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Electrochemical Determination of Tyrosine in Human Serum Based on Glycine Polymer and Multi-walled Carbon Nanotubes Modified Carbon Paste Electrode

Zhiqiang Wei, Yongjian Sun, Qianwen Yin, Liuxue Wang, Shuaigao Chen, Rui Sheng, Dengyun Pan, Hui Yang, Sanqiang Li**

Collage of Medical, Henan University of Science and Technology, Luoyang, 471023, China.
*E-mail: yanghui7761@163.com, sanqiangli2001@163.com

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A modified carbon paste electrode (CPE) for the sensitive detection of tyrosine (Tyr) in human serum has been fabricated by glycine polymer (Poly(Gly)) and multi-walled carbon nanotubes (MWCNTs). Poly(Gly) was prepared by in situ electrochemical polymeric deposition. The operational parameters of electrochemical polymeric deposition such as concentration of Gly solution and CV scan cycles which affected the analytical performance of the modified electrode were optimized. Compared to the bare CPE and MWCNTs modified CPE, Poly(Gly)/MWCNTs/CPE exhibited excellent electrooxidation towards Tyr. Under the optimized conditions, the value of the linear sweep voltammetry (LSV) oxidation peak current was linearly related with 0.2~400 μM Tyr, with the detection limit of 0.07 μM (S/N=3). Furthermore, Poly(Gly)/MWCNTs/CPE was applied to the determination of Tyr in diluted serum samples.

Keywords: Tyrosine; Glycine polymer; Multi-walled carbon nanotubes; Electrochemical polymeric deposition; Carbon paste electrode.

1. INTRODUCTION

Tyrosine (4-hydroxyphenylalanine, Tyr) is biochemical compounds which play important roles in various biological processes [1]. Its chemical structure is shown in Fig. 1. Tyr's biosynthesis in mammals occurs by hydroxylation of phenylalanine. And it is also the precursor for catecholamine synthesis (dopamine, norepinephrine, and epinephrine) [2]. Failures in the metabolic pathway of Tyr result in some inherited diseases such as phenilketonuria, tyrosinemia, and tyrosinosis [3]. Thus, Tyr is an essential biomarker which involves in the early detection of various diseases.

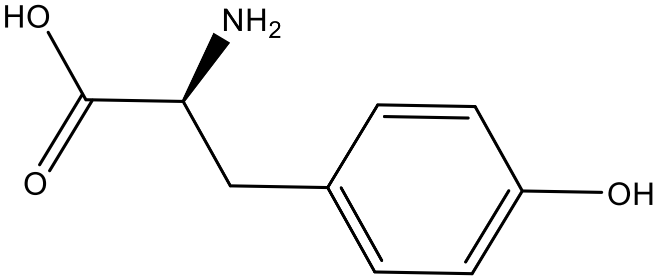


Figure 1. Chemical structure of tyrosine.

Many analytical methods such as chemiluminescence [4], fluorometric detection [5], high performance liquid chromatography [6], capillary electrophoresis [7] and electrochemical detection [8] have been reported for the determination of Tyr. Among them, electrochemical detection are ideal methods for directly and selectively getting information from a complex system, because of its advantages of simple, rapid, sensitive and cost effective [9]. The major factor hinders the electrochemical detection of Tyr is that amino acids have a poor electrochemical response at conventional working electrodes such as Au electrode, Pt electrode, glassy carbon electrode (GCE), graphite electrode and carbon paste electrode (CPE) [10]. Therefore, chemical modifications are employed to improve electrochemical response. The unique structures and properties, such as good electrical conductivity, high electrocatalytic effect, strong adsorptive ability, and low price, make multi-walled carbon nanotubes (MWCNTs) promising material for the task of modifying the electrode [11]. Many studies on fabrication of MWCNTs modified electrode and its application for Tyr determination have been reported [12-13].

Electrochemical polymeric deposition is a well-known technique used to immobilize materials to the modified electrode surface [14]. The thickness, permeation and charge transport characteristics of the polymeric films can be easily and precisely controlled by modulating the electrochemical parameters for various electrochemical techniques, such as chronoamperometry, chronopotentiometry and cyclic voltammetry (CV) [15]. Electrochemical polymeric deposition can provide larger surface area by forming homogeneous film, and it is able to promote electron transfer rates through strong adherence of the polymer to the electrode surface [16-18].

