

Electrocatalytic oxidation of L-tyrosine at carboxylic acid functionalized multi-walled carbon nanotubes modified carbon paste electrode

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Abstract The electrocatalytic oxidation of L-tyrosine (Tyr) was investigated on a carboxylic acid functionalised multi-walled carbon nanotubes modified carbon paste electrode using cyclic voltammetry and amperometry. The surface morphology of the electrodes was studied using field emission (FE)-SEM images, and the interface properties of bare and modified electrodes were investigated by electrochemical impedance spectroscopy (EIS). The influence of the amount of modifier loading and the variation of the pH of the solution on the electrochemical parameters have been investigated. Cyclic voltammetry was carried out to study the electrochemical oxidation mechanism of Tyr, which showed an irreversible oxidation process at a potential of 637.0 mV at modified electrode. The anodic peak current linearly increased with the scan rate, suggesting that the oxidation of Tyr at modified electrode is an adsorption-controlled process. A good linear relationship

between the oxidation peak current and the Tyr concentration in the range of 0.8–100.0 μM was obtained in a phosphate buffer solution at pH 7.0 with a detection limit of 14.0 ± 1.36 nM ($S/N=3$). The practical utility of the sensor was demonstrated by determining Tyr in spiked cow's milk and human blood serum. The modified electrode showed excellent reproducibility, long-term stability and antifouling effects.

Keywords L-Tyrosine · Carboxylic acid · Multi-walled carbon nanotubes · Bulk modification · Carbon paste electrode · Electrocatalytic oxidation

Introduction

Carbon nanotubes (CNTs) are one of the important allotropes of carbon and were first discovered by Iijima in 1991 [1]. It is a one-dimensional nanomaterial with excellent electrical properties, high surface-to-volume ratio, high chemical stability and biocompatibility. These properties enable them to be used as modifiers in the development of sensors [2]. The electrocatalytic nature of CNTs helps in promoting the electron transfer in reactions involving biologically significant molecules such as neurotransmitters, glucose and NADPH [3–6]. Functionalization of CNTs is necessary to prevent their aggregation which results in tampering of their unique properties. Carboxylic acid incorporation on CNTs is the most common method of functionalizing CNTs to prevent them from agglomeration. Carboxylated group increases the hydrophilicity of CNTs, so that they can be biocompatible in nature [7]. Carboxylic acid functionalized CNTs (COOH-CNTs) improve the interfacial interactions between the electrode surface and solution. Various types of COOH-CNTs modified electrodes, for instance, COOH-multi-walled CNTs (MWCNTs)-polymer-gelatin composite biosensor, COOH-single-walled CNTs (SWCNTs)-surfactant electrode and a

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polymer-COOH-MWCNTs composite gold electrode, have been used in the electrochemical analysis of some important compounds [8–11].

Amino acids are biologically significant organic compounds which play a vital role as building blocks of protein and are used as a source of energy in the human body. Tyrosine (Tyr) is an aromatic proteinogenic amino acid, which acts as a precursor to various neurotransmitters, namely L-dopa, dopamine, epinephrine and norepinephrine. These neurotransmitters are essential to maintain everyday life and functions [12]. Tyr is considered a non-essential amino acid because it is synthesized in the human body by another amino acid, phenylalanine [13]. Tyr imbalance causes Parkinson's disease, insomnia and other sleep disorders [14]. Tyr is necessary for the production of the thyroid hormones and is a major chemical component for the synthesis of melanin [15]. Since Tyr is a vital amino acid in human biological systems, it is necessary to determine its presence in dietary products and pharmaceuticals. Numerous methods have been employed for the detection of Tyr including spectrophotometry [16], chromatography [17] and fluorimetry [18]. But these methods involve expensive instrumentation, large material consumption, sample treatment and suffer from reaching lower detection limit. Therefore, there is a demand for a simple, fast, convenient and sensitive method to solve these difficulties. Electrochemical methods offer a convenient way of analysing biologically important molecules and environmentally significant samples. Biologically significant molecules like nifedipine, levodopa, methyl dopa and tiopronin are analysed using various modified electrodes [19–22]. Since Tyr is an electro-active species, electrochemical method is employed for its analysis. This method provides a simple, sensitive, accurate and fast way of analysing biomolecules in real samples. Tyr generally shows sluggish electron transfer at unmodified electrodes. Thus, it is desirable to modify the electrode with suitable materials which are electrocatalytic in nature in order to prevent high oxidation overpotential of Tyr.

