

Development of an Electrochemical Amperometric Sensor for the Determination of Epinephrine using MWCNT/ Dopamine Dithiocarbamate Modified Electrode

M. Devendiran^a, J. Kavitha^a, and S. Sriman Narayanan^{a,*}

^aDepartment of Analytical Chemistry, School of Chemical Sciences,

University of Madras, Guindy Campus, Chennai- 600 025, Tamil Nadu, India

*E-mail: sriman55@yahoo.com, sriman55@gmail.com, Phone: +91-44-22202717, Fax: +91-44-22352494

Abstract – Electrochemical determination of epinephrine (EP) was investigated with a new MWCNT/ Dopamine dithiocarbamate (DDTC) modified graphite electrode. The Dopamine dithiocarbamate was synthesized and characterized by FTIR spectroscopy. The performance of the modified electrode was studied at different electrolytes, scan rates and at different pHs. Under optimal conditions, the modified electrode shows good performance towards the electrochemical determination of epinephrine with the linear range from 3.32×10^{-5} M to 8.25×10^{-4} M and the detection limit was found to be 1.1×10^{-5} M (S/N = 3).

Keywords: Dopamine dithiocarbamate, MWCNT, Epinephrine, cyclic voltammetry.

I. INTRODUCTION

Epinephrine is a type of catecholamine also called as adrenaline which is synthesized in adrenal gland in human body. It is one of the important hormones that is released in result of pain, fear and anger. Epinephrine also known as a flight or fight hormone which provides rapid energy by catabolism of tissue glycogen[1]. Epinephrine is released in response to stress and also it enlarges the peripheral blood vessels thus cause a free flow of blood and hence it was used as emergency healthcare medicine during the cardiac arrest and asthma. The normal level of epinephrine in humans is 0.037 ± 0.006 ng/ml[2] and various physiologic conditions can alter the concentration of epinephrine. The abnormal levels of epinephrine may lead to adverse effects like Parkinson's disease as a result of low level of EP. Hence the determination of epinephrine concentration in human fluids such as urine and plasma plays a key role in the clinical diagnosis of some diseases.

There were number of methods available for the quantitative determination of epinephrine. Various methods like liquid chromatography[3], Spectrophotometry[4], HPLC with MS[5], Fluorescence[6], flow injection chemiluminescence[7], potentiometric[8], capillary electrophoresis[9] and electrochemical[10,11] methods are used to determine the EP. Among the various methods electrochemical method is considered to be the best method because of its sensitivity, selectivity, reliability and reproducibility. Other methods have some demerits with the determination like separation, derivatization, and time consuming procedure where as the electrochemical methods are rapid, accurate, ease of handling and economic.

The electrochemical method seems to be an emerging area for the determination of biologically importance compounds compared with other methods. The modification of electrode plays a key role in the sensitivity and selectivity. There were numerous mediators used for the modification of electrodes including Curcumin[12], Iron and Cobalt phthalocyanines[13,14], vinyl ferrocene[15], polymerized modification of electrodes with poly(p-xylenol sulfone phthalein), poly(L-arginine), poly(3,3'-bis[N,N-bis(carboxymethyl)aminomethyl]o-cresulfonephthalein), poly(p-aminophenol) and 1-butyl-4-methyl-pyridinium tetrafluoroborate ionic liquid[16-20] modified electrodes. The determination of EP by voltammetric method not only consist chemical mediators and it also expands to the biochemistry where the white rot fungi cells based microbial biosensor[21] also be used.

In the present work Dopamine dithiocarbamate was employed as a mediator for the determination of EP. The MWCNT provides larger surface area and also enhancing the current response of the mediator. The electrochemical behavior of the new MWCNT/DDTC modified electrode has been studied by cyclic voltammetry and the utility of the modified electrode for the determination of EP by cyclic voltammetry and chronoamperometry was also checked. The findings exhibit that the modified electrode can act as a better mediating platform for the electrocatalytic oxidation of EP.

