CNTs-COOH Paste Electrode for Detection of Temozolomide

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ABSTRACT: *The MWCNTs-COOH Paste Electrode (MCPE) was successfully used to study the electrochemical behavior of temozolomide in 0.2 M H_2SO_4 solution, phosphate buffer solution (0.1M PBS, pH 7.4) and 0.1M NaOH solution by Cyclic Voltammetry (CV) technique. The results exhibit that MCPE can remarkably enhance sensing and electrocatalytic activity towards the oxidation and reduction of temozolomide in acidic, neutral, and basic solutions. The effect of the scan rate exhibits the adsorption controlling process. The effect of pH range from 2 to 6 was investigated by cyclic voltammetry technique, from cyclic voltammetry study exhibits the peak current was pH dependent with a slope of 68 mV/pH. The detection limit (LOD)) at MCPE were found to be 0.056 mM,0.069 mM and 0.065 mM in 0.1M H_2SO_4, 0.1M PBS (pH 7.4) and 0.1M NaOH solutions respectively by Cyclic Voltammetric (CV) technique. Similarly, from Linear Scan Voltammetric (LSV) technique, the detection limits (LOD) were found to be 0.050 mM, 0.021mM and 0.036 mM in acidic, neutral, and basic solution respectively. The proposed method was successfully applied for the determination of temozolomide in the clinical sample.*

KEYWORDS: *Temozolomide, MWCNTs-COOH, MWCNTs-COOH paste electrode, Cyclic Voltammetry and Linear scan voltammetry.*

INTRODUCTION

Temozolomide chemical name is 3,4-dihydro-3 methyl-4-oxoimidazo[5,1-d]-as-tetrazine-8-carboxamide. Temozolomide (TMZ) is used to cure some brain cancers, TMZ is used for second-line treatment for astrocytoma

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and first line treatment for gliobastome [1, 2], TMZ drug also has been approved for the treatment of recurrent glioma, melanoma and some solid neoplasias. Temozolomide (TMZ) is an anticancer prodrug is stable in acidic pH (< 5), labile in pH > 7 that is suddenly hydrolyzed to the active 5-(3-methyltriazen-1-yl) imidazole-4-carboxamide (MTIC) in neutral and alkaline pH values, with hydrolysis taking place even more rapidly at alkaline pH. Thereafter, 5-(3-methyltriazen-1-yl) imidazole-4-carboxamide (MTIC) rapidly degrades to 5-aminoimidazole-4-carboxamide (AIC) and methyldiazonium ion [3,4] which is an active alkylating species [5]. These alkylating species inhibits DNA replication by methylating nucleotide bases with DNA of cancer cells and stopping to construct DNA, If the cancer cells can't construct DNA, these cancer cells can't split into 2 new cells, so the cancer can't continue to grow, it is also dependent upon formation of a reactive methyldiazonium cation from temozolomide [3,6]. Recently, the electrochemical interaction of TMZ with DNA sample was studied and a mechanism of TMZ–DNA electrochemical interaction was proposed [7]. Electrochemical investigation of the TMZ chemical degradation processes in aqueous medium [8] was reported.

Temozolomide (TMZ) is quickly and fully absorbed from the gastrointestinal tract after oral administration and the time required to reach peak plasma concentration (t_{max}) is 1 hour. It is quickly eliminated with a half-life ($t_{1/2}$ = ln 2/k, where k is the elimination rate constant) of 1.7 to 1.9 h and shows linear kinetics over the therapeutic dosing range [9, 10]. A TMZ half-life is nearly 15 min in vitro serum [11] and nearly 33 min and 28 min in water at pH = 7.9 [12] was reported. 5-(3-methyltriazen-1-yl) imidazole-4-carboxamide (MTIC) half-lives is about 1.9 hour in human plasma in vivo [13,14], nearly 25 min in human plasma in vitro [11,13], and 13 min in water nearly physiological pH= 7.9 [12] were reported. Several methods have been reported for the analysis of TMZ and its metabolites in aqueous media, such as reverse-phase high performance liquid chromatography (HPLC) with UV [11, 13, 15], micellar electrokinetic capillary electrophoresis (MEKC) [12, 14] and MS/MS detection [16], was reported. However, an electrochemical detection of TMZ in acidic, basic and neutral medium has not been carried out. The main uses of electrochemical detection techniques are its fast speed, low detection limits, low cost and high accuracy [17, 18].

Recently, carbon nanotube electrode is superior to other carbon material electrode. Because carbon nanotubes have large edge plane/basal plane ratio, and which exhibits rapid electrode kinetics. Therefore, carbon nanotube based sensors commonly show lower limits of detection and higher sensitivities [19, 20]. In addition, the modified carbon nanotube paste electrodes are widely used in electrochemical investigations of bioactive compounds [21, 22]. Furthermore, incorporation of carboxylic acid group on carbon nanotube is the most general technique of functionalizing carbon nanotube to avoid them from agglomeration. Carboxylic acid functionalized carbon tube (COOH-CNTs) increases the hydrophilicity of carbon nanotubes, so that they can be eco-friendly in nature [23]. The COOH-CNTs improves the interfacial exchanges between the electrode surface and solution. In addition, the COOH-CNTs and modified CCOOH-CNTs electrodes have been used in the electrochemical detection of some important compounds [24,25]. In the present work, our aim was to fabricate MWCNTs-COOH paste electrode (MCPE) and studied electrochemical investigation of temozolomide (TMZ).

