Anal. Bioanal. Electrochem., Vol. 10, No. 2, 2018, 175-191

Analytical & Bioanalytical Electrochemistry

2018 by CEE
www.abechem.com

Full Paper

Electrochemical Detection of Serotonin in Human Serum Sample and Simultaneous Resolution in Presence of Epinephrine

Kasetty Reddaiah,[1,*] Kummari Subba Venkata Krishna Rao[1] and Tukiakula Madhusudana Reddy[2,*]

[1]*Polymer Biomaterial Design and Synthesis Laboratory, Department of Chemistry, Yogi Vemana University, Kadapa-516003, Andhra Pradesh, India*
[2]*Electrochemical Research Laboratory, Department of Chemistry, SVU College of Sciences, Sri Venkateswara University, Tirupati- 517 502, Andhra Pradesh, India*

*Corresponding Author, Tel.: +91-8562-225410
E-Mail: k.reddyprasad4u@gmail.com
*Corresponding Author, Tel.: +91-877-2289303
E-Mail: tmsreddysvu@gmail.com

Received: 1 June 2017 / Accepted: 5 January 2018 / Published online: 28 February 2018

Abstract- In this paper, a new stable and sensitive electrochemical sensor was prepared by two-fold modification of glassy carbon electrode (GCE) with poly-alizarin red S (AzrS) and multiwalled carbon nanotubes (MWCNTs). The fabricated poly-AzrS/MWCNTs/GCE modified chemical sensor was employed towards the investigation of electrocatalytic oxidation of serotonin in 0.1 mol/dm^3 phosphate buffer (PBS) solution of pH 6.0 with the help of voltammetric techniques such as cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS). The developed electrochemical sensor exhibited a potent and persistent electron mediating behaviour towards the serotonin determination and also lavishly resolved oxidation peaks for serotonin and epinephrine (EP) simultaneously. The effect of experimental variables, such as potential scan rate, solution pH, simultaneous determination, accumulation time, impedance and concentration were examined. The limit of detection (LOD) and limit of quantification (LOQ) were found to be as 1.8×10^{-7} mol/dm^3 and 17.52×10^{-7} mol/dm^3, respectively with a dynamic range from 0.5×10^{-6} to 1.1×10^{-5} mol/dm^3. The fabricated electrochemical sensor was applied for the direct determination of serotonin in blood serum samples. Further the

fabricated two-fold modified electrochemical sensor exhibited good sensitivity, stability and reproducibility in comparison with bare GCE towards the determination of serotonin.

Keywords- Alizarin red S, Epinephrine, Glassy carbon electrode, Multiwalled carbon nanotubes, Simultaneous determination, Serotonin

1. INTRODUCTION

5-hydroxytryptamine (5HT) commonly known as serotonin, is produced from the enzymes tryptophan hydroxylase and aromatic amino acid decarboxylase. Serotonin is electrochemically active, but its detection is more difficult than epinephrine, because serotonin is a reactive oxidation product. The serotonin neurotransmitter system plays an important role within the cells of living organisms. i.e., the regulation of mood, attention, appetite, sleep, and other functions [1,2]. Eccentric levels of serotonin have been associated with several disorders such as exquisitely depression, Alzheimer's, Parkinson's, migraine, anxiety and synapse formation [3-5].

1-(3,4-dihydroxyphenyl)-2-methyloaminoethanol is otherwise known as epinephrine or adrenaline is an important catecholamine neurotransmitter in the mammalian central nervous system for transporting the information to biological cells [6-8]. Deficiency of EP may cause several diseases and neurological disorders such as shakiness, sweating, fast heart beat and high blood pressure and also Alzheimer's, Hyperactivity disorder, Schizophrenia, and Huntington's diseases. Medically, EP has been used as a common emergency healthcare medicine. Epinephrine plays an key role in increasing blood flow to muscles with fight-or-flight response, treatment in bronchial asthma, cardiac surgery and myocardial infarction [9-11].

Alizarin red S (3, 4-dihydroxy-9, 10-dioxo-2-anthracene sulfonic acid) is an anthraquinone derivative dye compound. It is used in biochemical assay and as a pH indicator. Alizarin red S on the surface of the GCE has higher concentration of negatively charged functional group $-SO_3^-$ and electron-rich oxygen atoms on its surface, these active sites facilitate the electrochemical deposition and strong cohesion of electro-active species [12]. Most of the redox dyes are artificial electron donors and can easily undergo electro-polymerization to generate stable redox active layers.

Carbon nanotubes (CNTs) are prime member of the carbon family offering attractive electronic, mechanical and electrochemical properties combined with chemical stability [13,14]. CNTs-modified electrodes are widely used in electroanalysis because, it exhibits π–π conjugating structure with high hydrophobic surface, ability to promote fast electron-transfer, and also exhibit unique properties, such as strong electrocatalytic activity, high effective surface area, and excellent electrical conductivity, it also has an additional assets, such as strong adherence to the electrode surface, more active sites and homogeneity in electrochemical deposition [15,16].

Voltammetry is a modest and low-cost electro analytical method with notable detection limit, suitable sensitivity, good reproducibility, and ease of miniaturization rather than other analytical methods such as chromatographic techniques [17], electrophoresis [18], mass-spectroscopy [19], spectrophotometry [20], capillary zone electrophoresis [21], chemiluminescence [22] and fluorescence [23].

Construction of electrochemical sensors towards the determination of neurotransmitters such as serotonin and EP continues to be of great interest, since the serotonin and EP are indistinguishable and influence each other in biological system and also exhibit very close oxidation potentials, hence the resolution of EP in the presence of serotonin is of great interest [24-26]. Therefore, the simultaneous firmness of EP and serotonin is of major importance in the field of neurochemistry, diagnostic and pathological research. Moreover, the redox reactions of these species at unmodified electrodes suffer from fouling effect due to the accumulation of oxidized products and insulating layers on the electrode surface which results in rather poor selectivity, sensitivity and reproducibility. To overcome these problems electrode surfaces were reformed with nanomaterials, carbon nanotubes, electro polymerization and self-assembled mono layers [27-29]. These modified electrodes reduce the over potential and helps in improving the mass transfer velocity of the analyte and enrich the active surface area of the electrode and prevent the passivation effect [30-32].

In the present investigation, the electrocatalytic oxidation of serotonin was examined at poly-AzrS/MWCNTs/GCE with the assistance of CV and DPV. The modification method was characterized by CV, DPV and EIS. The performance of the two-fold chemically modified sensor was checked towards the determination of serotonin in human blood serum samples. The two-fold chemically modified sensor was found to exhibit satisfactory result with good sensitivity, selectivity, and reproducibility.

2. EXPERIMENTAL

2.1. Instrumentation

A CHI 660D electrochemical analyser (CH Instrument, Austin, USA) 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 consisted of a modified GCE as a working electrode, saturated calomel electrode (SCE) as a reference electrode to measure cell potentials and glassy carbon rod as a counter electrode to measure the current. All potentials represented in this paper were reported against the calomel electrode. The pH values were measured with Elico U 120 pH meter with a combined pH CL 51 B electrode.