Various modified electrodes are reported in the literature for the detection of Tyr. A biosensor based on hemin immobilized reduced graphene oxide nanosheets on glassy carbon electrode, iron (III) doped zeolite modified carbon paste electrode, amperometric sensor based on tetroxide nanoparticles-graphene nanocomposite film modified glassy carbon electrode, chiral electrochemical sensor modified with cysteic acid and gold nanoparticles decorated graphene oxide glassy carbon electrode [23–27] are employed for the sensitive determination of Tyr. There is a report on the analysis of Tyr using SWCNTs modified glassy carbon electrode (GCE) [28], though the GCE requires pretreatment and Tyr suffers from oxidation overpotential at SWCNTs/GCE apart from it not being applied for real sample analysis directly. Moreover, the analysis is carried out at the physiological pH and the detection limit of Tyr is better than observed at SWCNTs/GCE. We report an

electrochemical oxidation characterized by lower activation energy at the carboxylic acid functionalised multi-walled carbon nanotubes modified carbon paste electrode.

Ever since their introduction by Ralph N. Adams in 1958, the carbon paste electrodes (CPE) have undergone tremendous progress as (bio)sensors in the electrochemical analysis of various biologically and environmentally significant analytes. Due to their beneficial physicochemical and electrochemical properties, CPEs are widely used for voltammetric, amperometric, coulometric and potentiometric measurements [29]. CPEs are still popular as (bio)sensors because of their unique characteristics such as ease of preparation, cost-effectiveness, wide potential window, low background current and biocompatibility [30]. Bulk modification involves the simplest fabrication technique in the modification of CPEs which can be achieved without any sophistication.

In our continual effort to employ CPE and modified CPE for electroanalytical applications [31–38], in the present work, our aim was to fabricate a non-enzymatic sensor for Tyr. To achieve this, we have modified CPE by bulk modifying it using COOH-MWCNTs in the CPE matrix and employed it for the sensitive determination of Tyr in the presence of interfering molecules. In addition, its analytical applicability as a sensor for the determination of Tyr in real sample has been validated. Though there are many reports on analysis of Tyr at various electrodes, these methods involve complicated procedures of modification. Hence, a simple, rapid and cost-effective method for fabrication of the sensor is desired. To overcome a sluggish electron transfer rate at CPE, we have incorporated electrocatalytic property of COOH-MWCNTs into the CPE matrix.

Materials and methods

Reagents

L-Tyrosine, uric acid (UA) (sourced from SRL), dopamine (DA) (sourced from Sigma-Aldrich), 4-aminophenol (4-AP) (sourced from Loba Chemie), KH_2PO_4 , NaOH pellets, H_3PO_4 , HClO_4 and silicone oil were bought from Merck. All the reagents were of analytical grade and were used without further purification. All aqueous solutions were prepared using ultrapure water ($>18.2 \text{ M}\Omega\text{-cm}$) from Milli-Q Plus system (Millipore). The stock solution of DA was prepared in 0.1 M HClO_4 while those of Tyr, UA and 4-AP were prepared in 0.1 M NaOH. The phosphate buffer solutions (PBS) were prepared from KH_2PO_4 and NaOH while the pH was adjusted using H_3PO_4 and NaOH. The graphite powder was obtained from Graphite India Ltd., and COOH-MWCNTs were prepared as discussed in “Generation of carboxylic acid functionalities on MWCNTs using diazonium grafting”.