EXPERIMENTAL SECTION

Reagents and chemicals

All analytical grade or high purity chemicals, namely, $K_3[Fe(CN)_6]$ from Xi Long Chemicals Industries (Shanghai, China). KCl, NaOH and H_2SO_4 from Strem Chemicals.Inc (Newburyport, USA). Na_2HPO_4 from Beijing Solarbio Science & Technology Co.Ltd (Beijing, China), Citric acid from and NaH_2PO_4 from Aladdin Industrial Corporation (Shanghai, China) were prepared by dissolving in Millipore water. MWCNTs-COOH from M.K Nano Co. LTD (Mssisauga, Canada) and silicon oil from Hang Ping Chemicals Industries (Fujian Sheng, China), Temozolomide (TMZ) from Tokyo Chemical Industry Co.LTD (Tokyo, Japan) TMZ stock solution was prepared by dissolving in acetate buffer solutions (pH 4.0). All aqueous solutions were prepared using Millipore water.

All electrochemical measurements were conducted on CHI 660E electrochemical workstation from Chen Hua Instruments Co., (Shanghai, China). A conventional three-electrode electrochemical system was used for all

the electrochemical experiments, a saturated calomel electrode (SCE) as reference electrode, a platinum wire as counter electrode, the MWCNTs-COOH paste electrode (MCPE) used as a working electrodes (Ø3.0mm). All potentials in the paper were reported versus SCE. In addition, the solution was purged with high-purity nitrogen for at least 10.0 min prior to each electrochemical measurement.

Preparation of the MWCNTs-COOH paste electrode

The multi walled carbon nanotube carboxylic acid-paste electrode (MCPE) was prepared by taking weight of 0.24g MWCNTs-COOH in 0.25 ml silicon oil. This mixture was thoroughly mixed in an agate mortar for about 30 min and packed into a homemade Teflon cavity current collector and polished using soft paper.

RESULTS AND DISCUSSION

Electrochemical response for ferrocyanide and temozolomide at MCPE

The electrochemical responses of 1mM $K_3Fe(CN)_6$ in 1M KCl and 2mM temozolomide in 0.1M H_2SO_4, 0.1PBS (pH 7.4), 0.1M NaOH solutions at the Glassy Carbon Electrode (GCE) and MWCNTs-COOH paste electrode (MCPE) were measured at a scan rate of 0.05 V/s.

The peak-potential differences (ΔEp) of 0097 V and 0.065V for the GCE and the MCPE respectively are shown in Fig.1 {A (a) & (b)}. The results exhibits greatly improved the cyclic voltammetry response for 1mM $K_3Fe(CN)_6$ at MCPE reflected by the enlargement of peak current and the decline of peak potential difference. This indicates that the MCPE exhibits good electrocatalytic activity than GCE.

Cyclic voltammograms (CVs) of freshly-prepared 2mM TMZ in the 0.1 M H_2SO_4, 0. 1 M PBS (pH 7.4) and 0.1 M NaOH solutions at GCE (curve a) and MCPE (curve b) are shown in Fig. 1 (B, C & D). The measured anodic peak potential (Epa) and cathodic peak potential (Epc) from Fig. 1 (B, C & D) were listed in the Table 1. The results exhibit enhancement of peak current and the decline of peak potential for 2mM TMZ in the 0.1 M H_2SO_4, 0.1 M PBS (pH 7.4) and 0.1 M NaOH at MWCNTs-COOH paste electrode (MCPE) as compared with GCE. This indicates that the MCPE exhibits good electrocatalytic activity and electrochemical sensing property for detection of TMZ in acidic, basic and neutral solution.

Effect of scan rate

The effect of scan rate for 1mM $K_3Fe(CN)_6$ in 1M KCl was studied by CV at MWCNTs-COOH paste electrode (MCPE). The results in Fig. 2 (a) shows an increase in the redox peak current at a scan rate of 0.05 to 0.500 V/s. The graph in Fig. 2 (b) exhibited good linearity between the scan rate (υ) and the anodic peak currents for the MCPE, with correlation coefficients of r^2=0.995.

Similarly, the effect of scan rate for 2mM TMZ in the following solutions: 0.1M H_2SO_4, 0.1 M PBS (pH 7.4) and 0.1 M NaOH solutions was studied by CVs at MWCNTs-COOH paste electrode (MCPE). The MCPE exhibits increase in the peak currents with increase in scan rate (0.05 to 0.500 V/s) are shown in Fig. 3. (A, B, C). The graph of anodic peak current (Ipa) versus scan rate (ν) was plotted. The graph obtained was good linearity between scan rate (ν) and anodic peak current (Ipa) are shown in Fig. 3 (a, b, c). The correlation coefficient of r^2= ~ 0.995, which indicate the MWCNTs-COOH paste electrode (MCPE) reaction was adsorption controlled process which was supported by previously reported literatures [26,27].

Effect of pH

The effect of pH on the determination of TMZ in buffer solution(0.2M Na_2HPO_4 and 0.1M Citric acid at the MWCNTs-COOH PE was carefully investigated in the pH range of 2–6.0. The pH of the supporting electrolyte has a significant influence on the TMZ electrocatalysis at the MWCNTs-COOH PE by affecting both peak current and peak potential. The effect of pH value on the determination of TMZ in buffer solution (0.2M Na_2HPO_4 and 0.1M Citric acid) at MWCNTs-COOH PE was carefully investigated in a wider pH range of 2.0 to 6.0 of TMZ in the different pH solutions. The cyclic voltammograms shown in Fig.4a and illustrate the dependency of the TMZ anodic peak current (Ipa) and anodic potential (Epa (V)) on the buffer solution pH. It can be seen that the variation of TMZ peak current with increasing pH value until it reaches 6.0 [shown in Fig.4b]. The anodic potential of TMZ shifts negatively with the increase of the pH value of solution and depends linearly on the pH value in the range of 2.0-6.0 with a slope of 0.068 V/pH (R^2 = 0.998) (shown in Fig.4c).