2.2. Reagents

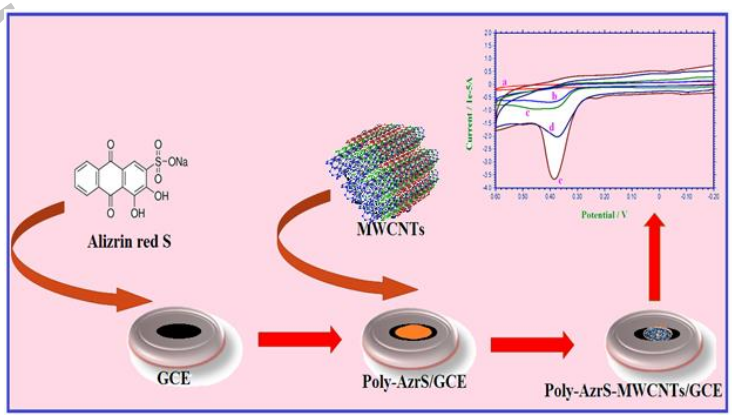
Serotonin and epinephrine were from Merck Specialities Pvt. Limited Mumbai, K4 [Fe $(CN)_6$] and KCl were from Qualigens Fine Chemicals, Mumbai. Alizarin red S was from Fisher Scientific Pvt. Limited, Mumbai, Multi-walled carbon nanotubes were from Dropsens, Edificio CEEI, Llanera (SPAIN). The stock solutions of 10×10^{-3} mol/dm^3 of serotonin and EP was prepared by dissolving in perchloric acid and working solution was prepared through diluting the stock solution with 0.1 mol/dm^3 phosphate buffer solution (PBS) which was prepared from $NaH_2PO_4\cdot2H_2O$ and Na_2HPO_4. All chemicals were of analytical grade and were used without further purification.

2.3. Preparation of poly-Alizarin red S modified glassy carbon electrode (poly-AzrS/GCE)

The GCE was polished to a mirror finished on chamois leather polishing pads with 1.0, 0.3, and 0.05 μm alumina (Al_2O_3) slurry and cleaned thoroughly with ultrapure distilled water without interruption. The aqueous solution of Alizarin red S was placed in the electrochemical cell, GCE was dipped in it and scanned for 15 multiple cycles between the potential windows from -0.6 V to +1.4 V at a scan rate of 100 mVs^{-1}. When the number of cycles was above 15, the peak currents started to decrease, this was due to the fact that the active electrode surface area does not change significantly after 15 cycles and the thickness of the poly-film increases, which hinders the electron transfer rate. After polymerization, the poly-AzrS film/GCE was rinsed with PBS buffer to remove the physically adsorbed material and was used for further experimental procedure.

2.4. Fabrication of poly-AzrS/MWCNTs/GCE

1 mg of MWCNTs was weighed accurately and solubilized in 2 mL of ethyl alcohol and sonicated by using ultra sonication bath (Toshiba, India, 1.5 L (H)) for about 10 min to get MWCNT dispersion.



Scheme 1. The electrode mechanism of poly-AzrS/MWCNTs/GCE towards serotonin

2.4. Fabrication of poly-AzrS/MWCNTs/GCE

1 mg of MWCNTs was weighed accurately and solubilized in 2 mL of ethyl alcohol and sonicated by using ultra sonication bath (Toshiba, India, 1.5 L (H)) for about 10 min to get MWCNT dispersion. The dispersion was stored in refrigerator for further use. 5 µL of MWCNTs dispersion was casted on to the surface of the poly-AzrS/GCE, and then it was allowed to dry to the natural air at room temperature for 5min and the resultant electrode was used as working electrode. The steps followed towards the fabrication of Poly-AzrS/MWCNTs/GCE were portrayed in Scheme 1.

3. RESULT AND DISCUSSION

3.1. Electrochemical fabrication of AzrS film on GCE

Alizarin red S is an anthraquinone redox dye and its electrochemical polymerization process onto the GCE surface was achieved between the potential window -0.6 and +1.4 V through CV for 15 multiple cycles. The potential window scan province was predominantly in the positive potential path and this was the most important factor in pursuing the poly-AzrS film. If the potential window was more than +1.4 V or lesser than -0.6 V, than it was observed that the development of poly-film on the GCE was not stable. The redox system of AzrS was gradually decreased up to the 3rd cycle and was steady there after. By this producer a stable poly film was obtained [33-35]. Fig.1a shows the 15 cycles of cyclic voltammograms which was responsible for the formation of Alizarin red S polymer film on the GCE. The electrode magnitude (poly-AzrS film) was adjusted by increasing the number of cycles of polymerization.

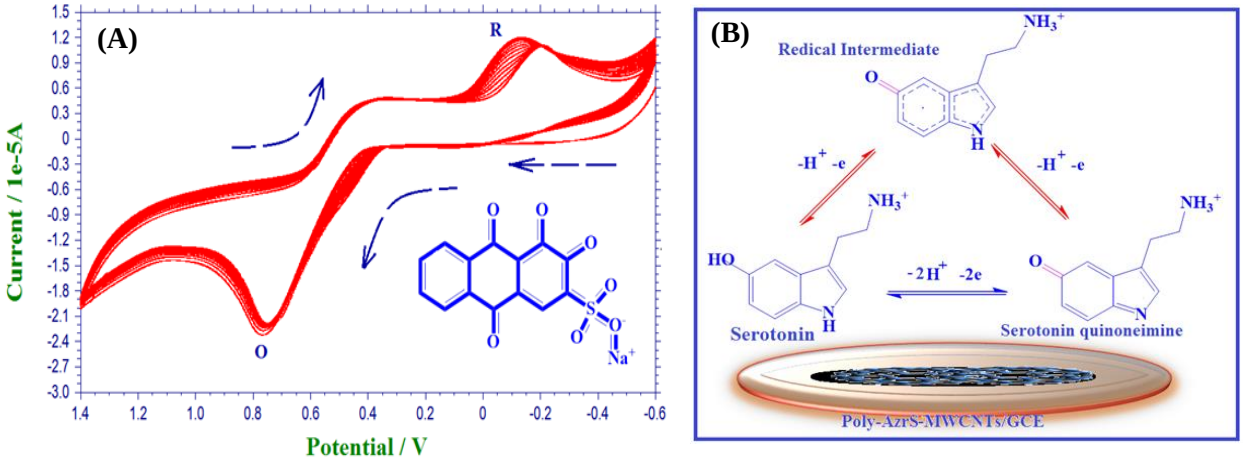


Fig. 1. (**A**) Cyclic voltammograms for the electrochemical polymerization of Alizarin red S at GCE in 0.1 mol/dm^3 PBS at a scan rate of 100 mV S^{-1} in PBS buffer of pH 7.0; (**B**) Electrochemical oxidation mechanism of serotonin at poly-AzrS/MWCNTs/GCE

The electro polymerization fashion of AzrS was similar to the radical polymerization of phenol. Alizarin red S having benzoquinonyl and hydroxyl groups can serve as a proton receptor that promotes neutral reaction and enhances the charge transfer rate between the electrode and reagent. The fabricated poly-AzrS electrode was again modified with MWCNT suspension to make poly-AzrS/MWCNTs/GCE in order to get better sensitivity towards the determination of analyte in comparison with poly-AzrS/GCE. The electrochemical oxidation mechanism of serotonin at poly-AzrS/MWCNTs/GCE was shown in Fig.1b, respectively.

