Iranian Journal of Analytical Chemistry

Journal home page: http://ijac.journals.pnu.ac.ir

Simultaneous Determination of Glutathione and Tryptophan at the Surface of Carbon Nanotube Paste Electrode in the Presence of Catechol

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Received 5 Jauanry 2014; Received in revised form 27 Jauanry 2014; Accepted 12 February 2014, Online published: 17 February 2014

ABSTRACT

The electrochemical oxidation and determination of tryptophan (Trp) and glutathione (GSH) in the presence of catechol as a homogeneous redox in the aqueous medium were investigated at the surface of a carbon paste electrode modified with multi-walled carbon nanotube (MWCNT-CPE) using cyclic voltammetry (CV), double step potential chronoamperometry and differential pulse voltammetry (DPV) techniques. The results of differential pulse voltammetry showed two well-resolved anodic peaks for glutathione and tryptophan which leads to voltammetric determination of each of them. The results shows the electrooxidation peak currents of GSH and Trp is linear in the concentration range of 2×10^{-6} M - 2.8×10^{-4} M and 4×10^{-6} M - 6×10^{-5} M, respectively. The kinetic parameters such as: the chemical reaction rate constant (k_h) and transfer coefficient (α) were calculated 2.01×10^{2} cm^3 mol^{-1} s^{-1} and 0.31, respectively. Also, the apparent diffusion coefficient, D_{app}, for GSH was found to be 1.95×10^{-6} cm^2 s^{-1} in aqueous buffered solution. The proposed method was successfully applied for determination of glutathione and tryptophan in real sample using standard addition method.

KEYWORDS: Glutathione; Tryptophan; Catechol; Multi-Walled Carbon Nanotubes.

1. INTRODUCTION

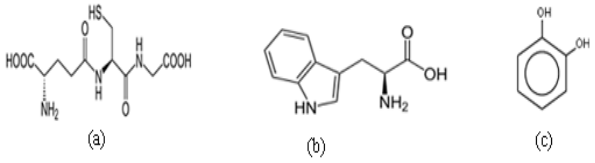
Glutathione (L-γ-glutamyl-L-cysteinyl-glycine) or (GSH) (Scheme 1, a) is the original non-protein compound, which exists in most mammalian cells [1-2]. The role of glutathione in the human metabolism consists protection versus oxidative stress and detoxification of xenobiotics [3]. Oxidative stress reflects imbalance between the systemic manifestation of reactive oxygen types (free radicals) and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage [4]. Many chemical and instrumental techniques have been reported for the determination of glutathione such as spectrofluorimetry [5], HPLC [6], Capillary electrophoresis [7] and Colorimetric [8]. Among these techniques, electrochemical methods [9-10] have the advantages of low cost, excellent sensitivity, simplicity and rapid detection.

Tryptophan (Trp) is a hydrophobic amino acid (Scheme 1, b), which a precursor for serotonin, niacin and melatonin. Also, it has been implicated as a possible agent of schizophrenia in persons who cannot metabolize it properly [11-12]. Therefore, a simple sensitive and less expensive method for detection of Trp is of great interest. Many methods have been proposed for determination of tryptophan such as high-performance liquid chromatography [13], capillary zone electrophoresis [14], florescence [15], Chemiluminescence [16], spectrophotometric [17] and electrochemical methods [12]. Compared with these methods, electrochemical analytic technique is an attractive method owning to low expense, simplicity and high sensitivity.

Glutathione is an important physiological antioxidant which significant role in neuronal processes and biological. Thus, every system in the body can be affected by the state of the glutathione system, especially the immune system and the nervous system [18]. The detection of tryptophan is very important due to its crucial roles in biological systems. Trp is also an essential amino acid for brain functions and neuronal regulatory mechanisms [19]. Both GSH and Trp have a critical role in biological and central nervous system [20]. In the other hand, the electrochemical oxidation of GSH and Trp at bare electrode results highly overlapped current responses which make their discrimination very

difficult and leads to poor selectivity and reproducibility of the electrode.

Catechol (1, 2- dihydroxy benzene or C) (Scheme 1, c), is one of the relatively important phenolic compounds which exists naturally in vegetables, fruits and it is extensively used in cosmetic, chemical materials and pharmaceutical industries [21-22]. Also catechol has a terrific importance in both environmental and biological analysis fields and this owning to its supreme electrochemical ability and can be utilized for the characterization of various analytical techniques [23]. However in this study, catechol was applied as a homogeneous electrocatalyst for electrochemical oxidation of GSH due of its considerable electroactive properties.