Table 1: Electrochemical oxidation and reduction peak potentials of temozolomide (TMZ) at GCE and MWCNTs-COOH paste electrode (MCPE) in acidic, basic and neutral electrolyte solution.

Electrodes	0.2 M H_2SO_4		0.1M PBS (pH 7.4)		0.1M NaOH	
	Epa (V)	Epc (V)	Epa (V)	Epc (V)	Epa (V)	Epc (V)
GCE	+ 0.48	- 0.50	0.31	-	0.22	-
MWCNTsCOOH PE	+0.24	-0.36	-0.04	-0.10	-0.045 & + 0.150	-

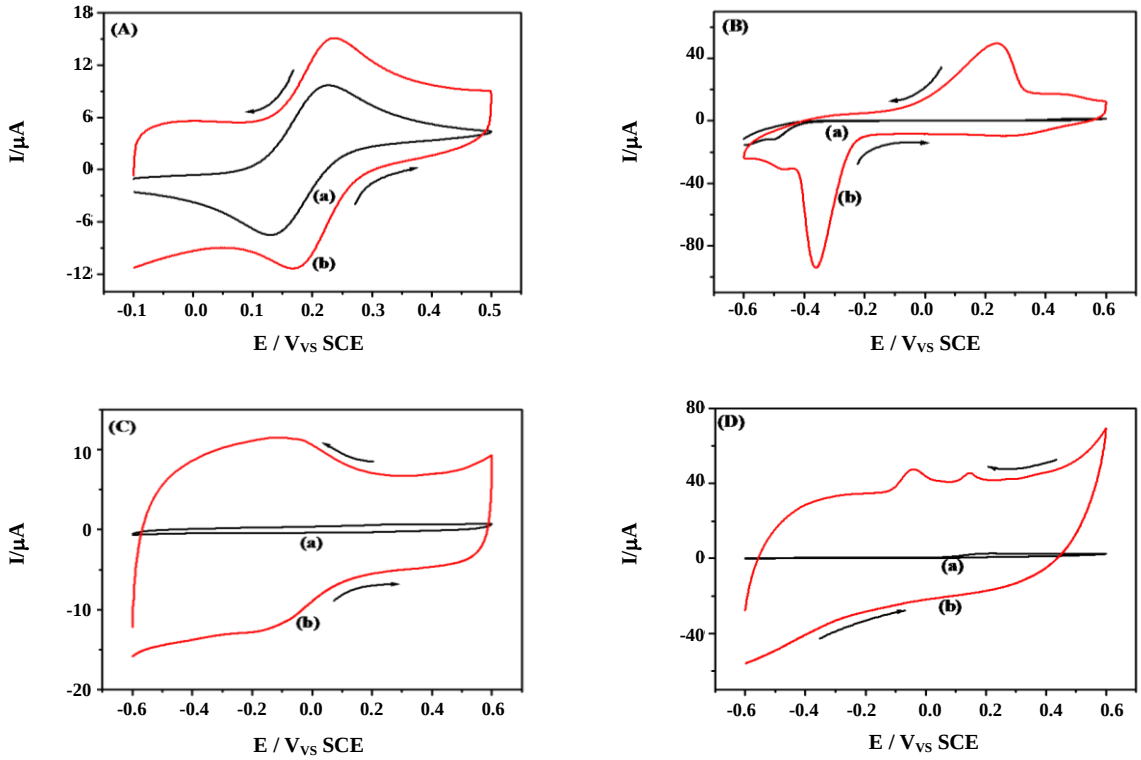

Fig. 1: Cyclic voltammograms with scan rate (0.05V/s) of (A) 1mM ferrocyanide in 0.1M KCl, (B) 2mM temozolomide in 0.1 M H_2SO_4, (C) 2mM temozolomide in 0. 1 M PBS (pH 7.4), (D) 2mM temozolomide in 0.1 M NaOH solutions at bare GCE (a) and MWCNTs-COOH paste electrode (MCPE) (b).

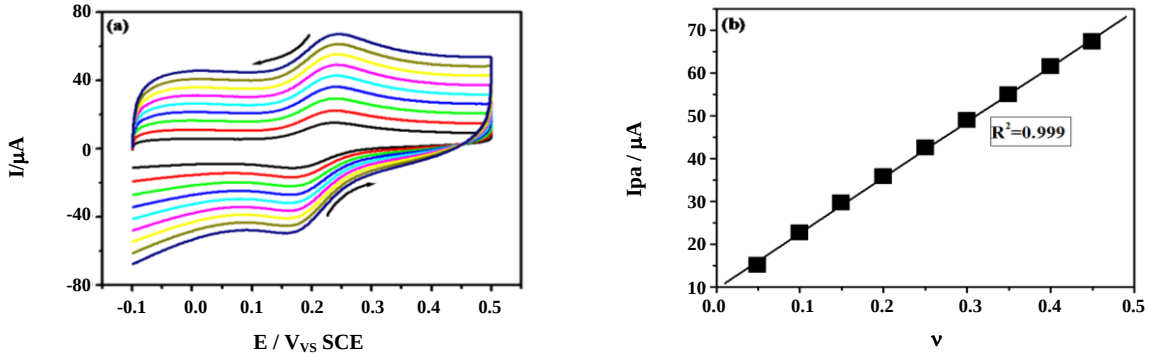

Fig. 2: (a) Cyclic voltammograms and (b) graphs (anodic peak current (Ipa) versus the scan rate) for 1mM ferrocyanide in 0.1 M KCl solutions of MWCNTs-COOH paste electrode (MCPE) at different scan rates (0.05 to 0.5 V/s).