II. EXPERIMENTAL

a. Chemicals and reagents

Graphite rods (3 mm) and Epinephrine were purchased from Aldrich, Germany. Dopamine hydrochloride and Carbondisulphide were purchased from Hi-media and Merck respectively. All other chemicals and reagents were of analytical grade and used without further purification. All aqueous solutions were prepared with doubly distilled water. Electrochemical determination of EP was carried out with Acetate buffer solution (ABS 0.1 M) of pH 6.0 as supporting electrolyte.

b. Instruments

Electrochemical measurements were performed using a CH Instruments 400A Electrochemical Workstation. A platinum wire and SCE electrode served as the counter and reference electrode, respectively. MWCNT/DDTC modified electrode was used as a working electrode with a geometric area of 0.07 cm^2 . pH measurements were made with an Elico pH meter (model LI 120, India). All experiments were carried out at ambient temperature.

c. Preparation of DDTC

Dopamine dithiocarbamate was synthesized with a slight modification of a reported procedure [22]. 0.5mmol of carbondisulphide (30 μ l) and 0.5mmol of Ammonium hydroxide were added to 10ml of ethanol and to the mixture was added to 0.5mmol of dopamine (94mg) and stirred for 2h in an ice bath then 3h at ambient temperature. The dopamine dithiocarbamate was formed in solution. Solid DDTC was obtained after evaporation of the solvent.

d. Preparation of MWCNT/DDTC modified electrode

The preparation of MWCNT/DDTC modification of electrode involves three steps as follows. In the first step PIGE was prepared from graphite rod as reported [23] and used for electrode modification. One end of the electrode was carefully polished with emery paper and then with 0.05 μ m alumina slurry, washed with distilled water and dried in air. Then, in the second step a pinch of MWCNT was mechanically immobilized on the electrode surface, washed with doubly distilled water and tried. Finally 10 μ l of dopamine dithiocarbamate in ethanol was drop casted on to the MWCNT/PIGE electrode and dried at room temperature. MWCNT/PIGE electrode and DDTC/PIGE were also prepared by immobilizing MWCNT and drop casting 10 μ l of DDTC on the PIGE respectively for comparison.

III. RESULTS AND DISCUSSION

a. Characterization of Dopamine dithiocarbamate

The DDTC was characterized by FTIR spectroscopy and the spectrum confirms the formation of dopamine dithiocarbamate. Fig.1 illustrates the FTIR spectrum of DDTC. The peak at 936 cm^{-1} for (C-S)_s, 1080 cm^{-1} for (C-S)_{as}, 2543 cm^{-1} for (S-H) and 1498 cm^{-1} for (C-N) of NCS₂ confirms the bond formation between the amine of dopamine with the carbon of CS₂.

b. Electrochemical behavior of the MWCNT/ DDTC modified electrode

Fig.2 shows the CV response of the bare (a), DDTC (b) and MWCNT/DDTC (c) modified electrodes in 0.1M ABS (pH 6.0) at a scan rate of 50mV/s. The DDTC modified electrode shows the oxidation and reduction peaks at 0.36V and -0.06V respectively. Whereas the MWCNT/ DDTC Modified electrode exhibits a well defined oxidation peak at 0.25V and a reduction peak at 0.105V with higher current. The above results clearly suggest that the current enhancement is due to the surface enrichment of MWCNT.

The comparative cyclic voltammetric responses of the modified electrode with different supporting electrolytes of the same concentration of 0.1M shown in Fig.3. Here, ABS, Ba(NO₃)₂, KCl, KNO₃, LiCl, NaCl, NaNO₃, NH₄Cl, NH₄NO₃ and PBS were used for this study at scan rate of 50mV/s. The result shows that the modified electrode exhibits a well defined redox peak in 0.1M ABS compared with other electrolytes. Hence further experiments were carried out with the 0.1M ABS as a background electrolyte.