Scheme 1. The structural formula of Glutathione (a) Tryptophan (b) and Catechol (c)

Carbon nanotubes (CNTs), as an attractive and new carbon material, have achieved multiplex theoretical and experimental studies [24]. Also, CNT is a type of inorganic material with a nano-structure, which has significant mechanical ability, high surface area, remarkable electrical conductivity and nice chemical stability [25]. They are built from sp^2 carbon units and present a monolithic structure with hexagonal honeycomb lattices [26]. To the best of our knowledge, there has been no report in the literature on the simultaneous determination of GSH and Trp at the surface of carbon nanotube paste electrodes (MWCNT-CPE) in the presence of catechol. Thus in this paper, we propose catechol as a new homogeneous electrocatlyst for the rapid, sensitive and highly selective simultaneous voltammetry determination of glutathione (GSH) and tryptophan (Trp) at the surface of MWCNT-CPE.

2. EXPERIMENTAL

2.1. Apparatus and Materials

The electrochemical experiments (cyclic voltammetry, chronoamperometry and differential pulse voltammetry) were carried out using a potentiostat/galvanostat (SAMA 500 Electroanalyzer System, I.R. Iran). Experiments were performed in a three-compartment cell. Ag| AgCl| KCl (3.0 M) was used as a reference electrode, a platinum wire was used as the auxiliary electrode. The working electrode was carbon paste electrode modified with multi-walled carbon nanotubes (MWCNT-CPE).

The solvent used for the electrochemical studies was twice distilled water. High purity graphite powder, sodium dihydrogen phosphate (NaH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) and glutathione were obtained from Merck. Multi-walled carbon nanotubes were obtained from Nano Star Tech. Co. Tehran, Iran. Paraffin oil (density=0.88 g cm^{-3}), tryptophan and catechol were obtained from Fluka. Buffer solutions were prepared from orthophosphoric acid and its salts in the pH ranges 4.0-9.0. All other reagents used were of analytical grade.

2.2. Working electrode

The prepared carbon paste electrode modified with multi-walled carbon nanotubes (MWCNT-CPE) was applied as a working electrode. Graphite powder (0.85 g) was hand mixed with 0.15 g multi-walled carbon nanotubes in a mortar and pestle. A syringe was used to add paraffin to the mixture, which was mixed well for 35 min until a uniformly wetted paste was obtained. The electrical connection was implemented by a copper wire lead fitted into a glass tube with Inner diameter 3.4 mm.

3. RESULT AND DISCUSSION

3.1. pH effect on GSH electrochemical behavior at MWCNT-CPE

It is well known that the electrochemical behavior of GSH and orthoquine obtained electrochemically at the surface of electrode are dependent on the pH value of the aqueous solution [27]. The effect of various pHs value ($4.0 \leq pH \leq 9.0$) on the reaction between GSH and ortoquine at the surface of MWCNT-CPE was studied by cyclic voltammetry (Fig. 1A).

Fig. 1B shows the variation of I_{pa} versus pH values for GSH electrooxidation at the surface of MWCNT-CPE electrode in the presence of catechol. As can be seen, the maximum current was obtained at pH=7.0. Therefore, pH=7.0 was obtained as the optimum pH.

In Fig. 1C, it can be seen the anodic peak potential of GSH in the presence of catechol at the surface of MWCNT-CPE was shifted to a less positive potential with enhancing of pH values. The variation of anodic peak potential with pH values showed a linear relationship with a slope of -0.0577 V per unit pH and a R^2=0.9882. The number of electron and proton in the electrochemical reaction of GSH in this condition is equal.

3.2. Electrochemical oxidation of glutathione

Fig. 2 depicts the cyclic voltammetry response of the electrochemical oxidation of GSH in the presence of catechol at the surface of unmodified and modified carbon paste electrode in 0.1 M phosphate buffered solution (pH=7.0). As can be seen, in the absence of GSH and catechol, the bare CPE and MWCNT-CPE do not show any anodic and cathodic peaks in 0.1 M PBS (Fig. 2. (a) and (b)). The cyclic voltammograms of 100 µM catechol at surface of CPE and MWCNT-CPE in phosphate buffered solution (pH=7.0) showed a well-behaved redox reaction of ortoquinine/catechol redox couple (curve c and d). On the other hand, Experimental results at the surface of modified electrode also show well-defined anodic and cathodic peaks with E_{pa} = 0.19 V, E_{pc} = 0.11 V, $E_{½}$ = 0.15 V vs. Ag| AgCl| KCl (3.0 M) and ΔE_p = 0.08 V. The electrode process was

quasi reversible, with ΔE_p greater than the (59/n) mV expected for a reversible system.