Apparatus

All the electrochemical experiments were performed using EA-201 Electro Analyzer (ChemiLink). A conventional three-electrode system was used for all the electrochemical experiments, which comprised a bare or modified CPE as the working electrode and a platinum wire as auxiliary electrode. The potentials were measured and applied using saturated calomel electrode (SCE) as a reference electrode. The tip of Luggin capillary was set approximately at a distance of 1 mm from the surface of the working electrode both bare and modified CPE, in order to minimize error due to IR drop in the electrolyte. Except for hydrodynamic amperometry measurements, all other voltammetry experiments were performed in quiescent solutions. All the voltammetry measurements were recorded at room temperature (~300 K). The charge transfer resistance value of the electrode was obtained by performing electrochemical impedance spectroscopy using CHI 660E (CH Instruments Inc.) electrochemical workstation. The surface morphology of the electrodes was studied using field emission (FE)-SEM using Quanta 200, FEI, Germany; SUPRA 40 VP, Gemini, Zeiss, Germany. A digital pH/mV meter (ELICO LI 614) was employed to measure the pH of the prepared buffer solutions.

Generation of carboxylic acid functionalities on MWCNTs using diazonium grafting

Of the NC7000 MWCNTs, 2.4 g is added to a mixture of 11.0 g of 4-aminobenzoic acid, 5.52 g of sodium nitrite and 8.24 ml of perchloric acid (70 %) in 1.2 l of water. Under these conditions, the aniline is transformed into diazonium salt which can be grafted onto the MWCNTs' surface. The solution is stirred for 2 h at 50 °C. The resulting compound is washed with deionized water, acetone, dichloromethane and finally pentane in order to remove the by-products.

Preparation of bare and the modified electrode

After optimization of the ratio of graphite powder to binder, the CPE was prepared by thoroughly hand-mixing the graphite powder and silicone oil in the ratio 70:30 (w/w) in an agate mortar using a pestle to obtain a homogeneous paste. A portion of the resulting homogeneous paste was packed into the cave of the Teflon tube. A copper wire fixed to a graphite rod and inserted into the Teflon tube served to establish electrical contact with the external circuit. Similarly, COOH-MWCNTs-modified CPE was prepared by bulk modification method by mixing carbon paste with COOH-MWCNTs in the ratio of 90:10 (w/w). The modified electrode was designated as modified carbon paste electrode (MCPE)/COOH-MWCNTs. For every reading, the surface was renewed by removing some amount of paste, fresh paste was filled and the surface was smoothened.

Results and discussion

Characterization of electrodes by (FE)-SEM

Comparison of morphological characteristics of the surfaces of CPE and MCPE/COOH-MWCNTs was done by (FE)-SEM. Figure 1a, b illustrates the (FE)-SEM images of CPE and MCPE/COOH-MWCNTs, respectively. In Fig. 1a, flakes of graphite are seen on the surface of CPE. It is obvious from Fig. 1b that the COOH-MWCNTs are homogeneously mixed into the CPE matrix and are exposed on the surface of MCPE.

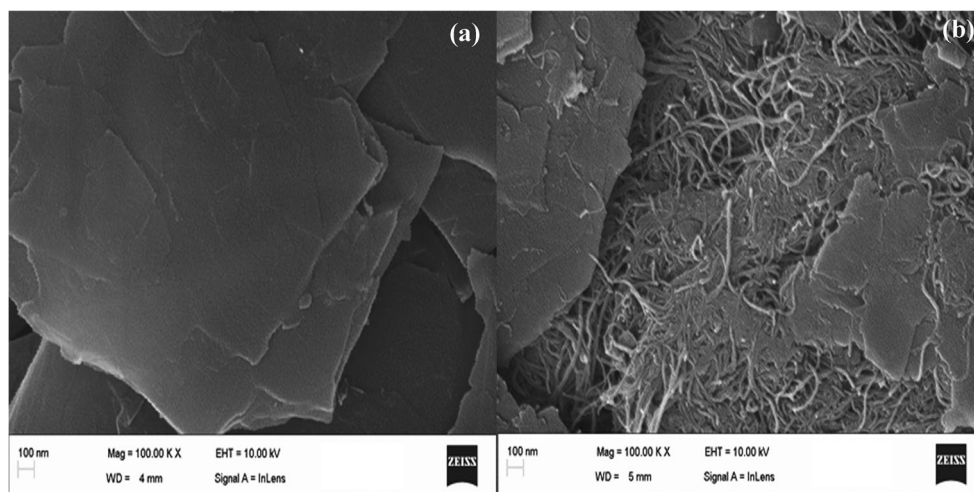
Characterization of the electrodes by electrochemical impedance spectroscopy