3.2. Voltammetric study of Serotonin

The voltammetric response of serotonin at the two-fold chemically modified electrode was studied by employing cyclic voltammetry. Fig. 2 represents CV of 2×10^{-4} mol/dm^3 serotonin in 0.1 mol/dm^3 PBS (pH 6.0) recorded at bare GCE (b), poly-AzrS/GCE (c), MWCNTs/GCE (d) and poly-AzrS/MWCNTs/GCE (e). At the bare GCE, serotonin showed an irreversible behaviour with relatively lesser current response. The CVs of serotonin at the poly-AzrS/MWCNTs/GCE modified electrode exhibited oxidation peak with a higher magnitude of current response in comparison to bare GCE. It can be seen from Fig. 2 that the serotonin exhibits a well-defined oxidation peak at the poly-AzrS/MWCNTs/GCE, and the over-potential of serotonin was reduced in comparison with bare GCE. The background current of poly-AzrS/MWCNTs/GCE (curve e) was higher than that of the bare GCE (curve b), which is ascribed to the larger surface area of the poly-AzrS/MWCNTs/GCE film. The results demonstrated that the poly-AzrS/MWCNTs/GCE modified electrode exhibited excellent electrocatalytic activity towards the oxidation of serotonin.

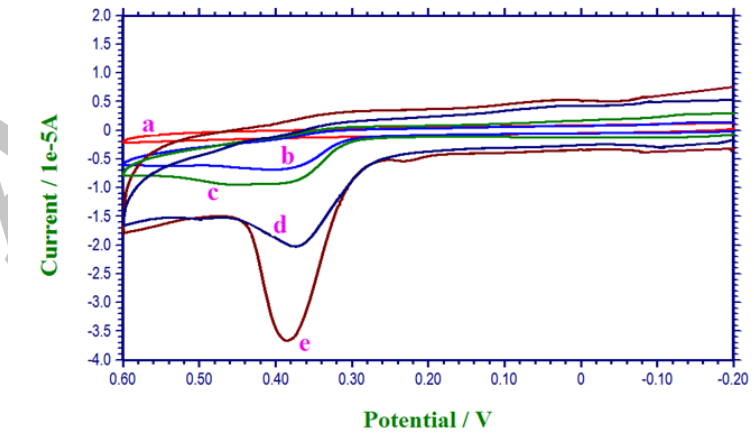


Fig. 2. Cyclic voltammograms for the electrochemical response of 2×10^{-4} mol/dm^{3}serotonin at (a) Blank(b) Bare GCE (c) poly-AzrS/GCE (d) MWCNTs/GCE (e) poly-AzrS/MWCNTs/GCE in 0.1 mol/dm^3 PBS (pH 6.0) at a scan rate of 100 mV S^{-1}

3.3. The effect of pH

The effect of solution pH on the electrochemical response of serotonin at poly-AzrS/ MWCNTs/GCE was investigated in 0.1 mol/dm^3 PBS solution of various pH values (5.5 to 8.0) with the help of CV and DPV techniques and was shown in Fig. 3a and 3b. As it can be seen from the figures, that the peak currents decreased with increasing in the solution pH ranging from 6.0 to 8.0, and also it was observed that the peak currents of the system shifted towards less positive side, and maximum peak current was observed at pH 6.0. Therefore PBS solution of pH 6.0 was selected for all subsequent electrochemical analysis of serotonin.

The effect of pH against peak currents and peak potentials at a scan rate of 100 mVs^{-1} was shown in Fig. 3c.

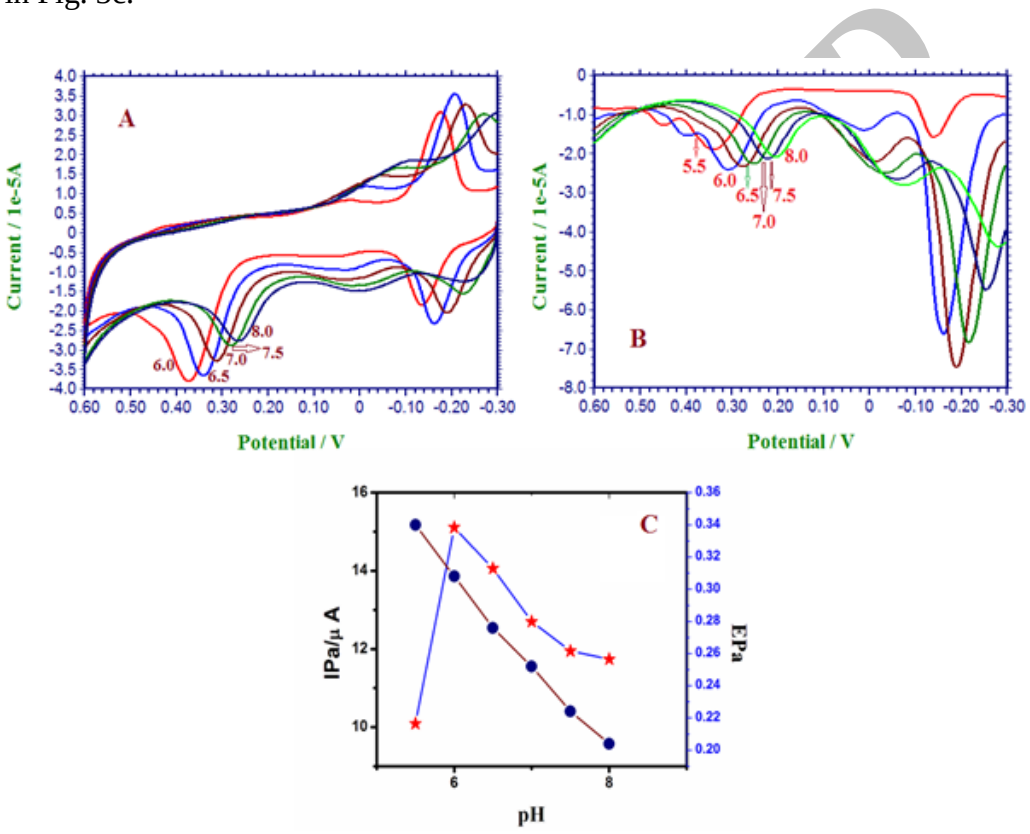


Fig. 3. **(A)** Cyclic voltammograms obtained at poly-AzrS/MWCNTs/GCE in 0.1 mol/dm^3 PBS solution in different pH values of (a) 6.0 (b) 6.5 (c) 7.0 (d) 7.5 (e) 8.0; (**B**) Differential pulse voltammograms obtained at poly-AzrS/MWCNTs/GCE in 0.1 mol/dm^3; PBS solution in different pH values of (a) 5.5 (b) 6.0 (c) 6.5 (d) 7.0 (e) 7.5 (f) 8.0; (**C**) A plot of serotonin oxidation peak current, peak potentials Vs pH