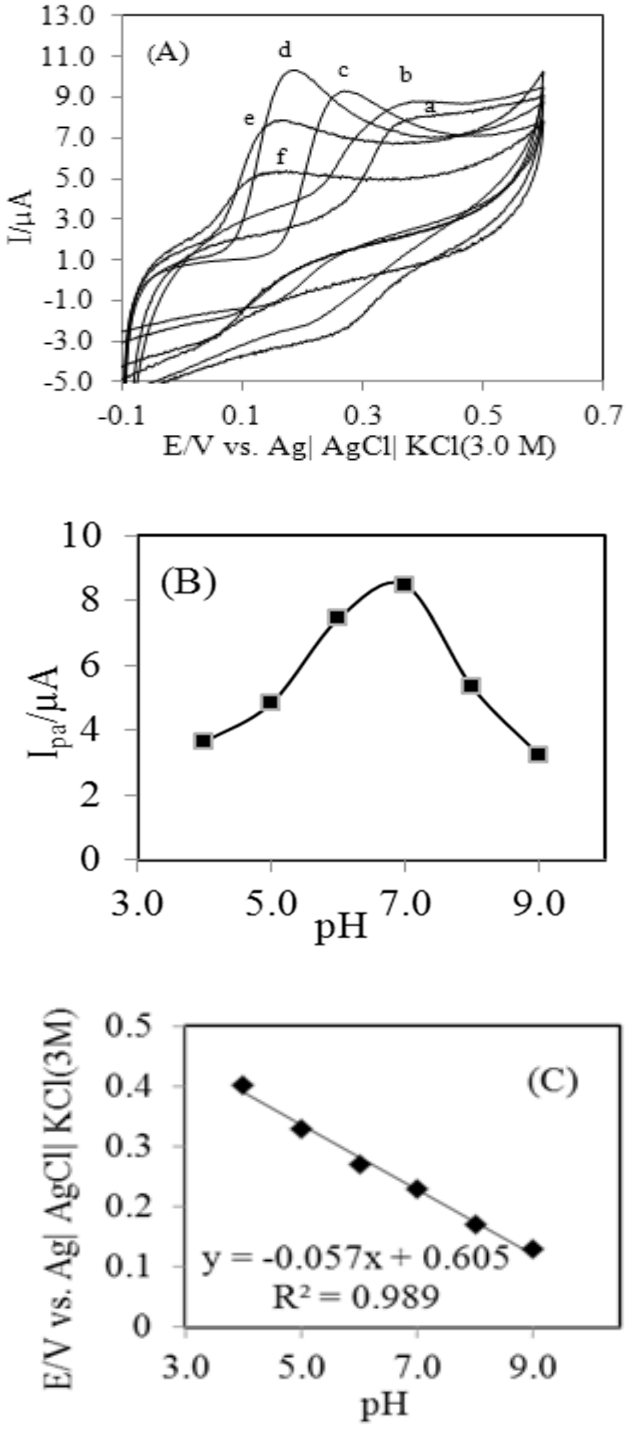


Fig. 1. (A) Cyclic voltammograms of 800 µM GSH in the presenc of 100 µM catechol in 0.1 M phosphate buffer soliution at the surface of MWCNT-CPE in the various pHs: (a) 4.0, (b) 5.0, (c) 6.0, (d) 7.0, (e) 8.0 and (f) 9.0 at scan rate 20 mV s^{-1}. (B) The variation of electrooxidation peak currents for 800 µM GSH in 0.1 M phosphate buffer solution at the surface of MWCNT-CPE versus pH values and (C) The variation of peak potential versus pH values.

Electrochemical oxidation of 800 µM GSH in the absence of catechol occurs irreversibly at the surface of CPE and MWCNT-CPE (Fig. 2. (e) and (f)). As can be seen, in the presence of catechol the anodic peak potential of GSH was shifted to a less positive potential. In fact the overpotential of GSH oxidation reduces about 820 mV and 900 mV at the surface of CPE and MWCNT-CPE, respectively (Fig. 2. (g) and (h)). On the other hands, the comparison between the cyclic voltammogarms (c), (g) at surface of CPE and (d), (h) at the surface of MWCNT-CPE show a dramatic enhancement of the anodic peak current at surface of modified electrode in the presence of 100 µM catechol relative to the value obtained at the surface of bare electrode this condition and the cathodic peak of GSH was disappeared in the reverse scan of potential.