Electrochemical impedance spectroscopy (EIS) is an efficient tool to study the properties of electrode/solution interface. Figure 2 illustrates the Nyquist plot of CPE and MCPE/COOH-MWCNTs. The EIS data of the electrodes were obtained in AC frequency range varying from 1.0 Hz to 1 MHz with a current voltage amplitude of 5 mV at the oxidation peak potential (E_{pa}) of Tyr in 0.1 M phosphate buffer solution of pH 7.0. The impedance spectrum consists of a semicircular portion at higher frequencies which corresponds to the electron transfer limiting process, and the other linear part at lower frequencies results from the diffusion limiting step of the electrochemical process. The diameter of the semicircle represents the charge transfer resistance (R_{ct}). The components of equivalent circuit are correlated to the resistance offered by supporting electrolyte (R_s), the charge transfer resistance (R_{ct}), the double layer capacitance (C_{dl}), the Warburg impedance resulting from the diffusion of ions from bulk solution to the electrode surface (W) and resistance due to double layer capacitance (R_{dl}). The R_{ct} values were obtained from the equivalent electrical circuit which matched well with impedance spectra. The R_{ct} values of 5.0×10^{-4} M Tyr at CPE and MCPE/COOH-MWCNTs were 261.6 and 186.3 Ω , respectively. The lower charge transfer resistance at MCPE/COOH-MWCNTs implies an effective increase in the active area of the electrode, higher charge transfer kinetics as well as the electrocatalytic activity of the sensor. Thus, the modification of CPE enhances the electrochemical response for Tyr. The impedance experiments further supported the results obtained by cyclic voltammetry (CV) experiments. A commercial saturated calomel electrode and platinum wire were used as reference and counter electrode, respectively.

Electrochemical behaviour of Tyr

The electrochemical behaviour of Tyr was studied at different electrodes using the CV. Figure 3 represents the cyclic voltammograms of 5.0×10^{-4} M Tyr in 0.1 M phosphate buffer solution of pH 7.0 at CPE (---), MCPE/MWCNTs (- · - · -) and

Fig. 1 (FE)-SEM images of **a** CPE and **b** MCPE/COOH-MWCNTs



MCPE/COOH-MWCNTs (—). Only an anodic peak was observed at both electrodes when the potential was scanned from 0 to 1000 mV. The oxidation process was not accompanied by a reduction wave, which implies that the oxidation reaction of Tyr is totally irreversible. The anodic peak potentials (E_{pa}) of Tyr at CPE, MCPE/MWCNTs and MCPE/COOH-MWCNTs were 683.0 ± 1.8 mV, 649.0 ± 2.3 mV and 637.0 ± 2.6 mV, respectively. The anodic peak currents (I_{pa}) of Tyr observed at CPE, MCPE/MWCNTs and MCPE/COOH-MWCNTs were 29.6 ± 4.3 μ A, 30.10 ± 1.4 μ A and 67.6 ± 1.9 μ A, respectively. It is evident from the cyclic voltammogram that the presence of carboxylated MWCNTs increases the current sensitivity of electrode by 2.3 times with 46.0 mV of less positive shift in E_{pa} compared to CPE. This enhancement in current and reduction in overpotential of Tyr oxidation is attributed to the large surface area of MCPE/COOH-MWCNTs. Such changes in voltammetric characteristics of Tyr indicate catalytic effect of COOH-MWCNTs and offer an increased level of mass transport which is in agreement with the data reported in the literature for glutamate biosensor and other phenolic compounds [39, 40].