It can be observed from the plot that the peak currents were maxima at pH 6.0. The number of electrons involved in the electrocatalytic oxidation of serotonin was calculated by using Nernst equation (Eqn.1). By substituting the slop obtained from the plot Ep_a Vs pH in eqn.1, the number of electrons and protons involved in the reaction process were calculated

and was found to be as 1.862 (≈2) [36]. This result corresponds to the Nernst equation for a two-electron and two-proton transfer reaction process.

$$E = E^0 - 0.059\ v\ pH/\ n \quad (1)$$

Where 'v' is the number of protons involved in the reaction, 'n' is the number of electrons involved in the complete reaction

3.4. The effect of scan rate

The effect of potential scan rate on the electrocatalytic current response of serotonin was investigated through the developed two-fold poly-AzrS/MWCNTs/GCE at various sweep rates (υ) in 0.1mol/dm^3 PBS pH (6.0) solution and it was shown in Fig 4a and 4b. As shown in the figure the oxidation peak currents increased linearly with increase in the potential scan rate for serotonin ranging from 30-150 mVs^{-1}. The investigation showed that the peak currents increased linearly with square root of scan rate (v) with a linear equations of I_{pa} (μA)=0.99792 $v^{1/2}$+0.02721 respectively. This indicates that the electron transfer process of serotonin was diffusion controlled.

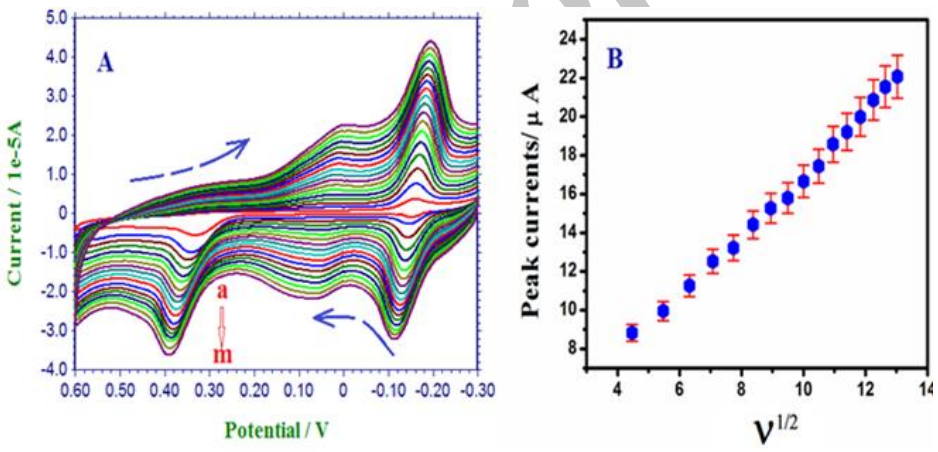


Fig. 4. (**A**) Cyclic voltammograms of different scan rates for serotonin at poly-AzrS/MWCNTs/GCE (a to m 30 –150 mV S^{-1}), in PBS solution of pH 6.0; (**B**) Calibration plots for the redox peak currents Vs the square root of scan rate

The area of the poly-AzrS/MWCNTs/GCE was calculated through cyclic voltammetry using $Fe(CN)_6^{3-/4-}$ solution, through Randles-Sevcik equation (2) [36] for the reversible process and was ascertain to be as 6.922×10^{-4} cm^2.

$$I_p=2.69\times10^5\ n^{3/2}\ A\ D^{1/2}\ C\ v^{1/2} \quad (2)$$

Where 'i_p' is the peak currents in µA, 'n' is the total number of electrons involved in the reaction, 'A' is the area of the working electrode, 'D' is the diffusion co-efficient in cm^2/s, (from graph I_p *vs* $\nu^{1/2}$) 'C' is the concentration of analyte and 'ν' is the scan rate.

3.5. Electrochemical impedance behaviour of serotonin

To examine the interface properties of the electrode surfaces, electrochemical impedance spectroscopy (EIS) was used as a powerful and emerging technique to characterize the different modified electrodes [25-29,33,37,38]. Nyquist diagram for 1×10^{-3} mol/dm^3 [Fe (CN_6)] $^{3-/4-}$ and 0.1 mol/dm^3 PBS (pH 7.0) at bare and modified electrodes were illustrated in Fig. 5a. At poly-AzrS/MWCNTs/GCE the interfacial electron transfer rate (R_{et}) increased greatly (curve c) in comparison with bare GCE (curve a) and poly-AzrS/GCE (curve b). This indicates that the modified electrode shows less resistances and more electron transfer rate. The bode plot was drawn between logarithm of frequencies in Hz's , logarithm of impedance in ohm's and phase in degree and was shown in Fig.5b. The red solid circles represent bare GCE, blue solid circles represent poly-AzrS/GCE and a brown solid circle represents poly-AzrS/MWCNTs/GCE.

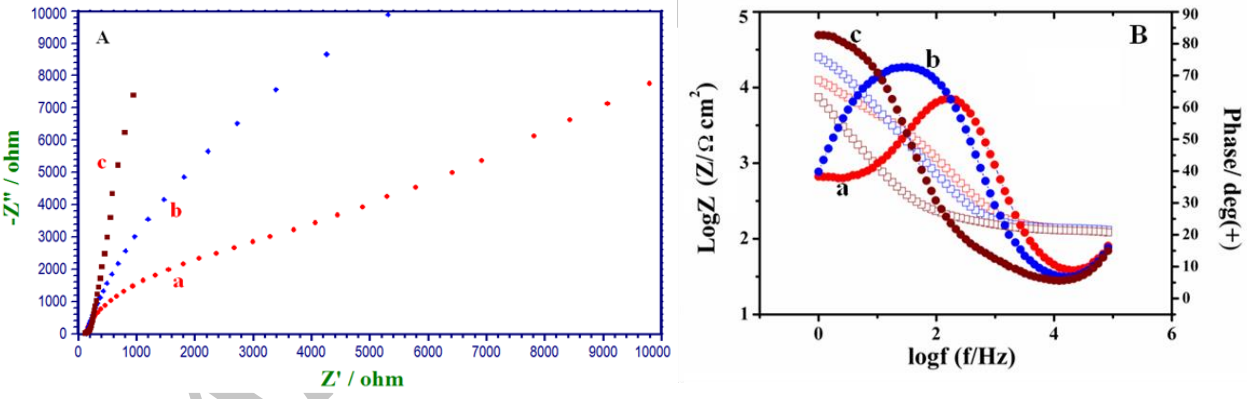


Fig. 5. (A) EIS spectrum (1 Hz to 1 MHz and pulse amplitude of 5 mV) in 0.1 mol/dm^3 M PBS of pH (6.0)/1mM K_3 [Fe $(CN)_6$]/ K_4 [Fe $(CN)_6$] (a) Bare GCE (b) at poly-AzrS/GCE (c) poly-AzrS/MWCNTs/GCE; (**B)** Bode plot of GCE (red solid circles), poly- AzrS/GCE (blue solid circles) and poly- AzrS/MWCNTs/GCE (brown solid circles)