From these results, it can be seen that the best electrocatalytic effect for GSH oxidation is observed in the presence of catechol at the surface of MWCNT-CPE (curve h).

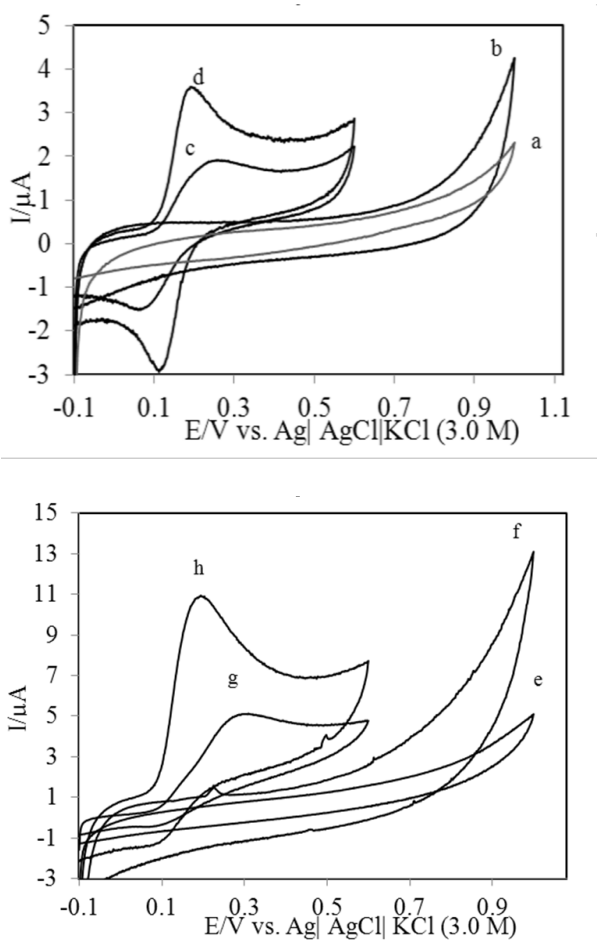
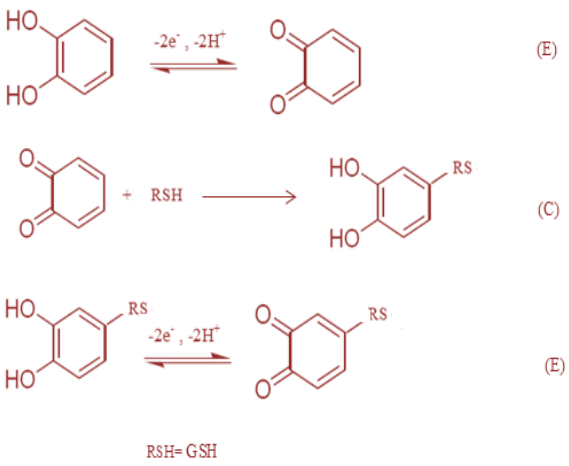


Fig. 2. Cyclic voltammograms of bare CPE (a) and MWCNT-CPE (b) in suporting electrolyte (0.1 M phosphate buffer soliution). (c) as (a) and (d) as (b) in the presence of 100 µM catechol. Cyclic voltammograms (e) as (a), (f) as (b) in the presence of 800 µM GSH, (g) as (a) and (h) as (b) in the presence of 800 µM GSH and 100 µM catechol in 0.1 M PBS (pH=7.0) at scan rate of potential 20 mV s^{-1}.

This result shows catechol acts as an effective redox mediator for the electrocatalysis of the GSH oxidation. On the basis of the information, we suggest the ECE

electrocatalytic mechanism for the oxidation of GSH (Scheme 2).



Scheme 2. Proposed mechanism for electrooxidation of catechol in the presence of GSH at the surface of MWCNT-CPE

3.3. Effect of scan rate

The effect of scan rate on the electrocatalytic oxidation of GSH in the presence of 100 µM catechol at the surface of MWCNT-CPE was investigated by cyclic voltammetry (Fig. 3A). As can be observed in Fig. 3 (A), the oxidation peak potential shifted to more positive potentials with enhancing scan rate, confirming the kinetic limitation in the electrochemical reaction. Also, a plot of anodic peak height (I_p) vs. the square root of scan rate ($\upsilon^{1/2}$) was found to be linear in the range of 5-120 mV s^{-1}, suggesting that at enough over potential, the process is diffusion controlled (Fig. 3B).