Glycine (2-aminoacetic acid, Gly) is the simplest amino acid, has acid and alkaline functional groups in the molecule. It can be ionized in water and has a strong hydrophilic property. At present, some studies on the electrochemical detection by Gly polymer (Poly(Gly)) modified electrode have been reported [19-30]. However, little research has been done about the electrochemical polymeric deposition of Gly on MWCNTs modified CPE (MWCNTs/CPE) and its application for electrochemical detection [23].

In the present study, we describe the fabrication of Poly(Gly) modified MWCNTs/CPE (Poly(Gly)/MWCNTs/CPE) and the determination of Tyr in human serum employing linear sweep voltammetry (LSV). Poly(Gly)/MWCNTs/CPE has displayed fast electron transfer and prominent electrocatalytic ability to Tyr. The oxidation current response of Tyr was significantly increased at Poly(Gly)/MWCNTs/CPE. Experimental results demonstrate that this proposed assay method has high sensitivity and stability, as well as satisfactory recoveries.

2. EXPERMENT

2.1. Apparatus and chemicals

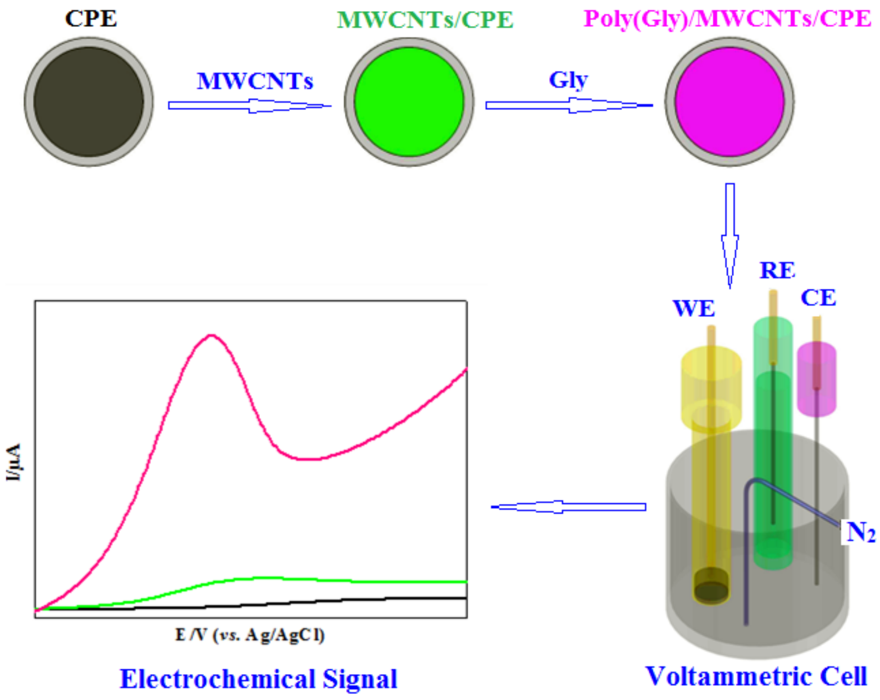
All electrochemical experiments were performed using CHI 660E electrochemical analyzer equipped with data processing software (CH Instrument, Shanghai, China). A conventional three-electrode system was used for all electrochemical experiments. Bare or modified CPE (diameter = 3 mm) was used as working electrode (WE), and platinum wire and Ag/AgCl (sat. KCl) were used as counter electrode (CE) and reference electrode (RE) (Gaoss Union, Wuhan, China) respectively. The pH measurements were performed with pHS-3C digital pH meter (Shanghai instrument electric science instrument, Shanghai, China).