3.6. Effect of concentration

The electrocatalytic impact of two-fold poly-AzrS/MWCNTs/GCE on serotonin oxidation was studied by varying the concentration from 0.5×10^{-6} mol/dm^3 to 1.1×10^{-5} mol/dm^3 by CV and DPV methods and it was shown in Fig. 6a & 6b. During the electrochemical measurement, the peak currents increased on increasing the volume of serotonin gradually. The result showed that a well-defined anodic peak of serotonin was observed at potential

0.325 V, due to the enhancement of the conductive surface area that influences the electron transfer rate. Two linear relationships were observed between the peak current and the serotonin concentration (Fig. 6c). The calibration plot of I_{pa} versus concentration of serotonin was described by the equation I_{pa} (10^{-6}A)=0.364 C (µM)+0.425 µA and I_{pa} (10^{-6}A)=0.1276 C (µM)+3.942 µA with a correlation coefficients of 0.98285 and 0.99408 respectively. The detection limit (LOD) and quantification limit (LOQ) of serotonin at poly-AzrS/MWCNTs/GCE was found to be as 1.8×10^{-7} mol/dm^3 and 17.52×10^{-7} mol/dm^3. The LOD and LOQ were calculated through the formulas LOD=3S/M and LOQ=10S/ M [39,40]. Where 'S' is the standard deviation of peak currents and 'M' is the slope obtained from the calibration plot. The LOD and linearity range of serotonin at various modified electrodes were compared with our present method and are shown in Table. 1.

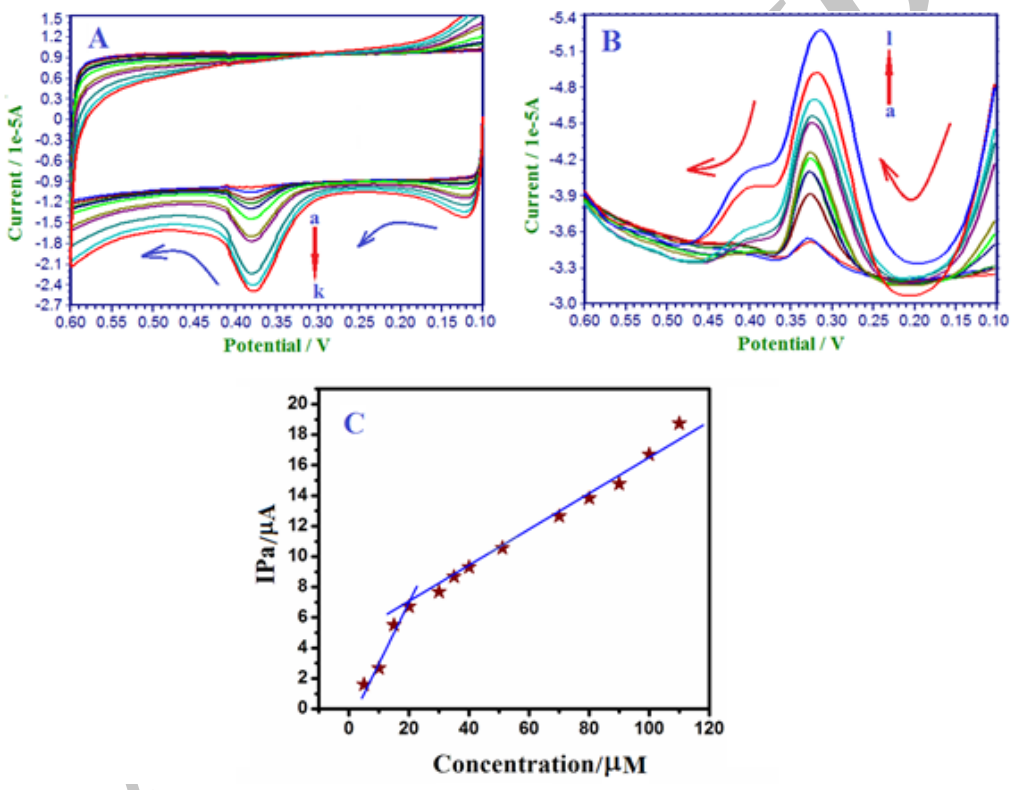


Fig. 6. (**A**) Cyclic voltammograms of serotonin for the different concentrations (a) Blank (b) 0.5×10^{-6} mol/dm^3 (c) 1×10^{-6} mol/dm^3 (d) 1.5×10^{-6} mol/dm^3 (e) 2.5×10^{-6} mol/dm^3 (f) 3×10^{-6} mol/dm^3 (g) 4×10^{-6} mol/dm^3 (h) 6×10^{-6} mol/dm^3 (i) 7×10^{-6} mol/dm^3 (j) 9×10^{-6} mol/dm^3 (k) 1.0×10^{-5} mol/dm^3 (l) 1.41×10^{-5} mol/dm^3; (**B**) A differential pulse voltammograms of serotonin for the different concentrations (a) Blank (b) 0.5×10^{-6} mol/dm^3 (c) 1×10^{-6} mol/dm^3 (d) 1.5×10^{-6} mol/dm^3 (e) 2×10^{-6} mol/dm^3 (f) 3×10^{-6} mol/dm^3 (g) 3.5×10^{-6} mol/dm^3 (h) 4×10^{-6} mol/dm^3 (i) 7×10^{-6} mol/dm^3 (j) 8×10^{-6} mol/dm^3 (k) 9×10^{-6} mol/dm^3 (l) 1.0×10^{-5} mol/dm^3 (m)1.1×10^{-5} mol/dm^3; (**C**) Calibration plot of serotonin concentration.

Table 1. Analytical performance of different electrochemical sensors for serotonin detection

Electrode	**Linearity range** mol/dm^3	**Detection limit** mol/dm^3	**Technique**	**Ref.**
Ach[a]/GCE[b]	1.0×10^{-6}- 3.0×10^{-5}	5.0×10^{-7}	DPV	[41]
Poly(SFO)[c]GCE	3×10^{-8} - 1.0×10^{-6}	5.0×10^{-9}	SW-AdSV	[42]
CNF[d] Electrode	1×10^{-6} - 10×10^{-6}	2.5×10^{-7}	DPV	[43]
Nano-Au[e]/PPyox[f]/GCE	0.07×10^{-6} - 2.2×10^{-6}	0.1×10^{-8}	DPV	[44]
MWCNT[g]-IE[h]	1×10^{-6} - 1.5×10^{-5}	0.2×10^{-6}	DPV	[45]
P(P3CA)[i]PGE	0.1×10^{-7} - 1.0×10^{-6}	0.25×10^{-8}	AdDPSV	[46]
Poly-AzrS[j]/MWCNTs/GCE	0.5×10^{-6} - 1.1×10^{-5}	1.80×10^{-7}	DPV	Present

a. Ach- Acetylcholine
b. GCE- Glassy carbon electrode
c. Poly(SFO)- Poly(safranine O)
d. CNF- Carbon nanofibers
e. Nano-Au- Gold nanocluster
f. PPyox- Over oxidized-polypyrrole
g. MWCNTs- Multiwall carbon nanotubes
h. IE- Intercalated graphite electrodes
i. P(P3CA)- Poly(pyrrole-3-carboxylic acid)
j. Poly-AzrS- poly- Alizarin red S