Also, we used the Tafel plot to determine the transfer coefficient (α) in the catalytic oxidation process (Fig 3. C). The slope of the Tafel plot was equal to n (1-α) F/2.3RT, which came up to 5.3514 V $decade^{-1}$. Therefore we obtained the value of nα equal to 0.31. Assuming that n=1, then α=0.31.

3.4. Chronoamperometric

The chronoamperometric behavior of GSH was examined at modified CPE in the presence of 100 µM catechol for the various concentration of GSH solution by setting the working electrode potential at 220 mV and 80 mV at versus Ag| AgCl| KCl (3M) at the first and second potential steps (Fig. 4. A).

The rate constant for the chemical reaction between GSH and catechol at the surface of MWCNT-CPE, k_h, can be evaluated by chronoamperometry according to the method of Gallus [28]:

$$I_C/I_L = \pi^{1/2} (k_h C_o t)^{1/2} \qquad (1)$$

Where I_C is the electrocatalytic current of GSH in the presence of catechol at the MWCNT-CPE, I_L is the limited current of GSH in the absence of catechol and $\gamma=k_h C_o t$, C_o is the initial concentration of GSH in bulk solution and t is elapsed time. The calculated k_h value for GSH was 2.01×10^{2} cm^3 $mol^{-1}s^{-1}$ using the slope of I_C/I_L versus $t^{1/2}$ plot.

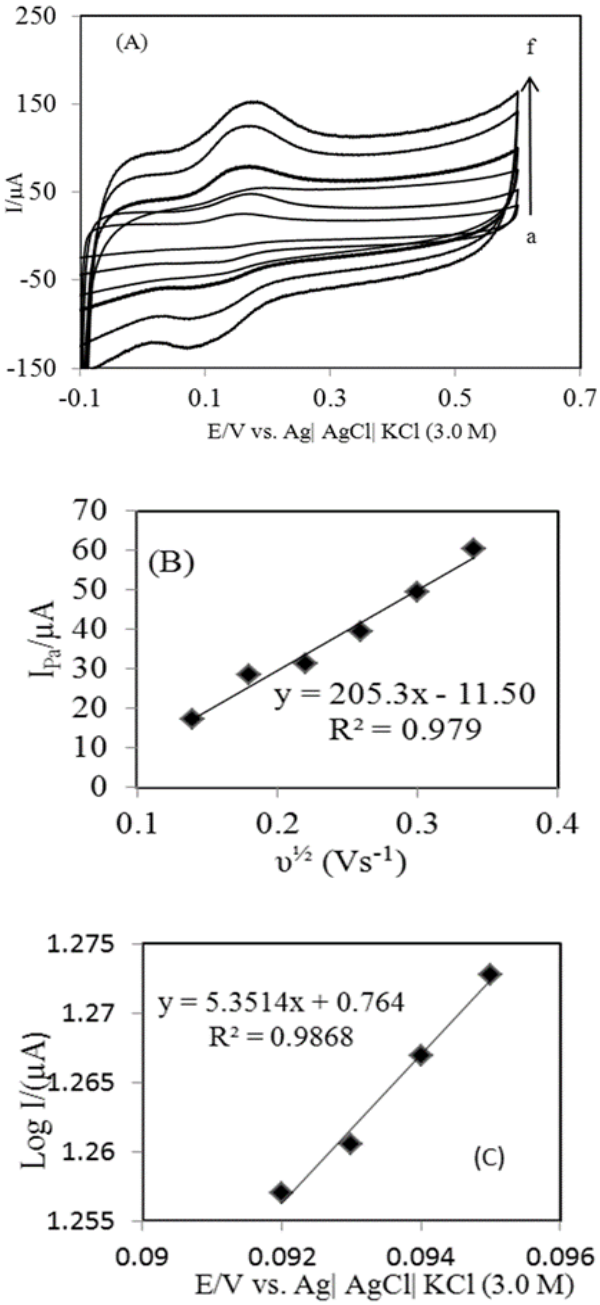


Fig. 3. (A) Cyclic voltamograms of GSH in the presence of catechol at MWCNT-CPE in 0.1 M phosphste buffer solution (pH=7.00) at various scan rates: (a) 0.02, (b) 0.035, (c) 0.05, (d) 0.75, (e) 0.09, (f) 0.12 Vs^{-1}. (B) plot of anodic peak currents versus $\upsilon^{1/2}$ from cyclic voltammograms of (A). (C) Variation of log I vs. anodic Peak potential (Tafel plot).