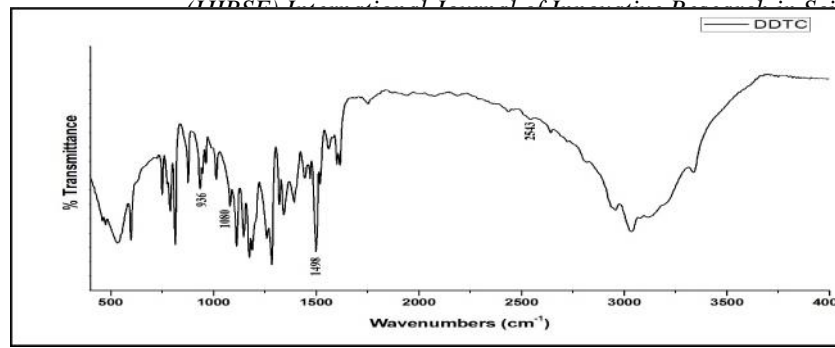


Fig.1 FTIR spectrum of Dopamine dithiocarbamate

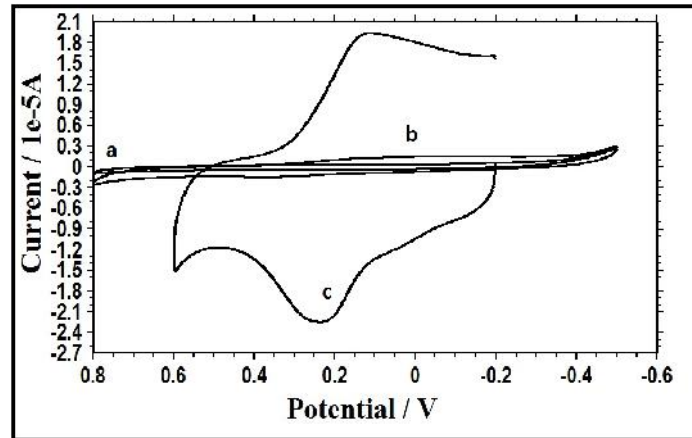


Fig.2 Cyclic voltammograms of (a)Bare, (b)DDTC and (c)MWCNT/DDTC modified electrode in 0.1M ABS (pH 6.0).