Tyrosine and glycine were obtained from Sigma-aldrich (Shanghai, China). Acetic acid, boric acid, phosphoric acid, graphite powder, potassium ferricyanide, and paraffin oil were obtained from Sinopharm Chemical Reagent (Shanghai, China). Sodium hydroxide, dipotassium hydrogen phosphate, potassium dihydrogen phosphate and potassium chloride were obtained from Tianjin Damao Chemical Reagent (Tianjin, China). Multi-walled carbon nanotubes (> 97% purity) with < 2μm length and 10~20 nm internal diameter were purchased from Nanotech Port (Shenzhen, China). All reagents were analytical grade and used as received otherwise specified statement. All required solutions were prepared using ultra pure water (Millipore, USA).

2.2. Fabrication of bare and modified CPEs

In order to remove the impurities, MWCNTs were pretreated by stirring for 10 h in 3 M HNO_3 at 60 °C then filtered and rinsed to neutral pH value with water, followed by drying in a vacuum oven [31-32]. Carbon paste was prepared by mixing 0.2 g graphite powder and 100 μL paraffin oil in a mortar to produce a homogeneous paste [33]. CPE was fabricated by inserting a portion of the resulting homogeneous paste into the bottom of a polyethylene tube (inside diameter = 3 mm) and polished carefully on a weighing paper. MWCNTs/CPE was fabricated according to the literature method [34]. Briefly, CPE was directly grinded with a suitable amount of pretreated MWCNTs powder, which was tiled on a weighing paper. A thin MWCNTs layer was formed on the CPE surface by intercalating the MWCNTs into the soft carbon paste with the aid of mechanical force and the action of chemical and physical absorption. Prior to the electrochemical experiment, MWCNTS/CPE was rinsed with water.

Poly(Gly)/MWCNTs/CPE was obtained by electrochemical polymeric deposition with CV. MWCNTs/CPE was immerged in a solution containing varied concentration of Gly, using 0.1 M PBS (pH 6.0) as supporting electrolyte. Cyclic scans were executed by applying definite consecutive potential cycles at the potential range of -0.45 to +1.85V (vs. Ag/AgCl), with initial potential of -0.45 V and scan rate of 100 $mV \cdot s^{-1}$. The prepared electrode was rinsed thoroughly with water after the electrochemical processing. All of the prepared electrodes were stored in refrigerator at 4 ◦C when not in use.



Scheme 1. Schematic illustration of Poly(Gly)/MWCNTs/CPE fabrication.

2.3. Electrochemical measurements

Stock solution of Tyr was prepared in Britton-Robinson buffer solution (B.R. buffer solution) (pH 2.0). Tyr solutions with different concentration were prepared by place required quantity of the stock solution into 25 ml volumetric flask and dilute it with buffer solution.

Linear sweep voltammetry (LSV) was carried out by applying potential from +0.7 to +1.1 V (vs. Ag/AgCl) with initial potential of +0.7 V at scan rate of 50 mV·s^{-1} in B.R. buffer solution (pH 2.0) for the electrochemical detection of Tyr at bare/modified CPE. The test solutions were thoroughly purged with nitrogen before experiments. All electrochemical measurements were performed at room temperature.

3. RESULTS AND DISCUSSION

3.1 Preparation and characterization of the Poly(Gly)/MWCNTs/CPE

3.1.1 Electrochemical polymeric deposition of Gly on the surface of MWCNTs/CPE

In this work, Poly(Gly)/MWCNTs/CPE was fabricated by electrochemical polymeric deposition. Carbon paste constituted the basic construction of the electrode, Poly(Gly) and MWCNTs served as electrocatalytic mediators. During the electrochemical polymeric deposition process of Gly, the amount of Poly(Gly) strongly affected the electrocatalytic capability of the modified electrode. So we firstly investigated the CV response of Tyr at the modified electrodes prepared in different concentration of Gly solution. As shown in Fig. 2A and Fig. 2B, CV response at different Poly(Gly)/MWCNTs/CPEs (prepared in 5~25 mM Gly solution) depended on the concentration of Gly solution. Peak current enhanced first with the increase of Gly concentration. The maximum CV response was obtained at the electrode which was prepared in 15 mM Gly solution. When Gly solution concentration was more than 15 mM, the CV response at the fabricated electrode was decreased. This phenomenon may be ascribed to that the excessive amounts of Poly(Gly) not only do not contribute to the improvement of electrocatalytic activity, but also affect the electroactive species access to the electron-conducting network of MWCNTs [32]. So the concentration of 15 mM Gly was used thereafter for Poly(Gly)/MWCNTs/CPE fabrication. As same as the influence of Gly solution concentration on electrochemical polymeric deposition, the CV scan cycles also have influence on the amount of Poly(Gly) deposited on the modified electrodes. Then we examined the CV response of Tyr at the electrodes prepared by different scan cycles. Fig. 2C and Fig. 2D illustrated the variations of peak current at the CV scan cycles range from 6 to 14. The maximum peak current was obtained at the scan cycles of 10. Therefore, the optimum CV scan cycles for Poly(Gly)/MWCNTs/CPE was 10.