For an electroactive compound (GSH in this case) with an apparent diffusion coefficient, D_{app}, the current observed for the electrochemical reaction at the mass transport limited condition is described by Cottrell equation [29]:

$$I = nFAD_{app}^{1/2} C_b \pi^{-1/2} t^{-1/2} \qquad (2)$$

The Cotrell plots of I vs. $t^{-1/2}$ were used, with the best fits for different concentrations of GSH (Fig. 4. A). The slopes of the resulting straight lines were then plotted vs. GSH concentrations (not shown). The mean

value of the D_{app} was found to be $1.95\times 10^{-6}cm^2\ s^{-1}$ from the resulting slope and Cottrell equation.

As can be seen in Fig 4B, the forward and backward potential step chronoamperometry for the mediator in the absence of GSH showed symmetrical chronoamperogram with an equal charge consumed for the reduction and oxidation of the catechol at the surface of MWCNT-CPE (Fig. 4B, a´). However, in the presence of GSH, the charge amount associated with forward chronoamperometry is significantly greater than that observed for backward chronoamperometry (Fig.4B, d´).

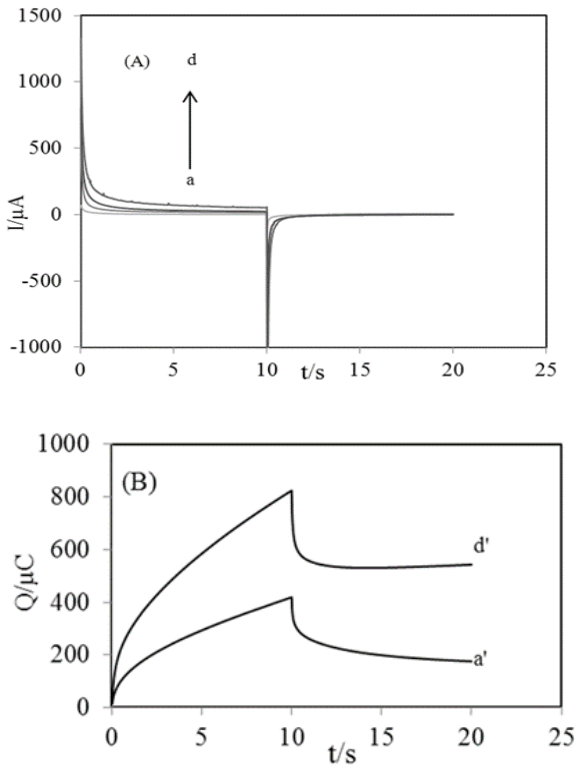


Fig. 4. (A) Chronoamperograms obtained for different concentration of GSH in the presence of 100 µM catechol in 0.1 M phosphate buffer solution (pH=7.00) at MWCNT-CPE first step and second step of potential are 220 and 80 mV vs, respencively. (Curve a-d correspond to 0.0, 9.0, 40 and 80 µM of GSH). (B) chronocolougrams of (a) and (d) obtained from chronoamprograms of (A).

3.5. Electrocatalytic determination of GSH in the presence of Trp

The electrooxidation of GSH was studied in the presence of 100 µM catechol, fixed concentration of Trp (20 µM) and different concentrations of GSH at the surface of MWCNT-CPE using differential pulse voltammetry (DPV) technique. The obtained result exhibited two anodic peak potentials at 330 mV (GSH anodic peak potential) and 810 mV (Trp anodic peak potential) versus Ag| AgCl| KCl (3.0 M) (Fig. 5A). The result showed a relatively large separation between anodic peak potentials of GSH and Trp. The calibration plot was linear in the GSH concentration range of 2×10^{-6} M-2.8×10^{-4} M with a correlation coefficient of R^2 = 0.99. The detection limit (3σ) was 7.7×10^{-7}M for GSH (Fig. 5B). This obtained value is comparable with values reported by other research groups (Table 1).