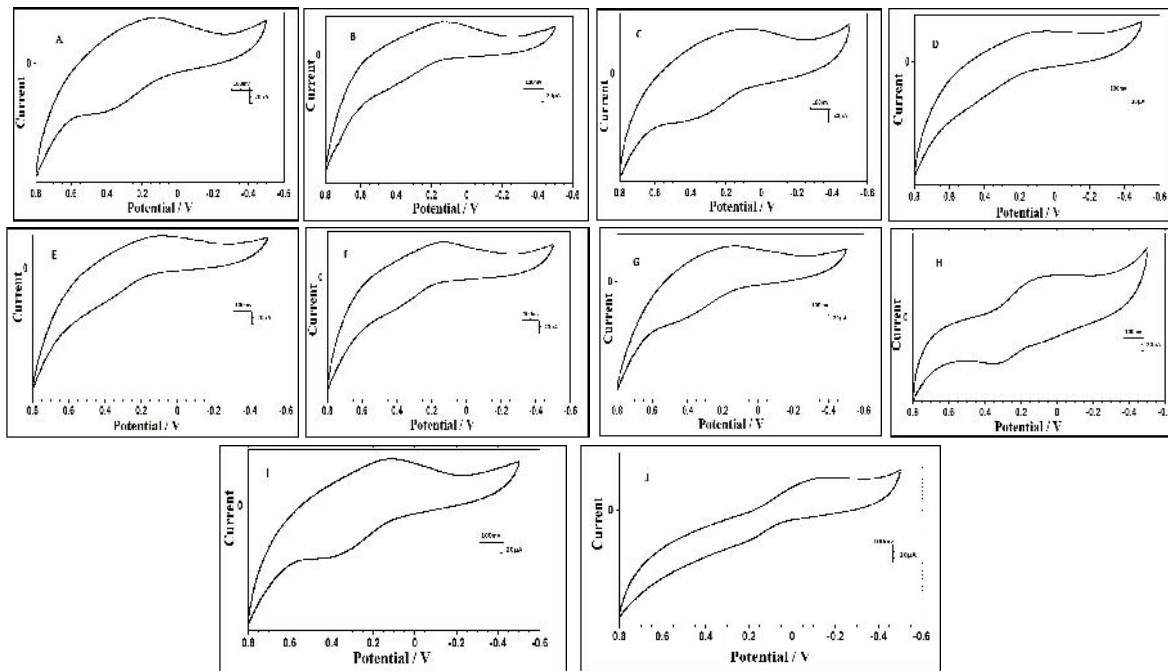


Fig.3. Cyclic voltammogram of the modified electrode in 0.1M (A)KNO₃, (B)NaNO₃, (C)NH₄Cl, (D)NaCl, (E)NH₄NO₃, (F)BaNO₃, (G)LiCl, (H) ABS, (I)KCl, (J)PBS.

The effect of scan rate was examined using CV for the MWCNT/DDTC modified electrode in 0.1M ABS as a background electrolyte. Fig. 4 indicates the effect of scan rate on the MWCNT/DDTC modified electrode by varying the scan rates from 2 to 150 mV/s in 0.1M ABS, The anodic and cathodic peak currents observed at the MWCNT/DDTC modified electrode increased with increases in the scan rate and from the calibration plot it can be seen that the electrode undergoes surface confined process.

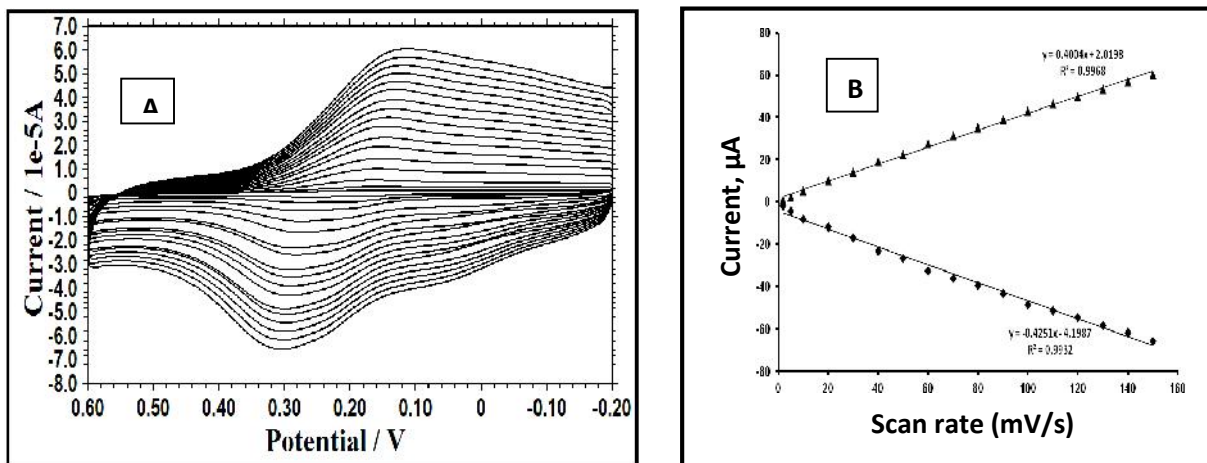


Fig. 4 (A) CVs of MWCNT/DDTC modified electrode at different scan rates in 0.1M ABS. The scan rates from inside to outer 2,5, 10 – 150 mV/s with increments of 10 mV/s. (B) Calibration plot of current vs scan rate.

c. Electrochemical oxidation of EP at MWCNT/DDTC modified electrode

The electrocatalytic oxidation of EP was examined using MWCNT/DDTC modified electrode by cyclic voltammetry technique. The modified electrode shows remarkable response for the electrocatalytic oxidation of Epinephrine in 0.1M ABS (pH 6.0). Fig.5 shows the CVs of bare (a), MWCNT/DDTC modified electrode (b) in 7.62×10^{-4} M EP in 0.1M ABS (pH 6.0) at a scan rate of 50 mV/s. The results demonstrate that the bare electrode shows the oxidation peak at 0.5V with lesser current but the modified electrode shows the oxidation at 0.3 V with enhanced current for the EP in 0.1M ABS. Compared with bare electrode, MWCNT/DDTC modified electrode shows a significant negative shift in the peak potential at 0.3 V with high current response. The above results show that the modified electrode is suitable towards the electrochemical oxidation of the EP in the lower potential range with enhanced current response.