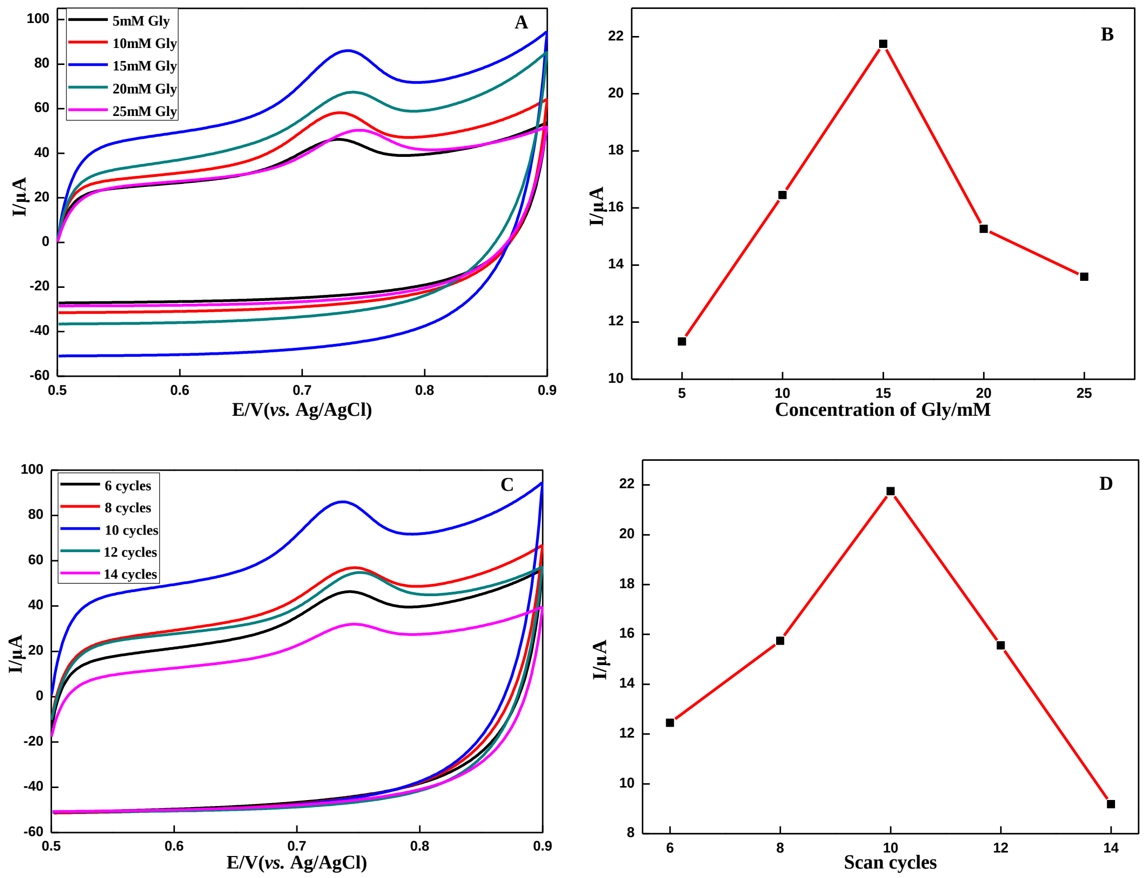


Figure 2. CV response of 40 μM Tyr at different Poly(Gly)/MWCNTs/CPEs (A and B: 10 CV scan cycles, 5~25 mM Gly electrochemical polymeric deposition solution; C and D: 10 mM Gly electrochemical polymeric deposition solution, 6~14 CV scan cycles) in B.R. buffer solution (pH 6.0). Scan rate: 50 mV·s^{-1}.