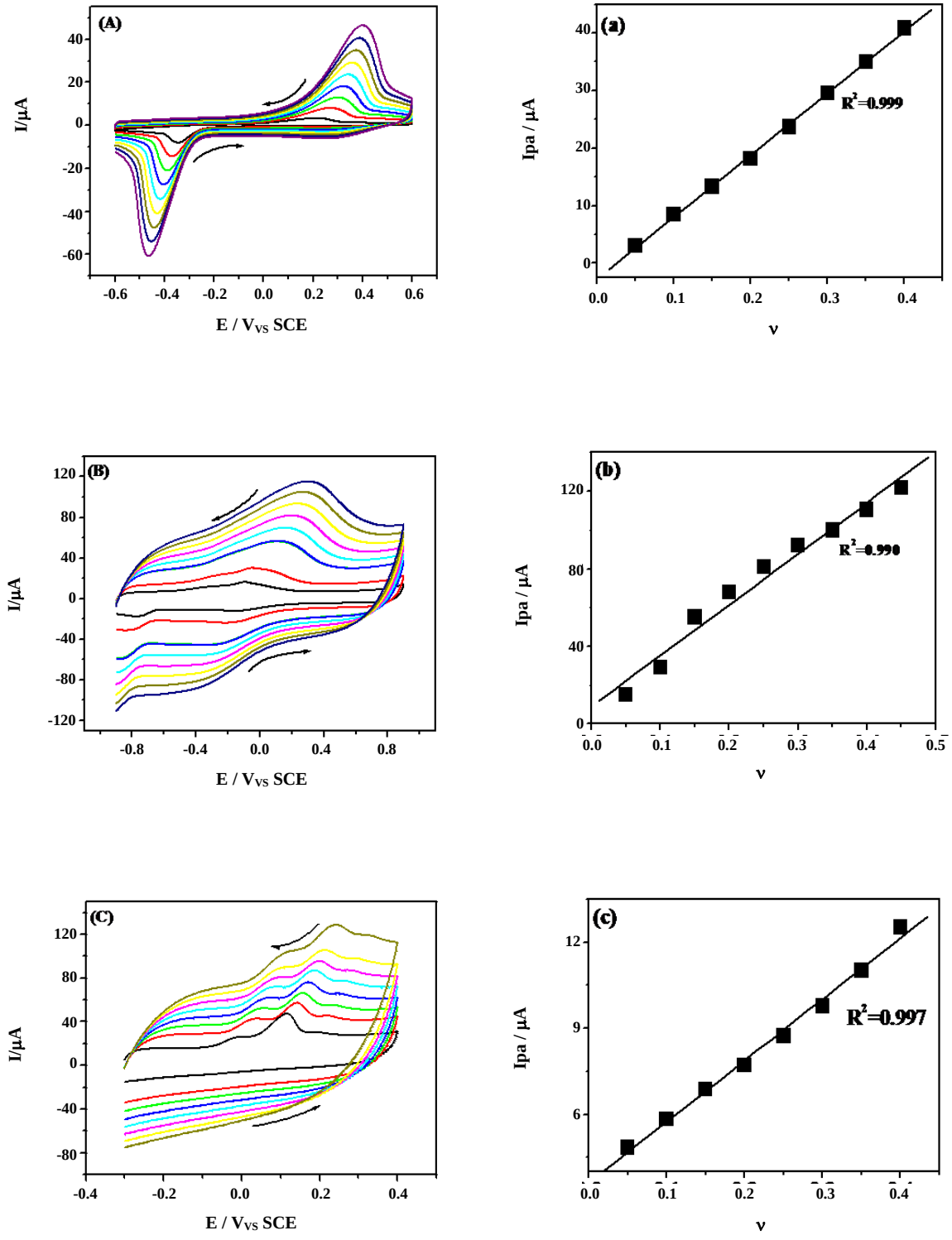

Fig. 3: Cyclic voltammograms and graphs (anodic peak current (Ipa) versus the scan rate) in 0.1 M H_2SO_4((A) & (a)), 0.1 M PBS (pH 7.4) ((B) & (b)), and 0.1 M NaOH solutions ((C)& (c)) of MWCNTs-COOH paste electrode (MCPE) at different scan rates (0.1 to 0.5 V/s).