The CV response of MWCNT/DDTC modified electrode in the presence of different concentration of EP in 0.1M ABS (pH 6.0) and the calibration plot are shown in Fig.6. The CVs suggest that the anodic current increases with the increase in the concentration of the EP. The calibration plot of current response against the concentration clearly states that the oxidation peak current increases linearly with the increase in the concentration of EP in the range from 3.32×10^{-5} to 8.25×10^{-4} M with a detection limit of 1.10×10^{-5} M (S/N = 3). The linear response suggests that the modified electrode can be employed for the determination of Epinephrine.

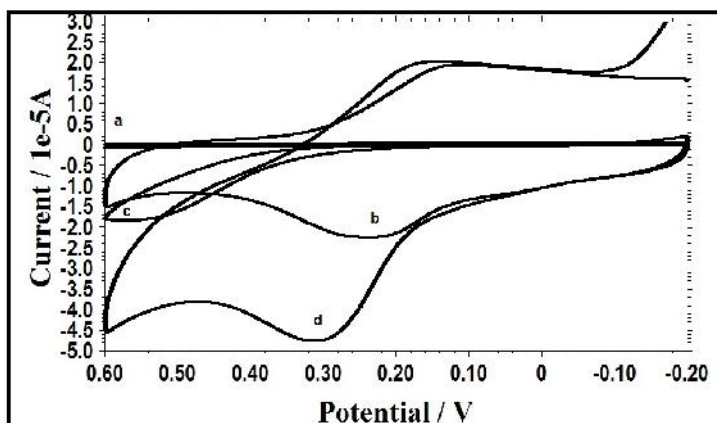


Fig.5 Cyclic voltammetric response of the (a)Bare, (b)MWCNT/DDTC modified electrode in the absence and (c)Bare, (d)MWCNT/DDTC modified electrode in the presence of 7.62×10^{-4} M EP at a scan rate of 50mV/s in 0.1M ABS (pH 6.0) as background electrolyte.