3.1.2 Electrochemical characterization of the Poly(Gly)/MWCNTs/CPE

To explore the electrochemical performance of Poly(Gly)/MWCNTs/CPE, the redox probe of $K_3[Fe(CN)_6]$ has been studied by CV [34]. As shown in Fig. 3, CV curves of bare CPE, MWCNTs/CPE and Poly(Gly)/MWCNTs/CPE were recorded in 2.0×10^{-4} M $K_3[Fe(CN)_6]$ (0.1 M KCl). Comparing the CV curves, it was found that the peak current ratio (Ipc/Ipa) is equal to 1.01 for Poly(Gly)/MWCNTs/CPE while bare CPE and MWCNTs/CPE exhibit Ipc/Ipa equal to 0.41 and 0.91 respectively. Therefore, Poly(Gly)/MWCNTs/CPE may be claimed to promote electron transfer reaction better than bare CPE and MWCNTs/CPE [34].

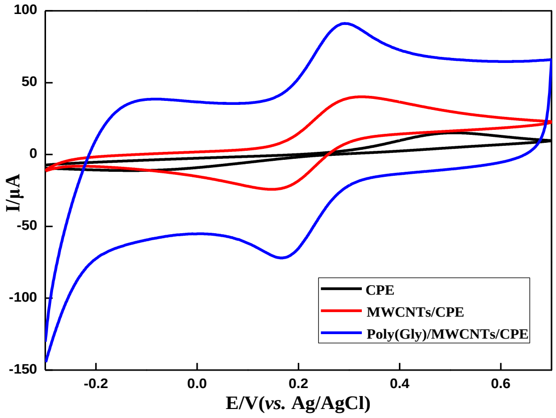


Figure 3. CV curves of bare CPE, MWCNTs/CPE and Poly(Gly)/MWCNTs/CPE in 2.0×10^{-4} M $K_3[Fe(CN)_6]$ (0.1 M KCl). Scan rate: 50 mV·s^{-1}.

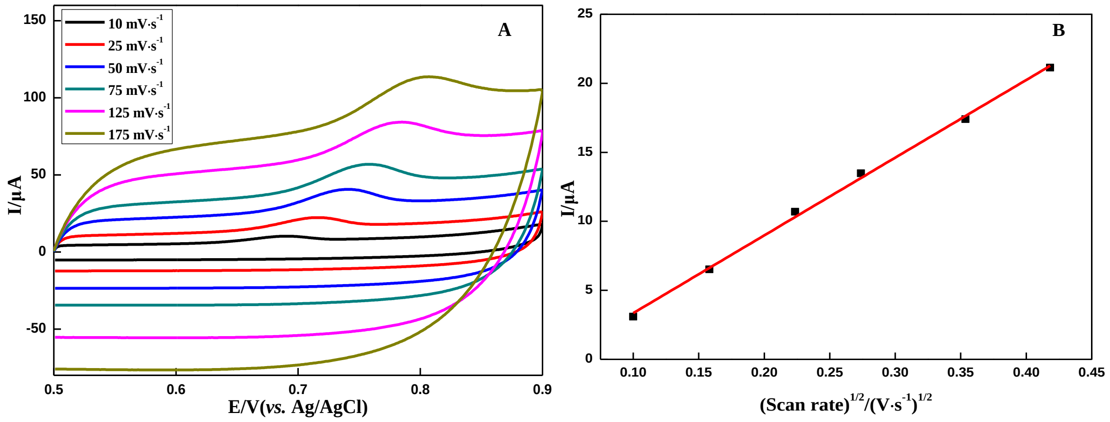


Figure 4. (A) Cyclic voltammograms of 20 μM Tyr at Poly(Gly)/MWCNTs/CPE at different scan rates in B.R. buffer solution (pH 6.0). (B) The linear relationship of current and the square root of the scan rate.