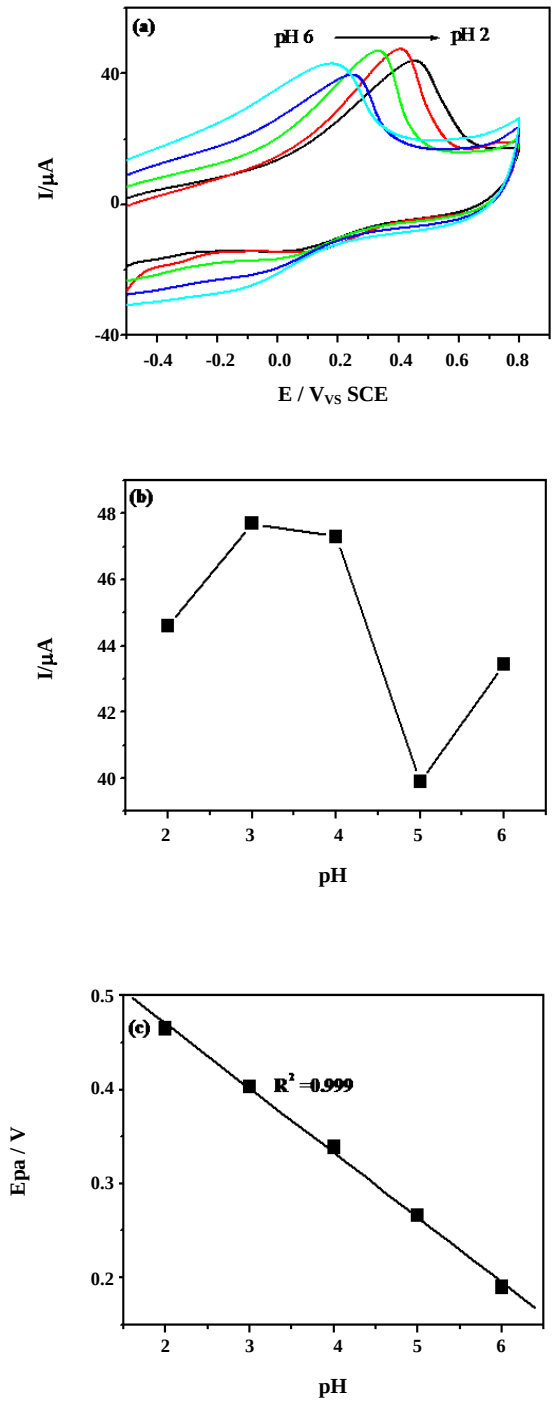

Fig. 4: (a) Cyclic voltammograms of different pH 2 to 6 solutions of 2mM temozolomide at the MWCNTs-COOH paste electrode (MCPE) (from right to left), (b) TMZ oxidation peak current versus pH and (c) anodic potential versus pH.

It demonstrates that the TMZ undergoes equal number of electron and proton transfer process, which is consistent with that reported in literature [28,29].

The effect of the concentration of TMZ

The Cyclic voltammetry (CV) and linear scanning voltammetric (LSV) technique was used for analysis of TMZ concentration which was varied from 0.25 to 4.25 mM TMZ in 0.1M H_2SO_4, 0.25 to 4.25 mM TMZ in 0.1MPBS (pH 7.4) and 0.25 to 4.25 mM TMZ in 0.1M NaOH solutions at the MWCNTs-COOH paste electrode (MCPE). The obtained cyclic voltammograms and linear scan voltammograms are shown in Fig.5 (A,B,C) & Fig. 6 (A,B,C) respectively. The corresponding graphs of anodic peak current versus concentration of TMZ shows linear relationship ranges 0.25 to 4.25 mM.

The correlation coefficient for the linearity was r^2=~0.990 are shown in Fig. 5 (a,b,c) & Fig. 6 (a,b,c). The detection limit of TMZ in 0.1M H_2SO_4, 0.1 MPBS (pH 7.4), 0.1M NaOH solutions at MCPE was calculated from Fig.5 (a,b,c) & Fig. 6 (a,b,c) and obtained results were listed in Table.2. The detection limit was calculated according to the equation of LOD= K S°/S, and K was a constant related to the confidence level. According to the suggestion of the IUPAC, the value of K is 3 at the 99% confidence level, S^0 is the standard deviation of ten blank-solution measurements (no added TMZ) and S is the slope of the calibration graph, the proposed electrode showed relatively lower detection limit than the recent reported [28,29] and calculated by using same formula. The sensitivity from the slope of calibration plot from {Fig.5 (a,b,c) & Fig. 6(a,b,c)} were calculated and obtained results were listed in the Table 2.

Application to real system

The practical application of the MWCNTs-COOH paste electrode (MCPE) was demonstrated by the quantitative determination of temozolomide (TMZ) in human blood serum sample. The procedure followed is as follows; 10 mL of human serum sample without any pretreatment was diluted to 100 mL with 0.1M H_2S0_4 solution. Each experiment was carried out at least 5 times and the results are presented in Table 3. The obtained recovery and Relative Standard Deviation (RSD) seems to be very good, indicating the performance of the MWCNTs-COOH paste electrode (MCPE).