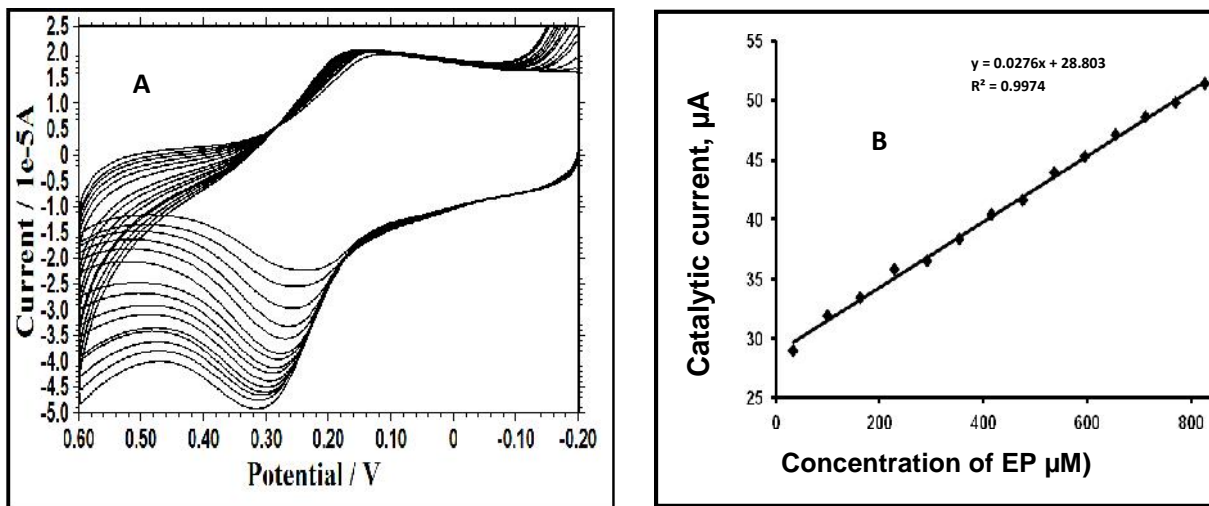


Fig.6 (A) Cyclic Voltammograms of various concentrations of EP from 0 to 8.25×10^{-4} M in 0.1M ABS, Scan rate – 50 mV/s. (B) Calibration plot of catalytic current vs concentration

d. Amperometric determination of EP at MWCNT/DDTC modified electrode

In order to ensure the applicability of the modified electrode for the determination of EP under dynamic conditions, hydrodynamic studies were carried in the potential range of -0.2 to 0.6 V and the current response for the oxidation of EP at a concentration of 7.62×10^{-4} M was measured in a stirring solution of 0.1 M ABS. It can be observed that the modified electrode exhibited maximum current response for EP at a potential of 0.3 V under dynamic conditions. Based on the hydrodynamic studies, an operational potential of 0.35V was chosen for the chronoamperometric determination.

The electrocatalytic oxidation of the EP with MWCNT/DDTC modified electrode for the oxidation of EP was studied by chronoamperometric technique at an applied potential of 0.35 V. The current response of the modified electrode in a stirred solution for successive increments of $49\mu\text{M}$ of EP in 0.1M ABS shown in Fig.7. On each addition of EP the oxidation current increases linearly implying that the modified electrode can be used for the determination of EP in the dynamic conditions.

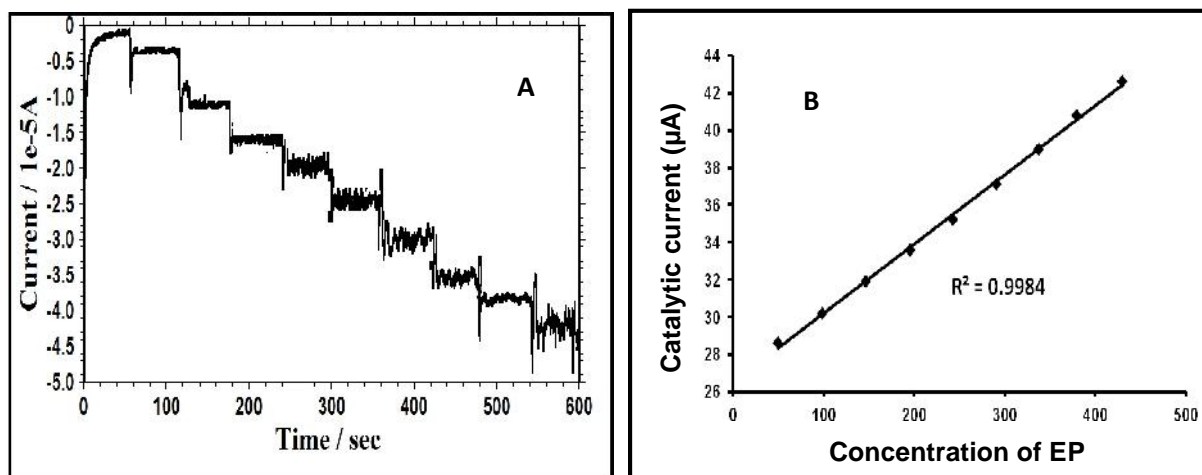


Fig.7(A) Amperometric response of MWCNT/DDTC modified electrode for the successive addition of $49 \mu\text{M}$ of EP in 0.1 M ABS(pH 6.0). (B) Calibration plot for amperometric determination of EP

f. Stability and reproducibility

The modified electrode was used for repetitive determination of EP with the concentration of 7.62×10^{-4} M in 0.1M ABS and the result shows that the modified electrode can reproduce almost the same current for same concentration. The result also implies the modified electrode does not undergo any surface fouling and leaching during the experiment. Also 5 modified electrodes were employed for the determination of 7.62×10^{-4} M EP and all the electrodes exhibit almost identical results indicating the appreciable stability and reproducibility towards the determination of EP.