Next, the effect of scan rate upon the electrochemical oxidation behaviors of Tyr was explored in B.R. buffer solution (pH 6.0). As shown in Fig. 4A, cyclic voltammograms of 20 μM Tyr were obtained at different scan rates from 10 to 175 mV·s^{-1}. No reduction peak has been observed in the cyclic voltammograms of Tyr. There was a positive shift of peak potential with the increase of scan rates (Fig. 4B). The analysis of peak height against the square root of the scan rate was found to be linear (Ipa (μA) = $56.299v^{1/2}(mV\cdot s^{-1})^{1/2} - 2.278$ ($R^2 = 0.9982$)). This indicates that the electrochemical reaction of Tyr at Poly(Gly)/MWCNTs/CPE is diffusion controlled process.

3.2 Influence of detection buffer solution pH

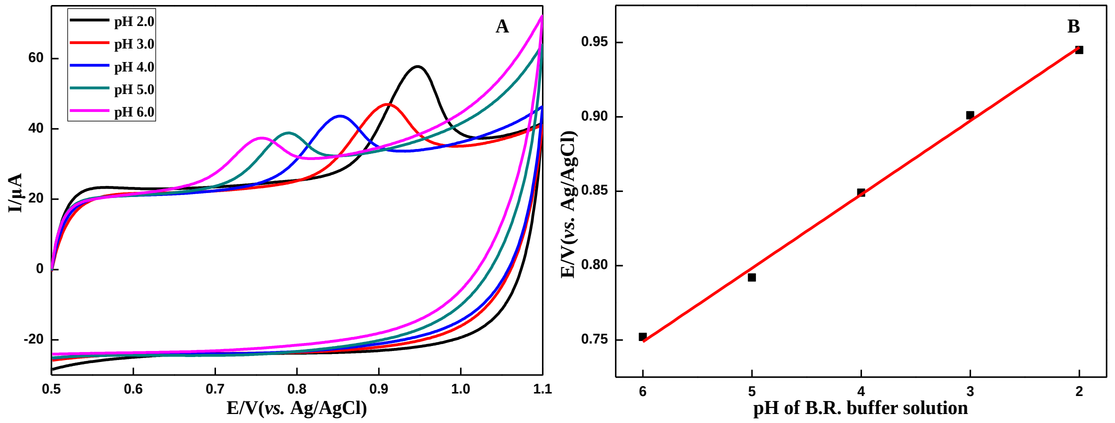
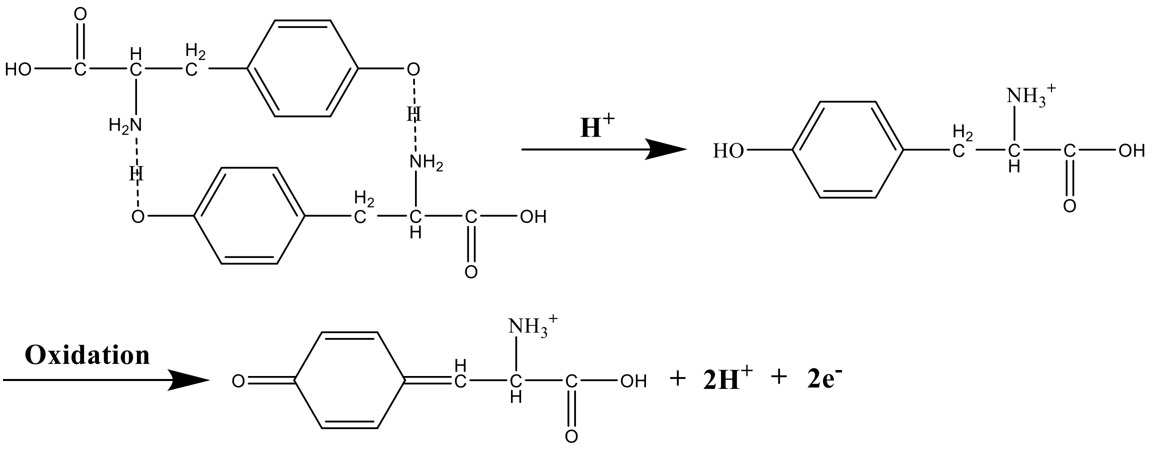


Figure 5. (A) CV curves of 20 μM Tyr at the Poly(Gly)/MWCNTs/CPE in B.R. buffer solution with different pH value; (B) The linear relationship of potential and the pH value of detection buffer solution. Scan rate: 50 mV·s^{-1}.