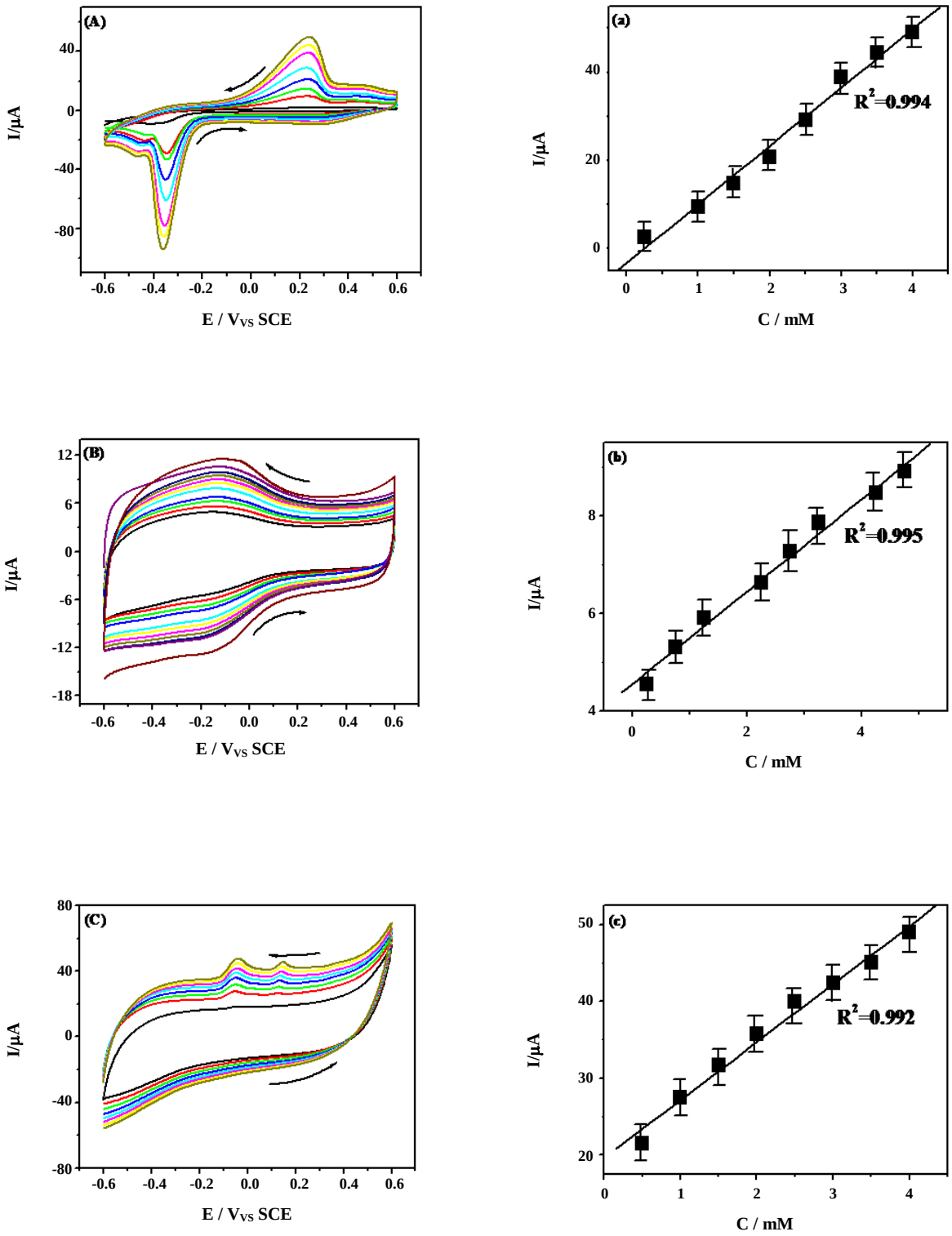

Fig. 5: Cyclic voltammograms and graphs (peak current versus concentration) of 0.25 to 4.25 mM TMZ in 0.1 M H_2SO_4 ((A) & (a)), 0.25 to 4.25 mM TMZ in 0.1 M PBS (pH 7.4) ((B) & (b)) and 0.25 to 4.25 mM TMZ in 0.1 M NaOH solutions ((C)&(c)) at MWCNTs-COOH paste electrode (MCPE).