Conclusion

A new electrode based on MWCNT/DDTC was prepared and characterized. The DDTC has been synthesized and confirmed by FTIR spectroscopy. The modified electrode was successfully employed for the electrochemical determination of EP. The modified electrode exhibited good current response for the electrocatalytic oxidation of EP and the oxidation current increase linearly with increase in concentration of EP in the range of 3.32×10^{-5} to 8.25×10^{-4} M with a detection limit of 1.10×10^{-5} M (S/N = 3). The modified electrode showed good stability and reproducibility.

ACKNOWLEDGEMENTS

The authors acknowledge the financial assistance from Department of Science and Technology, Delhi through INSPIRE programme.

REFERENCE

- [1]. D.L. Wong, T. C. Tai, R. Claycomb, D.C.W. Faull, and R. Kvetnansky, "Adrenergic Responses to Stress Transcriptional and Post-transcriptional Changes", *Ann. N.Y. Acad. Sci.*, vol. 1148, pp. 249–256, 2008.
- [2]. J. R. Stratton, M. A. Pfeifer, J. L. Ritchie, J. B. Halter, "Hemodynamic effects of epinephrine: concentration-effect study in humans", *J Appl physiol.*, vol. 58, pp. 1199-1206, 1985.
- [3]. A. Sanchez, E.A.T. Pinto, M.L. Menezes, O.C.M. Pereira, "A simple high-performance liquid chromatography assay for on-line determination of catecholamines in adrenal gland by direct injection on an ISRP column", *Pharmacological Research*, vol. 50, pp. 481–485, 2004.
- [4]. A.V. Bulatov, A.V. Petrova, A.B. Vishnikin, A.L. Moskvina, L.N. Moskvina, "Stepwise injection spectrophotometric determination of epinephrine", *Talanta*, vol. 96, pp. 62-67, 2012.
- [5]. V. Carrera, E. Sabater, E. Vilanova, M.A. Sogorb, "A simple and rapid HPLC–MS method for the simultaneous determination of epinephrine, norepinephrine, dopamine and 5-hydroxytryptamine: Application to the secretion of bovine chromaffin cell cultures", *J. Chromatogr. B*, vol. 847, pp. 88-94, 2007.
- [6]. M.A. Fotopoulou, P.C. Ioannou, "Post-column terbium complexation and sensitized fluorescence detection for the determination of norepinephrine, epinephrine and dopamine using high-performance liquid chromatography", *Anal Chim Acta.*, vol. 462, pp. 179-185, 2002.
- [7]. X. Zheng, Z. Guo, Z. Zhang, "Flow-injection electrogenerated chemiluminescence determination of epinephrine using luminol", *Anal Chim Acta.*, vol. 441, pp. 81-86, 2001.
- [8]. C.G. Amorim, A.N. Araujo, M.C.B.S.M. Montenegro, "Exploiting sequential injection analysis with lab-on-valve and miniaturized potentiometric detection Epinephrine determination in pharmaceutical products", *Talanta*, vol. 72, pp.1255-1260, 2007.
- [9]. S. Wei, G. Song, J.M. Lin, "Separation and determination of norepinephrine, epinephrine and isoprenaline enantiomers by capillary electrophoresis in pharmaceutical formulation and human serum", *J. Chromatogr. A*, vol. 1098, pp. 166-171, 2005.
- [10]. F. Valentini, E. Ciambella, V. Conte, L. Sabatini, N. Ditaranto, G. Palleschi, M. Bonchio, F. Giacalone, Z. Syrgiannis, M. Prato, "Highly selective detection of Epinephrine at oxidized Single-Wall Carbon Nanohorns modified Screen Printed Electrodes (SPEs)", *Biosens. and Bioelectron.*, vol. 59, pp. 94-98, 2014.
- [11]. X. Liu, D. Ye, L. Luo, Y. Ding, Y. Wang, Y. Chu, "Highly sensitive determination of epinephrine by a MnO₂/Nafion modified glassy carbon electrode", *J. Electroanal Chem.*, vol. 665, pp.1-5, 2012.
- [12]. B. Devadas, M. Rajkumar, S.M. Chen, "Electropolymerization of curcumin on glassy carbon electrode and its electrocatalytic application for the voltammetric determination of epinephrine and p-acetoaminophenol", *Colloids and Surfaces B: Biointerfaces*, vol. 116, pp. 674-680, 2014.