The surface coverage concentration of poly-AzrS/MWCNTs/GCE was predicted by using the Laviron's equation (3) [47].

$$I_p = n^2F^2A\,\Gamma\nu / 4\,RT \qquad (3)$$

Where 'n' is the number of electrons involved, 'F' is the Faraday constant (96,500 C mol^{-1}), 'Γ'is the surface coverage concentration (mole/cm^2). 'A' is the surface area of the electrode, 'ν' is the scan rate (V.s^{-1}), 'R' is the gas constant (8.314 J mol^{-1} K^{-1}) and 'T' is the absolute temperature (300 K). The value of the surface coverage concentration (Γ on the electrode was found to be as 3.3154×10^{-11} mole/cm^2

3.7. Effect of accumulation time

The results retrieved that the accumulation potential did not affect the oxidation peak currents, and hence open-circuit accumulation was performed in this study. The influence of accumulation time on the oxidation peak currents of serotonin at poly-AzrS/MWCNTs/GCE was investigated from 0 to 300 sec. The oxidation peak currents increased obviously within the first 185s, and then continued with moderate slope up to 260 sec., with further increase in the accumulation time, the oxidation peak current increased very slightly, Finally the oxidation peak current tended to be constant (data not shown), suggesting that the amount of

serotonin at two-fold poly-AzrS/MWCNTs/GCE surface tends to a regulating value. Considering sensitivity, speed and an extended dynamic range, an accumulation time of 185 sec was employed.

3.8. Stability, Repeatability and Reproducibility of poly-AzrS/MWCNTs/GCE

The stability of the two-fold poly-AzrS/MWCNTs/GCE chemical sensor was investigated by the successive recording of serotonin (2×10^{-4} mol/dm^3) in the potential range from -0.3 to +0.6 V at a scan rate of 100 mVs^{-1} in PBS (pH 6.0) solution. After 2nd successive potential scan disturbance peak currents of serotonin was observed up to 30th cycle. It can be convincing that the peak current of serotonin remains constant and it was shown in Fig.7a. Another important aspect of the two-fold poly-AzrS/MWCNTs/GCE chemical sensor is long term durability. The stability of the proposed chemical sensor was examined by storing in 0.1 M PBS (pH 6.0) at cool condition in a refrigerator for not less than two weeks and it was found that 96% of the initial current response was retained. A plot was drawn between repetitive measurements against peak currents, and it was found that the modified chemical sensor has good stability (Fig. 7b).

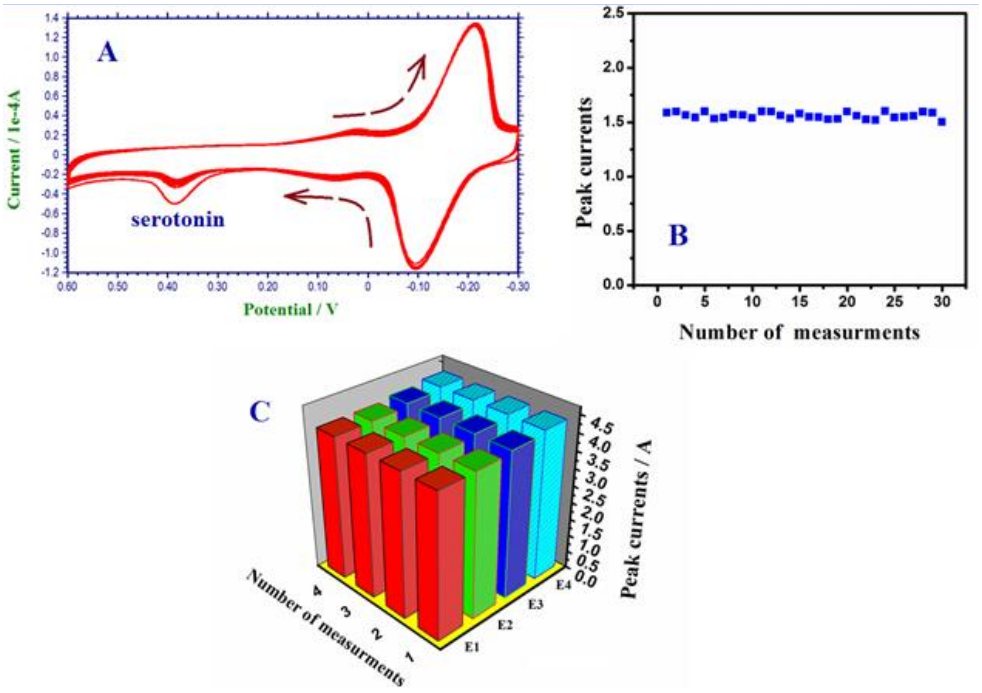


Fig. 7. (**A**) Cyclic voltammograms for 30 multiple cycle of 2×10^{-4} mol/dm^3 serotonin in 0.1 mol/dm^3 PBS solution of pH 6.0 at a scan rate of 100 mV S^{-1}; (**B**) A plot of no. of repetitive cycles Vs I_p; (**C**) A 3D plot of four different poly-AzrS/MWCNTs/GCE (E_1, E_2, E_3, E_4) Vs peak currents.

The robustness of two-fold poly-AzrS/MWCNTs/GCE chemical sensor surface was reviewed by the concept of reproducibility (Fig 7c). The concept describes and validates the preparation of newly electrode, fresh electrolyte, fresh analyte, new analyst and number of assays. The results suggested that the two-fold poly-AzrS/MWCNTs/GCE chemical sensor possess good stability and reproducibility in determining the serotonin [48,49].

3.9. Simultaneous determination and resolution of serotonin in the presences of EP

The fabricated sensor was applied in determining the biological compounds such as serotonin and EP simultaneously. Fig 8a & 8b shows the CV and DPV for the mixture of serotonin (3×10^{-5} mol/dm^3) and EP (3×10^{-5} mol/dm^3 M) at the bare GCE (curve b), poly-AzrS/GCE (curve c) and poly-AzrS/MWCNTs/GCE (curve d).