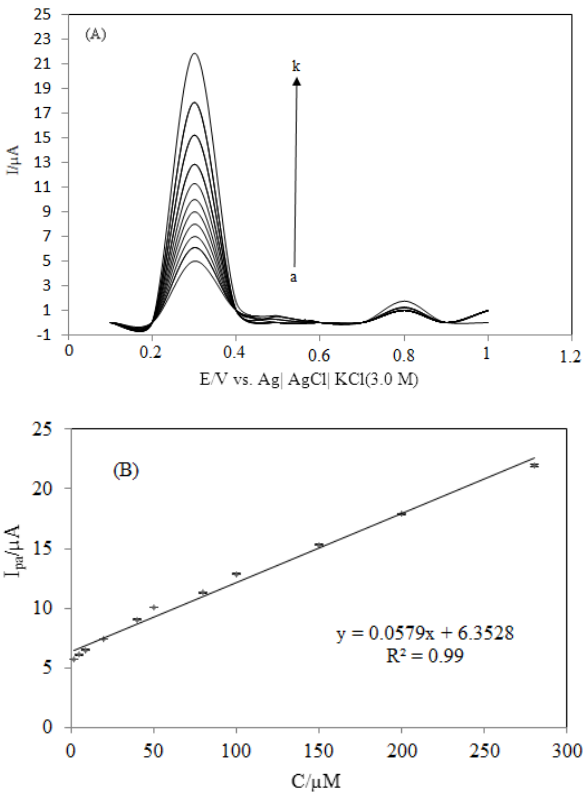


Fig. 5. (A) Differential pulse voltammograms of fixed concentration of Trp (20 µM) and different concentrations of GSH: (a) 2 µM, (b) 5 µM, (c) 9 µM, (d) 20 µM, (e) 40 µM and (f) 50, (g) 80, (h) 100, (i) 150, (j) 200 and (k) 280 µM in the presence of 100 µM catechol at the surface of MWCNT-CPE in 0.1 M phosphate buffer solution (pH= 7.0). (B) Calibration plot for GSH voltammetric determination obtained from its DPV data.

3.6. Electrocatalytic determination of Trp in the presence of GSH

Dependency of the oxidation peak current of Trp to its concentration in the presence of a fixed concentration of 50 µM GSH and 100 µM of catechol was investigated using differential pulse voltammetry method at the surface of modified electrode (Fig. 6A). The calibration plot was linear in the concentration range of 4×10^{-6} M-6×10^{-5} M of Trp with a correlation coefficient of R^2= 0.996. The detection limit (3σ) was 1.28×10^{-7}M (Fig. 6B) for Trp. This obtained value is comparable with values reported by other research groups (Table 2).

Those results clearly demonstrate and confirm the capability of the 100 µM catechol as a homogeneous electrocatalyst in the voltammetric determination of GSH and Trp with high selectivity, precision, and well reproducibility at the surface of MWCNT-CPE.

3.7. Determination of GSH and Trp in real samples

In order to indicate the electrocatalytic oxidation of GSH and Trp in real samples, we examined this ability in the voltammetric determination of GSH and Trp in

Table 1. Comparison of the efficiency of some methods in the determination of GSH

Electrode	Modifier	Methods	PH	LOD[c](M)	LDR[d](M)	Ref
CPE	TTF-TCNQ[a]	AMPb	7.00	3×10^{-7}	5×10^{-6}-3.4×10^{-4}	[30]
GCE	MWCNT	AMP	-	3.3×10^{-6}	5×10^{-6}-1×10^{-4}	[31]
MWCNT_CPE	Catechol	DPV	7.00	7.7×10^{-7}	2×10^{-6}-2.8×10^{-4}	This work

[a] Tetra thiafulvalene (TTF)-tetra cyano quinodim ethane (TCNQ)
[b] Amprometry
[c] Limit of detection
[d] Linear dynamic range

Table 2. Comparison of the efficiency of some methods in the electrocatalitical determination of Trp.