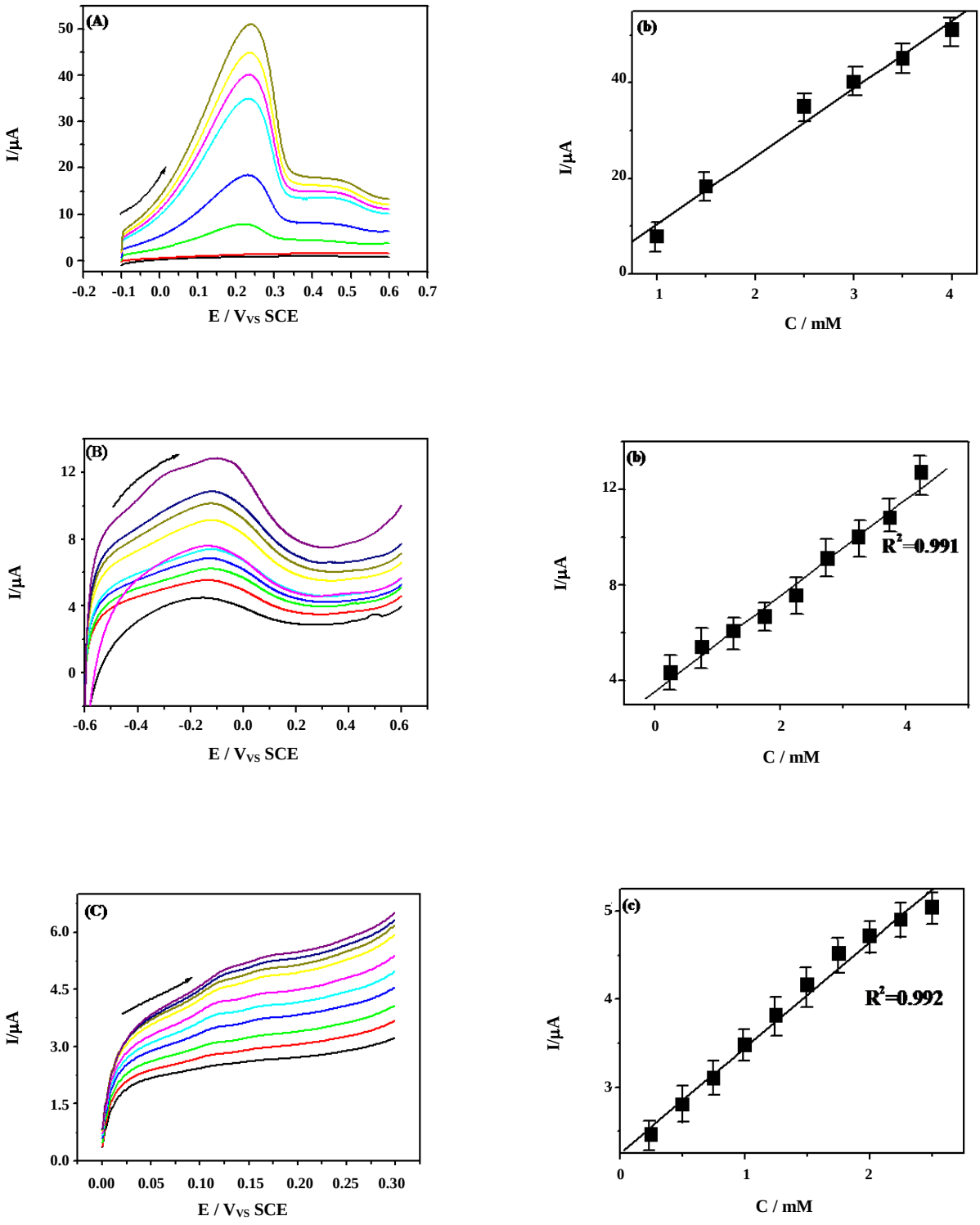

Fig. 6: Linear scanning voltammograms (LSV) and graphs (peak current versus concentration) of 0.25 to 4.25 mM TMZ in 0.1 M H_2SO_4 ((A) & (a)), 0.25 to 4.25 mM TMZ in 0.1 M PBS (pH 7.4) ((B) & (b)) and 0.25 to 4.25 mM TMZ in 0.1 M NaOH solutions ((C)&(c)) at MWCNTs-COOH paste electrode (MCPE).

Table 2: Listed analytical performance such as detection limit (LOD) and sensitivity of MWNTS- COOH paste electrode for Temozolomide detection.

Analytical Parameters	$0.2MH_2SO_4$ solutions		0.1M PBS solutions (pH 7.4)		0.1M NaOH Solutions	
	CV	LSV	CV	LSV	CV	LSV
LOD (mM)	0.056	0.051	0.069	0.021	0.065	0.036
Sensitivity (μA/mM)	0.0132	0.0142	0.001	0.002	0.075	0.0012

Table 3: Determination of TMZ in human blood serum by using MWNTS- COOH paste electrode (number of trial = 5).

Samples	Spiked TMZ sample (mM)	Found (mM)	Recovery (%)	RSD (%)
Blood Serum	0.3	0.3	100	1.80
	0.4	0.398	99.5	0.88
	0.5	0.497	99.4	2.41

CONCLUSIONS

The MWCNTs-COOH paste electrode (MCPE) exhibits remarkable enhancement of peak currents response, low detection and high sensitivity for detection of temozolomide (TMZ). The MCPE is found to be very effective when applied to clinical sample. The MCPE show high electrocatalytic activity and electrochemical sensing towards detection of TMZ. It is expected that the MCPE based method may found application as an electrochemical sensor for detection of TMZ concentration in the real samples. In addition, due to the easiest of the preparation procedures, fast routine detection of TMZ can be achieved by this method.

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REFERENCES

[1] Stupp R., Mason W.P., van den Bent M.J., Weller M., Fisher B., Taphoorn M.J., Belanger K., Brandes A.A., Marosi C., Bogdahn U., Curschmann J., Janzer R.C., Ludwin S.K., Gorlia T., Allgeier A., Lacombe D., Cairncross J.G., Eisenhauer E., Mirimanoff R.O., Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma, *N. Engl. J. Med.*, **352**: 987-996 (2005).

[2] NICE, NICE Technology Appraisal Guidance [TA23]: Guidance on the Use of Temozolomide for the Treatment of Recurrent Malignant Glioma Brain Cancer), https://www.nice.org.uk/guidance/ta23/resources/ guidance -on- the- use-of- temozolomide-for-the- treatment- of- recurrent- malignant -glioma-brain-cancer-pdf-2294454507973, Published date: April (2001).

[3] Denny B.J., Wheelhouse R.T., Stevens M.F., Tsang L.L., Slack J.A., NMR and Molecular Modeling Investigation of the Mechanism of Activation of the Antitumor Drug Temozolomide and Its Interaction with DNA, *Biochemistry*, **33**: 9045- 9051(1994).

[4] Friedman H.S., Kerby T., Calvert H., Temozolomide and Treatment of Malignant Glioma, *Clin. Cancer Res.* **6**: 2585–2597 (2000).

[5] Darkes M.J.M., Plosker G.L., Jarvis B., Temozolomide, A Review of its Use in the Treatment of Malignant Gliomas, Malignant Melanoma and Other Advanced Cancers, *Am. J. Cancer.*, **1**: 55-80 (20020.

[6] Clark A.S., Stevens M.F.G., Sansom C.E., Schwalbe C.H., Anti-Tumour Imidazotetrazines. Part XXI. Mitozolomide and Temozolomide: Probes for the Major Ggroove of DNA, *Anti-Cancer Drug Des.*, **5**: 63-68 (1990).

[7] Lopes I.C., Oliveira S.C.B., Oliveira-Brett A.M., In Situ Electrochemical Evaluation of Anticancer Drug Temozolomide and Its Metabolites–DNA Interaction, *Anal. Bioanal. Chem.* **405**: 3783–3790 (2013).

[8] Lopes I.C., de Oliveira S.C.B., Oliveira-Brett A.M., Temozolomide Chemical Degradation to 5-Aminoimidazole-4-Carboxamide – Electrochemical Study, *J. Electroanal. Chem.*, **704**: 183-189 (2013).

[9] Britten C.D., Rowinsky E.K., Baker S.D., Agarwala S.S., Eckardt J.R., Barrington R., Diab S.G., Hammond L.A., Johnson T., Villalona-Calero M., Fraass U., Statkevich P., Von Hoff D.D., Eckhardt S.G., A Phase I and Pharmacokinetic Study of Temozolomide and Cisplatin in Patients with Advanced Solid Malignancies, *Clin. Cancer Res.*, **5**: 1629–1637 (1999).

[10] Park D.M., Shah D.D., Egorin M.J., Beumer J.H., Disposition of Temozolomide in a Patient with Glioblastoma Multiforme After Gastric Bypass Surgery, *J. Neuro-Oncol.*, **93**: 279 –283 (2009).