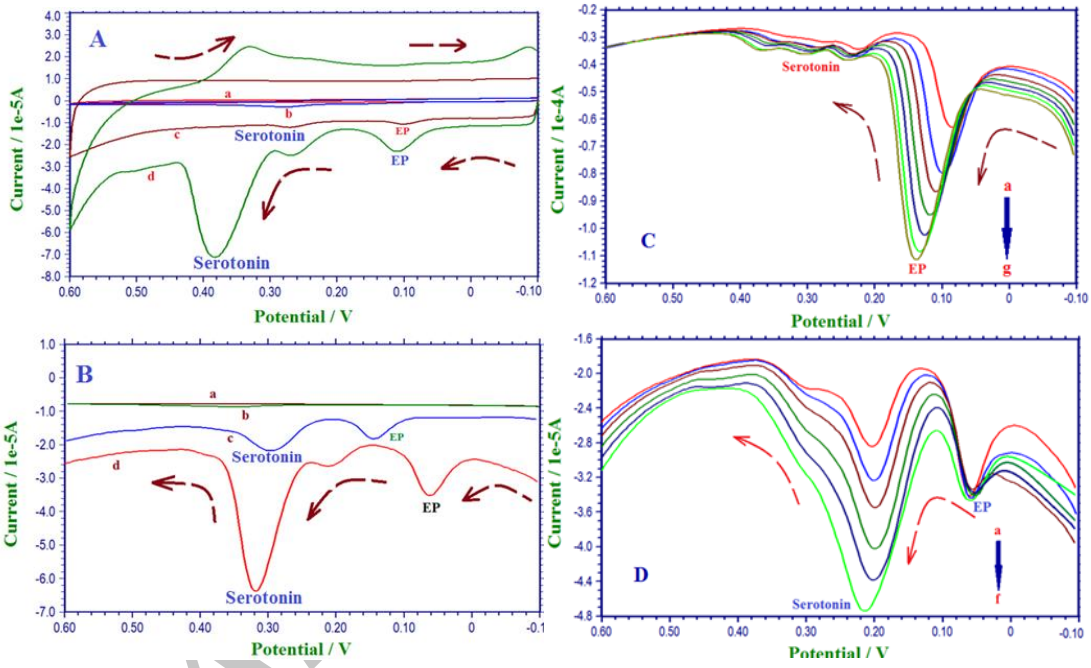


Fig. 8. Simultaneous determination of 3.0×10^{-5} mol/dm^3 serotonin and 3×10^{-5} mol/dm^3 EP, at poly-AzrS/MWCNTs/GCE (A) CV (B) DPV, (C) Differential pulse voltammograms of (a) 4×10^{-5} mol/dm^3 (b) 6×10^{-5} mol/dm^3 (c) 8×10^{-5} mol/dm^3 (d) 1×10^{-4} mol/dm^3 (e) 1.2×10^{-4} mol/dm^3 (f) 1.4×10^{-4} mol/dm^3 (g) 1.6×10^{-4} mol/dm^3 of EP in PBS of pH 6.0 in the presence of 3×10^{-5} mol/dm^3 serotonin at poly-AzrS/MWCNTs/GCE, **(**D**)** Differential pulse voltammograms of (a) 4×10^{-5} mol/dm^3 (b) 6×10^{-5} mol/dm^3 (c) 8×10^{-5} mol/dm^3 (d) 1×10^{-4} mol/dm^3 (e) 1.4×10^{-4} mol/dm^3(f) 1.8×10^{-4} mol/dm^3 of serotonin in PBS of pH 6.0 in the presence of 3×10^{-5} mol/dm^3 EP at poly-AzrS/MWCNTs/GCE

The oxidation peaks of serotonin, and EP was unable to be separated at bare GCE. The modified chemical sensor reduces the over potential, required for the oxidation of serotonin, and EP. The CV and DPV at poly-AzrS/MWCNTs/GCE modified chemical sensor showed good separated well defined peaks for the serotonin and EP with different oxidation potentials. The separation of oxidation peak potentials between serotonin and EP plays an important role for the analysis of serotonin. This result was sufficient to recognize the serotonin in the presence of EP at poly-AzrS/MWCNTs/GCE [50,51].

Serotonin usually coexistent with EP in the extra cellular fluid of central nervous system and their concentrations is very nearer to EP. The resolution of serotonin in the presence of EP at bare GCE was not an easy process, since the oxidation potentials of these biological compounds were nearer, but were as at the poly-AzrS/MWCNTs/GCE the resolution was easier, since these two compounds have well separated oxidation potentials. The resolution of serotonin and EP in the mixture solution was investigated by DPV technique because; it provides a better peak resolution and sensitivity in comparison with CV. The investigation was carried out by changing the concentration of each individual and keeping the concentration of the other species constant.

Under the suitable conditions, the DPV currents were proportional to the EP concentrations, over the concentration range from 4.0×10^{-5} mol/dm^3 to 1.6×10^{-4} mol/dm^3, when keeping the concentration of serotonin (3.0×10^{-5} mol/dm^3) constant and this was shown in Fig 8c. It can be seen that, there was no change in peak currents of serotonin. Similarly, in the same way the Fig 8d shows various concentrations of serotonin (4×10^{-5} mol/dm^3 to 1.8×10^{-4} mol/dm^3) with constant EP (3.0×10^{-5} mol/dm^3) concentration. These results show that at poly-AzrS/MWCNTs/GCE overlapping and inference from serotonin was greatly reduced in determining EP, [26,28,35,52].

3.10. Determination of serotonin in Human Serum Samples

Drug free human blood samples were collected from healthy individuals. These samples were centrifuged (2000 rpm) for 1 hour at room temperature to remove proteins and separate serum residues.

Table 2. Detection of serotonin in human blood serum samples

Sample	Content(μM)	Found (μM)	Recovery (%)	Bias
1	20	19.56	97.80	-2.20
2	40	38.94	97.35	-2.65
3	60	59.31	98.85	-1.15
4	80	78.62	98.28	-1.72

This was stored in cold condition until assay. After gentle dispersing, an aliquot volume of serum sample was spiked with serotonin. The separated supernatant of the sample was taken in to the electrolytic cell containing 0.1 mol/dm^3 PBS buffer of pH 6.0. The differential pulse voltammograms were recovered with standard addition method and the results were shown in Table 2. The recovery values suggested that the proposed chemical sensor was appeasing.

4. CONCLUSION

In the present work a novel poly-AzrS/MWCNTs/GCE modified chemical sensor was developed and successfully applied for the simultaneous resolution of epinephrine and serotonin. The developed method was simple, rapid, and accurate and opens new avenues for quick electrocatalytic activity towards determination of serotonin. The electrochemical characterization of two-fold modified electrochemical sensor has shown promising results in determining serotonin concentrations. Moreover, the proposed chemical sensor exhibits low detection limit, good potential applications, effective linearity range, admirable stability and better selectivity towards the determination of serotonin.

Acknowledgments

One of author K. Reddaiah, is grateful to the Department of Science and Technology-Science and Engineering Research Board (DST-SERB) New Delhi, India, for the financial support in the form of National Postdoctoral Fellowship project (PDF/2015/000214).