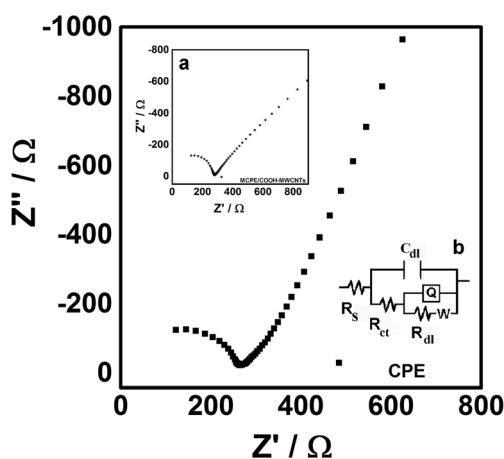


Fig. 2 Nyquist plot of 5.0×10^{-4} M Tyr in 0.1 M PBS of pH 7.0 at CPE. Inset a Nyquist plot of 5.0×10^{-4} M Tyr in 0.1 M PBS of pH 7.0 at MCPE/COOH-MWCNTs and inset b equivalent circuit

Additionally, electrocatalytic effect of COOH-MWCNTs is caused by the presence of oxygen functionalities on MWCNTs as a result of carboxylation of MWCNTs.

Effect of scan rate

The effect of variation of scan rate on the electrochemical parameter of Tyr was studied using 5.0×10^{-4} M Tyr in 0.1 M PBS of pH 7.0. Cyclic voltammograms of Tyr at various scan rates ranging between 25 and 150 mV s^{-1} in 0.1 M PBS of pH 7.0 at MCPE/COOH-MWCNTs are represented in Fig. S1. The I_{pa} of Tyr was found to vary linearly with the scan rates as shown. The linear regression equation for the variation I_{pa} with scan rate is given by I_{pa} (μ A) = $29.54 + 0.494 v$ (mV s^{-1}) with squared correlation coefficient $R^2 = 0.9934$. This indicates that the electrode reaction of Tyr at MCPE/COOH-MWCNTs is controlled by the adsorption process [41].

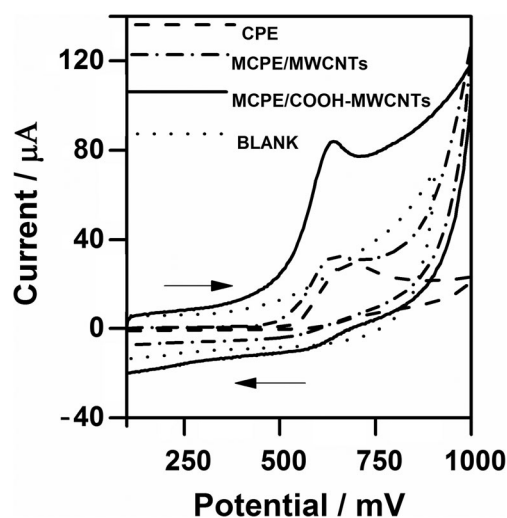


Fig. 3 Cyclic voltammograms of 5.0×10^{-4} M Tyr in 0.1 M PBS of pH 7.0 at CPE (---), MCPE-MWCNTs (- · - · -) and MCPE/COOH-MWCNTs (—) at a scan rate of 50 mV s^{-1} . Dotted line represents blank

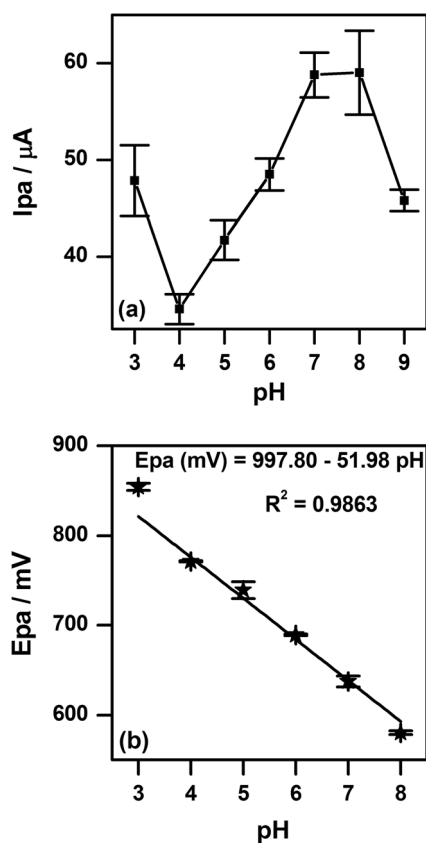


Fig. 4 Plot of variation of **a** I_{pa} and **b** E_{pa} of 5.0×10^{-4} M Tyr in 0.1 M PBS of different pH at MCPE/COOH-MWCNTs at a scan rate 50 mV s^{-1}