Scheme 2. Probable oxidation mechanism for Tyr.

The pH value of detection buffer solution is very important for Tyr detection [8]. Therefore, the current response and oxidation potential of Tyr at Poly(Gly)/MWCNTs/CPE were investigated in B.R. buffer solution with different pH value range from 2.0 to 6.0 by CV. As shown in Fig. 5A, by decreasing the pH value, the oxidation peak currents of Tyr increased and the maxi-mum of the oxidation peak current was observed in pH 2.0. This is ascribed to the fact that hydrogen bonds are formed due to the presence of phenolic hydroxyl groups for tyrosine and hydrogen bonds are more easily disrupted and replaced by solvation of water in strong acid solutions. Scheme 2 is the probable oxidation mechanism for Tyr according to the literature [12]. Hence B.R. buffer solution with pH 2.0 was chosen for further experiments. As shown in Fig. 5B, the oxidation potentials of Tyr became more positive upon the decrease of buffer pH value. The relationship of potential and the pH value was linear, and the regression equation was as follows: E_{pa} (V) = 1.0458 – 0.0495 pH (R^2 = 0.9963). The slope of the equation is close to the theoretical Nernstian value of −59 mV·pH^{-1}, and was consistent with a two electron-two proton mechanism for the electrooxidation of Tyr [22].

3.3 Calibration curve and detection limit

LSV was performed for the different concentration of Tyr at Poly(Gly) /MWCNTs/CPE in B.R. buffer solution (pH 2.0) (Fig. 6A). The LSV oxidation peak current and the concentration of Tyr showed a good linear relationship in the range from 0.2 to 70 μM and 70 to 400 μM, the linear regression equations were Ipa (μA) = 1.031c (μM) + 0.723 (R^2 = 0.9987) and Ipa (μA) = 0.106c (μM) + 63.209 (R^2 = 0.9928) respectively (Fig. 6B). The detection limit for Tyr was obtained as 0.07 μM (S/N=3).

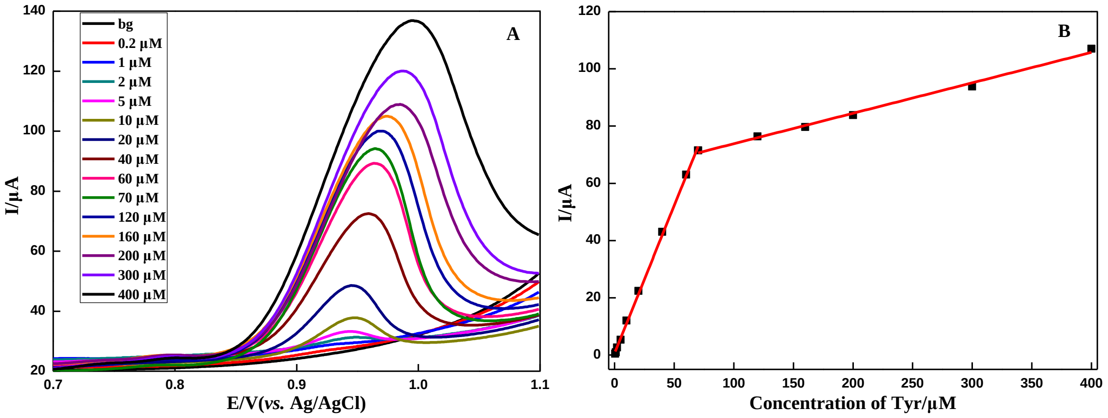


Figure 6. (A) LSV curves of different concentration of Tyr at Poly(Gly)/MWCNTs/CPE in B.R. buffer solution (pH 2.0); (B) The linear relationship of current and the concentration of Tyr. Scan rate: 50 mV·s^{-1}.