REFERENCES

[1] M. Perry, Q. Li, and R.T. Kennedy, Anal. Chim. Acta 653 (2009) 1.
[2] S. K. Kim, D. Kim, and S. Jeon, Sens. Actuator B 174 (2012) 285.
[3] I. P. Kema, W. G. Meijer, G. Meiborg, B. Ooms, P. H. B. Willemse, and E. G. E. de Vries, Clin. Chem. 47 (2001) 1811.
[4] D. L. Murphy, Q. Li, S. Engel, C. Wichems, A. Andrews, K.P. Lesch, and G. Uhl, Brain. Res. Bull. 56 (2001) 487.
[5] O. Hornykiewicz, Neurology 51 (1998) S2.
[6] B. N. Chandrashekar , B. E. Kumara Swamy , N. B. Ashoka , M. Pandurangachar, J. Mol.Liq. 165 (2012) 168.
[7] L. Wang, J. Bai, P. Huang, H. Wang, L. Zhang, and Y. Zhao, Int. J. Electrochem. Sci. 1 (2006) 238.
[8] X. Li, Int. J. Chem. 1 (2006) 206.

[9] B. N. Chandrashekar, B. E. Kumara Swami, N. B. Ashoka, and M. Pandurangachar, J. Mol. Liq.165 (2012) 168.

[10] M. Taei, H. Hadadzadeh, F. Hasanpour, N. Tavakkoli, and M. Hadadi Dolatabadi, Ionics 21 (2015) 3267.

[11] N. Lavanyaa, E. Faziob, F. Nerib, A. Bonavitac, S. G. Leonardic, G. Neric, and C. Sekara, Sens. Actuator B 221 (2015) 1412.

[12] A. N. Golikand, J. B. Raoof, M. Baghayeri, M. Asgari, and L. Irannejad, Russ. J. Electrochem. 45 (2009) 881.

[13] G. G. Wildgoose, C. E. Banks, H. C. Leventis, and R. G. Compton, Microchim. Acta 152 (2006) 187.

[14] Pumera, S. Sanchez, I. Ichinose, and J. Tang, Sens. Actuator B 123 (2007) 1195.

[15] S. Wang, Y. Wang, Q. Min, T. Shu, X. Zhu, A. Peng, and H. Ding, Int. J. Electrochem. Sci.11 (2016) 2360.

[16] U. Yogeswaran, and S. M. Chen, Electrochim. Acta 52 (2006) 5985.

[17] B. A. Patel, M. Arundell, K. H. Parker, M. S. Yeoman, D. O. Hare, J. Chromatogr. B 818 (2015) 269.

[18] M. Du, V. Flanigan, and Y. Ma, Electrophoresis 25 (2004) 1496.

[19] Z. D. Peterson, M. L. Lee, and S. W Graves, J. Chromatogr. B 810 (2004) 101.

[20] F. B. Salem, Talanta 34 (1987) 810.

[21] M. M. Hsieh, and H. T. Chang, Electrophoresis 26 (2005) 187.

[22] M. Israel, Neurochem. Int. 42 (2003) 215.

[23] T. Yoshitake, J. Kehr, K. Todoroki, H. Nohta, and M. Yamaguchi, Biomed. Chrom. 20 (2006) 267.

[24] E. Dremencov, I. Gispan-Herman, M. Rosenstein, A. Mendelman, D. H. Overstreet, J. Zohar, and G. Yadid, Prog.Neuro-Psychoph. 28 (2004) 141.

[25] K. Reddaiah, T. Madhusudana Reddy, and P. Raghu, J. Electroanal. Chem. 682 (2012) 164.

[26] K. Reddaiah, M. Mohan Reddy, P. Raghu, and T. Madhusudana Reddy, Colloid. Surface B 106 (2013) 145.

[27] N. F. Atta, M. F. El-Kady, and A. Galal, Anal. Biochem. 400 (2010) 78.

[28] K. Reddaiah, T. Madhusudana Reddy, K. Mallikarjuna, and G. Narasimha, Anal. Methods 5 (2013) 5627.

[29] K. Reddaiah, T. Madhusudana Reddy, M. Mohan Reddy, and P. Raghu, Sensor Lett. 11 (2013) 2272.

[30] M. Baghayeri, M. Barazandeh Tehrani, A. Amiri, B. Maleki, and S. Farhadi, Mat. Sci. Eng. C 66 (2016) 77.

[31] H. Beitollahi, A. Mohadesi, M. Mostafavi, H. Karimi-Maleh, M. Baghayeri, and A. Akbari, Ionics 20 (2014) 729.

[32] M. Qin, K. Cao, X. Wang, H. Xu, and Z. Yu, Ionics 19 (2013) 1891.
[33] P. Raghu, T. Madhusudana Reddy, K. Reddaiah, L. R. Jaidev, and G. Narasimha, Enzyme. Microb. Tech. 52 (2013) 377.
[34] W. Hu, D. Sun, and W. Ma, Electroanalysis 22 (2010) 584.
[35] P. V. Narayana, T. Madhusudana Reddy, P. Gopal, M. Mohan Reddy, and G. R. K. Naidu,
Mat. Sci. Eng. C 66 (2015) 57.
[36] A. J. Bard, and L. R. Faulkner, Electrochemical Methods: Fundamentals and Applications, John Wiley, New York (2001).
[37] P. Gupta, and R. N. Goyal, Talanta 120 (2014)17.
[38] S. Ahmad Mozaffari, T. Chang, and S. Moon Park, Biosens. Bioelectron. 26 (2010) 74.
[39] T. Madhusudana Reddy, M. Sreedhar, and S. Jayarama Reddy, J. Pharm. Biomed. Anal. 31 (2003) 811.
[40] K. Reddaiah, T. Madhusudana Reddy, Y. Subba Rao, P. Raghu, and P. Gopal, Mat. Sci. Eng. B 183 (2014) 69.
[41] G. P. Jin, X. Q. Lin, and J. M. Gong, J. Electroanal. Chem. 569 (2004) 135.
[42] H. Filik, A. A. Avan, and S. Aydar, Int. J. Electrochem. Sci. 9 (2014) 2922.
[43] E. Rand, A. Periyakaruppan, Z. Tanaka, D. A. Zhang, M. P. Marsh, R. J. Andrews, K. H. Lee, B. Chen, M. Meyyappan, and J. E. Koehne, Biosens. Bioelectron. 42 (2013) 434.
[44] J. Li, and X. Lin, Sens. Actuator B 124 (2007) 486.
[45] Z. H. Wang, Q. L. Liang, Y. M. Wang, and G. A. Luo, J. Electroanal. Chem. 540 (2003) 129.
[46] A. Ozcan, and S. Ilkbaş, Sensor. Actuator B 215 (2015) 518.
[47] E. Laviron, J. Electroanal. Chem.100 (1979) 263.
[48] P. Raghu, T. Madhusudana Reddy, P. Gopal, K. Reddaiah, and N. Y. Sreedhar, Enzyme. Microb. Tech. 57 (2014) 8.
[49] P. Gopal, T. Madhusudana Reddy, K. Reddaiah, P. Raghu, and P. V. Narayana, J. Mol. Liq. 178 (2013) 168.
[50] H. Liu, G. Zhao L. Wen, and B. Ye, J. Anal. Chem. 61 (2006) 1104.
[51] S. Sharath Shankar, and B. E. Kumara Swamy, Int. J. Electrochem. Sci. 9 (2014) 1321.
[52] N. F. Atta, A. Galal, and R.A. Ahmed, J. Electrochem. Soc. 158 (2011) F52.