Effect of pH on the supporting electrolyte

Amino acid can have several forms depending on the solution pH. In the acid medium, the amino group gets protonated by the addition of the proton (H^+) from the acid and hence has a positive charge. In the basic medium, the carboxylic acid ($-\text{COOH}$) gets deprotonated by the removal of a proton and gets a negative charge.

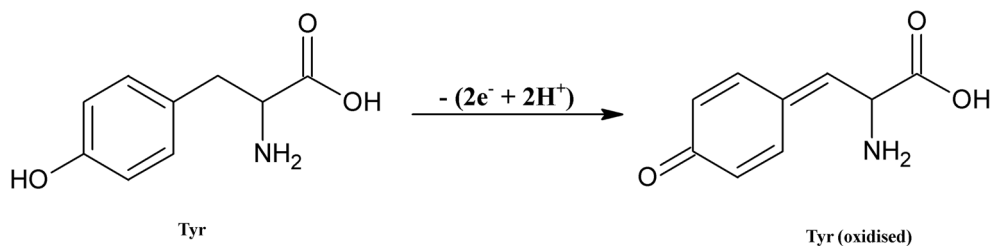
Since the pH plays a vital role, the effect of variation of pH on E_{pa} and I_{pa} of 5.0×10^{-4} M Tyr in 0.1 M PBS was investigated using CV at a scan rate of 50 mV s^{-1} . The pH was adjusted in the range between 3.0 and 9.0 using H_3PO_4 or NaOH. Figure S2 represents the cyclic voltammograms of Tyr at MCPE/COOH-MWCNTs recorded at various pH. The variation of I_{pa} and E_{pa} with pH are displayed in Fig. 4a, b, respectively. The peak current

decreased with an increase in pH from 3.0 to 4.0 on account of decreased electrostatic attraction between the electrode surface and the molecule; above pH 4.0 current raised to maximum value till pH 8.0, and thereafter, it decreased. At solution pH of 3.0 (close to $\text{pK}_{a1}=2.20$), Tyr acquires a net positive charge due to the protonation of the amino group. In alkaline pH from 8.0 to 9.0 (close to $\text{pK}_{a2}=9.11$), carboxylic acid groups of both Tyr and electrode surface undergo deprotonation to acquire net negative charge. I_{pa} at pH 7.0 and 8.0 was almost the same, but on account of the advantage of carrying out the study in physiological pH, we chose pH 7.0 as an optimum pH value for the electrochemical detection of Tyr. The maximum current response observed at pH 7.0 was possibly due to the π - π stacking between aromatic rings of Tyr and COOH-MWCNTs. Also, the formation of hydrogen bonding between the hydroxyl hydrogen of Tyr and oxygen functionalities of COOH-MWCNTs contributes to the rise in current. At pH 7.0, most of Tyr is neutral in nature and the electrostatic force of attraction between Tyr and electrode surface is ruled out. As depicted in Fig. 4b, the E_{pa} of Tyr was observed to shift linearly towards negative potential with increase in pH, which can be described by linear regression equation $E_{pa} \text{ (mV)} = 997.80 - 51.98 \text{ pH}$, with a coefficient of determination, $R^2 = 0.9863$. A slope of $-51.98 \text{ (mV pH}^{-1})$ implies involvement of equal number of protons and electrons in redox reaction of Tyr. Hence, the overall oxidation process of Tyr involves two electrons and two protons as shown in Scheme 1. Our results are consistent with the mechanism reported in the literature [42].