- [13]. S. Shahrokhian, M. Ghalkhani, M.K. Amini, "Application of carbon-paste electrode modified with iron phthalocyanine for voltammetric determination of epinephrine in the presence of ascorbic acid and uric acid", *Sensors and Actuators B*, vol. 137, pp. 669-675, 2009.
- [14]. F.C. Moraes, D.L.C. Golinelli, L.H. Mascaro, "Determination of epinephrine in urine using multi-walled carbon nanotube modified with cobalt phthalocyanine in a paraffin composite electrode", *Sensors and Actuators B*, vol. 148, pp.492-497, 2010.
- [15]. H. Beitollahi, M.A. Taher, A. Hossein, "Fabrication of a nanostructure-based electrochemical sensor for simultaneous determination of epinephrine and tryptophan", *Measurement*, vol. 51, pp.156-163, 2014.
- [16]. A.A. Ensafi, M. Taei, T. Khayamian, "Simultaneous determination of ascorbic acid, epinephrine, and uric acid by differential pulse voltammetry using poly(p-xylene)sulfonephthalein modified glassy carbon electrode", *Colloids and Surfaces B: Biointerfaces*, vol. 79, pp. 480-487, 2010.
- [17]. M. Wei, S.D. Ming, "Simultaneous Determination of Epinephrine and Dopamine with Poly(L-arginine) Modified Electrode", *Chin J Anal Chem*, vol. 35, pp. 66-70, 2007.
- [18]. A.A. Ensafi, B. Rezaei, S.Z.M. Zare, M. Taei, "Simultaneous determination of ascorbic acid, epinephrine, and uric acid by differential pulse voltammetry using poly(3,3'-bis[N,N-bis(carboxymethyl)aminomethyl]-o-cresolsulfonephthalein) modified glassy carbon electrode", *Sensors and Actuators B*, vol.150, pp.321-329, 2010.
- [19]. Ç.C. Koçak, Z. Dursun, "Simultaneous determination of ascorbic acid, epinephrine and uric acid at over-oxidized poly(p-aminophenol) film modified electrode", *J Electroanal Chem.*, vol. 694, pp. 94–103, 2013.
- [20]. B.N. Chandrashekar, B.E. Kumara Swamy, N.B. Ashoka, M. Pandurangachar, "Simultaneous electrochemical determination of epinephrine and uric acid at 1-butyl-4-methyl-pyridinium tetrafluoroborate ionic liquid modified carbon paste electrode: A voltammetric study", *J Molecular Liquids.*, vol.165, pp. 168–172, 2012.
- [21]. E. Akyilmaz, M. Turemis, I. Yasa, "Voltammetric determination of epinephrine by White rot fungi (*Phanerochaete chrysosporium* ME446) cells based microbial biosensor", *Biosens. and Bioelectron.*, vol.26, pp. 2590–2594, 2011.
- [22]. S.K. Kailasa, H.F. Wu, "One-pot synthesis of dopamine dithiocarbamate functionalized gold nanoparticles for quantitative analysis of small molecules and phosphopeptides in SALDI- and MALDI-MS", *Analyst*, vol. 137, pp. 1629–1638, 2012.
- [23]. F. Scholz and B. Lange, "Abrasive stripping voltammetry - an electrochemical solid of wide applicability", *Trend Anal. Chem.* vol.11, pp. 359-367, 1992.