3.4 Stability of Poly(Gly)/MWCNTs/CPE

In order to investigate the stability of the fabricated electrode, three Poly(Gly)/MWCNTs/CPEs were prepared individually, and the LSV voltammograms of 20 μM Tyr in B.R. buffer solution (pH 2.0) were recorded for 7 consecutive days. As shown in Fig. 7, although the peak current decreased gradually, it was found to retain 94.1% of its initial peak current response by the end of 7 days. The results indicate a good stability of Poly(Gly)/MWCNTs/CPE.

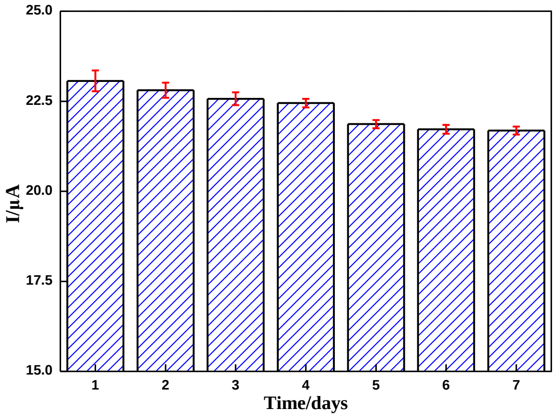


Figure 7. Investigation of the stability of Poly(Gly)/MWCNTs/CPE (n=3).

3.5 Comparison of proposed method with literature methods

A comparison between the analytical performances of the proposed method with previously reported methods for determination of Tyr is shown in Table 1. The data reveals that Poly(Gly)/MWCNTs/CPE has superior analytical performance in terms of wide linear dynamic range and low detection limit over other modified electrodes reported in literatures.

Table 1. Comparison with other modified electrodes for the determination of Tyr.

Modified electrode	Linear range/(μM)	LOD/(μM)	Method	Reference
EuHCF film	10~600	8	CV	[35]
MWCNTs-GNS/GCE	0.90~95	0.19	CV	[8]
ERGO	0.5~80.0	0.2	DPV	[36]
AuNPs/MWCNTs/GCE	0.4~80	0.2	DPV	[12]
AuNPs/poly(trisamine)/GCE	3.9~61.8	0.9	DPV	[37]
AuNPs/PTAT/GCE	10~560	2	DPV	[38]
MW-FEs	25~750	8	DPV	[39]
PbO_2/CCE	5~1458	0.77	Amperometry	[40]
Poly(Gly)/MWCNTs/CPE	0.2~400	0.07	LSV	This work

3.6 Analytical applications

The fabricated Poly(Gly)/MWCNTs/CPE was further applied to the determination of Tyr in human serum. Human blood obtained from volunteers was centrifuged at 15000 rpm for 10 min to separate serum. The serum was diluted 10 times with B.R. buffer solution (pH 2.0) without any pretreatment. The samples were spiked with known amounts of Tyr and measured by LSV as described before. As shown in Table 2, the quantitative recoveries for Tyr ranged from 98.6% to 99.5% with the RSDs were lower than 4.2%. This suggests that the accuracy and selectivity of the developed method were satisfactory.

Table 2. Analytical results for Tyr in diluted serum samples (n=5).

Method	Added/(μM)	Determined/(μM)	Recovery/%	RSD/%
LSV	10	9.86	98.6	4.2
	20	19.84	99.2	3.7
	50	49.76	99.5	3.4

4. CONCLUSION

In this work, Poly(Gly)/MWCNTs modified CPE was fabricated by electrochemical polymeric deposition of Gly and applied to the determination of Tyr. The fabricated electrode shows excellent electrocatalytic activity, large linear dynamic range and low detection limit. What's more, the test results of Tyr at Poly(Gly)/MWCNTs/CPE in mixture solution formed by human serum sample demonstrated the high accuracy and selectivity of electrode. Therefore, the developed method is feasible for Tyr detection in practical samples.

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