acid and uric acid with electrospun carbon nanofibers modified electrode, Electrochem. Commun. 10 (2008) 1431–1434.
[31] P. Kannan, S.A. John, Determination of nanomolar uric and ascorbic acids using enlarged gold nanoparticles modified electrode, Anal. Biochem. 386 (2009) 65–72.
[32] S. Chitravathi, B.E. Kumara Swamy, E. Niranjana, Umesh Chandra, G.P. Mamatha, B.S. Sherigara, Electrochemical studies of sodium levothyroxine at surfactant modified carbon paste electrode, Int. J. Electrochem. Sci. 4 (2009) 223–237.
[33] U. Chandra, O. Gilbert, B.E. Kumara Swamy, Y.D. Bodke, B.S. Sherigara, Electrochemical studies of eriochrome black T at carbon paste electrode and Immobilized by SDS surfactant: a cyclic voltammetric study, Int. J. Electrochem. Sci. 3 (2008) 1044–1054.
[34] M. Pandurangachar, E. Niranjana, B.E. Kumara Swamy, B.S. Sherigara, K.V. Pai, Electrochemical studies of amaranth at surfactant modified carbon paste electrode: a cyclic voltammetry, Int. J. Electrochem. Sci. 3 (2008) 588–596.
[35] H.R. Zare, N. Nasirizadeh, M.M. Ardakani, Electrochemical properties of a tetrabromo-p-benzoquinone modified carbon paste electrode, application to the simultaneous determination of ascorbic acid, dopamine and uric acid, J. Electroanal. Chem. 577 (2005) 25–33.
[36] P. Kalimuthu, S.A. John, Selective electrochemical sensor for folic acid at physiological pH using ultrathin electropolymerized film of functionalized thiadiazole modified glassy carbon electrode, Biosens. Bioelectron. 24 (2009) 3575–3580.
[37] S.E. Qouatli, R. Najih, V. Hambate, A. Chtaini, Electrochemical studies and square wave voltammetry of paracetamol at manganese modified carbon paste electrode, Pharm. Anal. Acta 4 (2013) 2.
[38] M. M.-Ardakani, H. Beitollahi, M.K. Amini, F. Mirkhalaf, M. A.-Alibeik, New strategy for simultaneous and selective voltammetric determination of norepinephrine, acetaminophen and folic acid using ZrO_2 nanoparticles-modified carbon paste electrode, Sensors Actuators B 151 (2010) 243–249.
[39] Z.A. Alothman, N. Bukhari, S.M. Wabaidur, S. Haider, Simultaneous electrochemical determination of dopamine and acetaminophen using multiwall carbon nanotubes modified glassy carbon electrode, Sensors Actuators B 146 (2010) 314–320.
[40] J. Zhang, X. Tan, D. Zhao, S. Tan, Z. Huang, Y. Mi, Z. Huang, Study of nimesulide and its determination using multi-walled carbon nanotubes modified glassy carbon electrodes, Electrochim. Acta 55 (2010) 2522–2526.
[41] H. Shang, C. Liu, Fei Wei, FT-IR study of carbon nanotube supported Co–Mo catalysts, J. Nat. Gas Chem. 13 (2004) 95–100.
[42] Y.J. Liu, F. Yin, Y.M. Long, Z.H. Zhang, S.Z. Yao, Study of the immobilization of alcohol dehydrogenase on Au-colloid modified gold electrode by piezoelectric quartz crystal sensor, cyclic voltammetry, and electrochemical impedance techniques, J. Colloid Interface Sci. 258 (2003) 75–81.
[43] C.P. Andrieux, O. Haas, J.M. SavGant, Catalysis of electrochemical reactions at redox-polymer-coated electrodes. Mediation of the iron(III)/iron(II) oxido-reduction by a polyvinylpyridine polymer containing coordinatively attached bisbipyridine chlororuthenium redox centers, J. Am. Chem. Soc. 108 (1986) 8175–8182.
[44] C. Li, Voltammetric determination of tyrosine based on an L-serine polymer film electrode, Colloids Surf. B 50 (2006) 147–151.
[45] B.J. Sanghavi, S.M. Mobin, P. Mathur, G.K. Lahiri, A.K. Srivastava, Biomimetic sensor for certain catecholamines employing copper(II) complex and silver nanoparticle modified glassy carbon paste electrode, Biosens. Bioelectron. 39 (2013) 124–132.
[46] B.D. Jones, J.D. Ingle Jr., Evaluation of immobilized redox indicators as reversible, in situ redox sensors for determining Fe(III)-reducing conditions in environmental samples, Talanta 55 (2001) 699–714.
[47] K. Aoki, K. Akimoto, K. Tokuda, H. Matsuda, J. Osteryoung, Linear sweep voltammetry at very small stationary disc electrodes, J. Electroanal. Chem. 171 (1984) 219–220.
[48] P.V. Narayana, T. Madhusudana Reddy, P. Gopal, G. Ramakrishna Naidu, Electrochemical sensing of paracetamol and its simultaneous resolution in the presence

of dopamine and folic acid at a multi-walled carbon nanotubes/poly(glycine) composite modified electrode, Anal. Methods 6 (2014) 9459–9468.

[49] P.V. Narayana, T. Madhusudana Reddy, P. Gopal, P. Raghu, K. Reddaiah, M. Srinivasulu, Development of trypan blue polymer film based electrochemical sensor for the determination of dopamine and its simultaneous detection in presence of ascorbic acid and uric acid: a voltammetric method, Anal. Bioanal. Electrochem. 6 (2014) 485–500.

[50] G.-P. Jin, Q.-Z. Chen, Y.-F. Ding, J.-B. He, Electrochemistry behavior of adrenalin, serotonin and ascorbic acid at novel poly rutin modified paraffin-impregnated graphite electrode, Electrochim. Acta 52 (2007) 2535–2541.

[51] X.H. Wei, F. Wang, Y.M. Yin, Q.Y. Liu, L.N. Zou, B.X. Ye, Selective detection of neurotransmitter serotonin by a gold nanoparticle-modified glassy carbon electrode, Analyst 135 (2010) 2286–2290.

[52] F. Valentini, D. Romanazzo, M. Carbone, G. Palleschi, Modified screen-printed electrodes based on oxidized graphene nanoribbons for the selective electrochemical detection of several molecules, Electroanalysis 24 (2012) 872–881.

[53] S. Mohammadi, H. Beitollahi, A. Mohadesi, Electrochemical behaviour of a modified carbon nanotube paste electrode and its application for simultaneous determination of epinephrine, uric acid and folic acid, Sens. Lett. 11 (2013) 388–394.

[54] K.I. Ozoemena, D. Nkosi, J. Pillay, Influence of solution pH on the electron transport of the self-assembled nanoarrays of single-walled carbon nanotube-cobalt tetraaminophthalocyanine on gold electrodes: electrocatalytic detection of epinephrine, Electrochim. Acta 53 (2008) 2844–2851.