Electrode	Modifier	Methods	PH	LOD (M)	LDR (M)	Ref
GCE	Poly (Metyl Red) film	SWV	7.0	4.0×10^{-8}	1×10^{-7}-1×10^{-4}	[32]
GCE	Carbon nanofibers	-	-	1×10^{-7}	119×10^{-6}-1×10^{-5}	[33]
MWCNT-CPE	Catechol	DPV	7.00	1.28×10^{-7}	4×10^{-6}-6×10^{-5}	This work

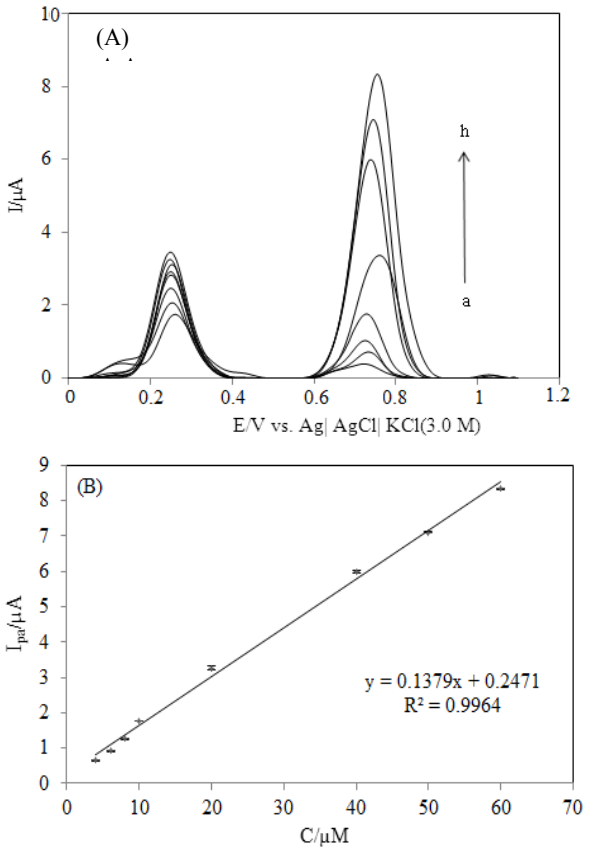


Fig. 6. (A) Differential pulse voltammograms of fixed concentration of GSH (50 μM) and different concentrations of Trp: (a) 4 μM, (b) 6 μM, (c) 8 μM, (d) 10 μM, (e) 20 μM (f) 40 μM, (g) 50 μM (h) 60 μM in the presence of 100 μM catechol at the surface of MWCNT-CPE in 0.1M phosphate buffer solution (pH= 7.0). (B) Calibration plot for Trp voltammetric determination obtained from its DPV data.

the presence of 100 μM catechol human plasma at the surface of MWCNT-CPE by utilization of standard addition method. In this method, we added a known amount of GSH or Trp to buffer phosphate solution (pH=7.0) containing a deliberated amount of plasma. The results are presented in Table 3.

Table 3. Voltammetric determination of GSH and Trp in real sample n=3.

Sample		added (μM)	found (μM)	Recovery	RSD%
Serum	GSH	4.0	4.2	105	0.78
		5.0	5.06	101	2.5
	Trp	3.0	2.9	96	1.26
		6.0	6.28	104	2.8

3.8. Interference study in GSH and Trp determination

The interference effect has been studied on the analysis signal of GSH and Trp. Various possible interfering substances, such as cysteine and ascorbic acid were examined for their effect on the determination of GSH and Trp under the optimum conditions. The results showed there was no substantial change in the peak current of GSH and Trp in the absence and presence of these interfering agents (data not shown). The data is summarized in Table 4.

Table 4. Effect of different interferents on the voltammetric signal of NAC at MWCNT/GCE in the presence of 4-chlorocatechol

Interfering agent	Concentration (μM)	Signal change (%)	
		NAC	Trp
Cysteine	30	7.61	-4
Ascorbic acid	50	-5.98	9.8

4. CONCLUSIONS

In this work, catechol was used as a redox mediator for the homogeneous electrocatalysis of glutathione in presence of tryptophan at the surface of MWCNT-CPE electrode. This mediator showed to be promising for GSH voltammetric determination in the presence of Trp with many desirable properties such as low detection limit and high selectivity. Also the electrode was reliable, simple to prepare, low cost, and did not re-

quire extensive preliminary sample treatment. The electrochemical oxidation peak current of glutathione was linearly depended on its concentrations and the calibration curve was obtained in the ranges 2×10^{-6} M-2.8×10^{-4} M with differential pulse voltammetry (DPV) method, and detection limit (3σ) was determined as 7.7×10^{-7} M. Also, voltammetric determination of Trp in the presence of GSH was liner in the range 4×10^{-6} $M^{-6}\times$-10^{-5} M with DPV method and the detection limit (3σ) was determined 1.28×10^{-7} M. Finally, the modified electrode was successfully applied to determination of glutathione and tryptophan in real samples.

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