[11] Hong K., Likhari P., Parker D., Statkevich P., Marco A., Lin C.C., Nomeir A.A., High-Performance Liquid Chromatographic Analysis and Stability of Anti-Tumor Agent Temozolomide in Human Plasma, *J. Pharm. Biomed. Anal.*, **24**: 461-468 (2001).

[12] Andrasi M., Bustos R., Gaspar A., Gomez F.A., Klekner A., Analysis and Stability Study of Temozolomide Using Capillary Electrophoresis, *J. Chromatogr. B*, **878**: 1801-1808 (2010).

[13] Kim H.K., Lin C.C., Parker D., Veals J., Lim J., Likhari P., Statkevich P., Marco A., Nomeir A.A., High-Performance Liquid Chromatographic Determination and Stability of 5-(3-methyltriazen-1-yl)-imidazo-4-carboximide, the Biologically Active Product of the Antitumor Agent Temozolomide in Human Plasma, *J. Chromatogr. B*, **703**: 225-233 (1997).

[14] Andrási M., Törzsök B., Klekner A., Gáspár A., Determination of Temozolomide in Serum and Brain Tumor with Micellar Electrokinetic Capillary Chromatography, *J. Chromatogr. B*, **879**: 2229-2233 (2011).

[15] Shen F., Decosterd L.A., Gander M., Leyvraz S., Biollax J., Lejeune F., Determination of Temozolomide in Human Plasma and Urine by High-Performance Liquid Chromatography after Solid-Phase Extraction, *J. Chromatogr. B Biomed. Appl.*, **667**: 291-300 (1995).

[16] Chowdhury S.K., Laudicina D., Blumenkrantz N., Wirth M., Alton K.B., An LC/MS/MS Method for the Quantitation of MTIC (5-(3-*N*-methyltriazen-1-yl)-imidazole-4-carboxamide), a Bioconversion Product of Temozolomide, in Rat and Dog Plasma, *J. Pharm. Biomed. Anal.*, **19**: 659-668 (1999).

[17] Xiao L.F., Chen J., Cha C.S., Elimination of the Interference of Ascorbic acid in the Amperometric Detection of Biomolecules in Body Fluid Samples and the Simple Detection of Uric Acid in Human Serum and Urine by Using the Powder Microelectrode Technique, *J. Electroanal. Chem.*, **495**: 27-35 (2000).

[18] Reddy S., Swamy B. E. K., Jayadevappa H., CuO Nanoparticle Sensor for the Electrochemical Determination of Dopamine, *Electrochim. Acta*, **61**: 78-86 (2012).

[19] Jacobs C.B., Peairs M.J., Venton J.B., Review: Carbon Nanotube Based Electrochemical Sensors for Biomolecules, *Anal. Chim. Acta*, **662**: 105-127 (2010).

[20] Banks C.E., Crossley A., Salter C., Wilkins S.J., Compton R.G., Carbon Nanotubes Contain, Metal Impurities which are Responsible for the "electrocatalysis" Seen at Some Nanotube-Modified Electrodes, *Angew. Chem. Int. Ed.*, **45**: 2533–2537 (2006).

[21] Reddy S., Du R., Xu H., Wu J., Mao N., Zhang J., Carbon Nanotube/gold Nanoparticles Sensor for Detection of Hydrazine in the Pyrogallol Red Solution, *Anal. Bioanal. Electrochem.*, **7**: 277-290 (2015).

[22] Raoof J. Bh., Chekin F., Ojani R., Barari S., Carbon Paste Electrode Incorporating Multi-Walled Carbon Nanotube/Ferrocene as a Sensor for the Electroanalytical Determination of N-Acetyl-L-Cysteine in the Presence of Tryptophan, *J. Chem. Sci.*, **125**: 283-289 (2013).

[23] Mattson M.P., Haddon R.C., Rao A.M., Molecular Functionalization of Carbon Nanotubes and Use as Substrates for Neuronal Growth, *J. Mol. Neurosci.*, **14**: 175-182 (2000).

[24] Hua M.Y., Chen H.C., Tsai R.Y., Tseng S.J., Hu S.C., Chiang C.D, Chang P.J., Preparation of Polybenzimidazole- Carboxylated Multiwalled Carbon Nanotube Composite for Intrinsic Sensing of Hydrogen Peroxide, *J. Phys Chem. C*, **115**: 15182-15190 (2011).

[25] DSouza O.J., Mascarenhas R.J., Satpati A.K., Aiman L.V., Mekhalif Z., Electrocatalytic Oxidation of L-Tyrosine at Carboxylic Acid Functionalized Multi-Walled Carbon Nanotubes Modified Carbon Paste Electrode, *Ionics*, (2015).
DOI 10.1007/s11581-015-1552-6.

[26] Bard A.J., Faulkner L.R., Electrochemical Methods: Fundamental and Application, (Wiley, New York), (2000).

[27] Gosser Jr. D.K., Cyclic Voltammetry Simulation and Analysis of Reaction Mechanisms, (VCH, Weinheim), (1993).

[28] Xia C., Ning W., Long W., Lin G., Synthesis of Nanochain-Assembled ZnO Flowers and Their Application to Dopamine Sensing, *Sens. Actuat. B.*, **147**: 629-634 (2010).

[29] Zhu Z., Qu L., Guo Y., Zeng Y., Sun W., Huang X., Electrochemical Detection of Dopamine on a Ni/Al Layered Double Hydroxide Modified Carbon Ionic Liquid Electrode, *Sens. Actuators B*, **151**: 146-152 (2010).