Effect of variation of COOH-MWCNTs loading in the carbon paste matrix

The amount of modifier is also one of the parameters which affect the current sensitivity. Figure S3 shows the effect of loading different weight of the modifier COOH-MWCNTs on the anodic peak current. The weight of COOH-MWCNTs with respect to weight of graphite powder is varied from 1.0 to 8.0 mg. It is evident from Fig. S3 that, with an increase in the weight of COOH-MWCNTs, the anodic peak current increased and attained a maximum value at 6.0 mg. This may be ascribed to an increase in both, the surface area as well as

Scheme 1 Electrochemical oxidation of Tyr



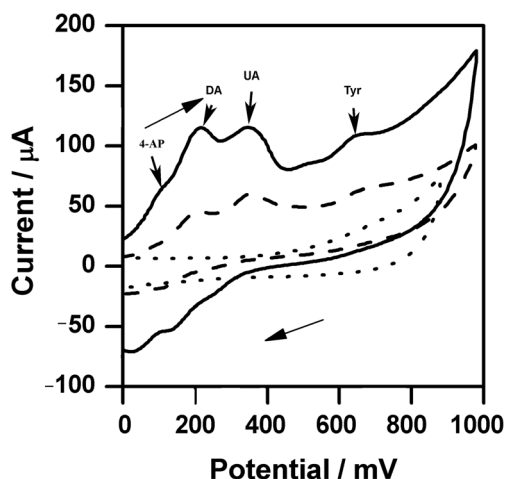


Fig. 5 Cyclic voltammograms of a mixture of 4-AP (2×10^{-3} M), DA, UA and Tyr (1×10^{-3} M) at CPE (---) and at MCPE/COOH-MWCNTs (—) in 0.1 M PBS of pH 7.0 at a scan rate of 50 mV s^{-1} . Dotted line represents blank

the catalytic sites with an increase in the weight of COOH-MWCNTs. The I_{pa} was decreased and finally found to remain constant beyond a loading of 6.0 mg of COOH-MWCNTs, which could be possibly due to the saturation reached at the surface of the electrode. So, an optimum of 6.0 mg weight of COOH-MWCNTs was used for further experiments since it gave the best response.

Electrochemical behaviour of a mixture of 4-AP, DA, UA and Tyr at MCPE/COOH-MWCNTs

In order to evaluate the selectivity of the proposed electrochemical sensor in the determination of Tyr, its determination was carried out in the presence of common and biologically important molecules using CV. Figure 5 depicts the cyclic

voltammograms of 2.0×10^{-3} M 4-AP and 1.0×10^{-3} M each of DA, UA and Tyr in 0.1 M PBS of pH 7.0 at CPE and MCPE/COOH-MWCNTs. The results illustrate the failure of CPE (---) to distinguish the responses of different molecules with adequate sensitivity and selectivity. In addition, peaks of 4-AP and DA are merged at CPE whereas MCPE/COOH-MWCNTs is able to distinguish them. Peak separations of 499.0, 401.0 and 276.0 mV were observed for Tyr-4-AP, Tyr-DA and Tyr-UA, respectively. Improved value of measured current evidently suggests an electrocatalytic activity at the modified electrode surface. The peak potentials of each of the analyte mixtures remains the same as when studied independently, indicating that the reactions of analytes at the electrode surface are not affected in the presence of each other. Hence, it can be concluded that none of the three biological molecules interfere in the determination of Tyr. Hence, MCPE/COOH-MWCNTs can be safely used for the selective determination of Tyr.

Amperometric determination of Tyr

The calibration curve and the detection limit of Tyr in 0.1 M PBS of pH 7.0 was determined using amperometry technique under hydrodynamic conditions. The advantage of hydrodynamic conditions is the enhancement of mass transport towards the surface of the electrode which accounts for the higher sensitivity and enhanced current. Figure 6a shows the amperometric responses of various concentrations of Tyr at MCPE/COOH-MWCNTs. Aliquots of Tyr were added to the buffer solution under hydrodynamic conditions, and MCPE/COOH-MWCNTs gave quick responses. The responses attained stability in 6.0 s.

The corresponding calibration plot of I_{pa} against various concentrations of Tyr is represented in Fig. 6b. The least

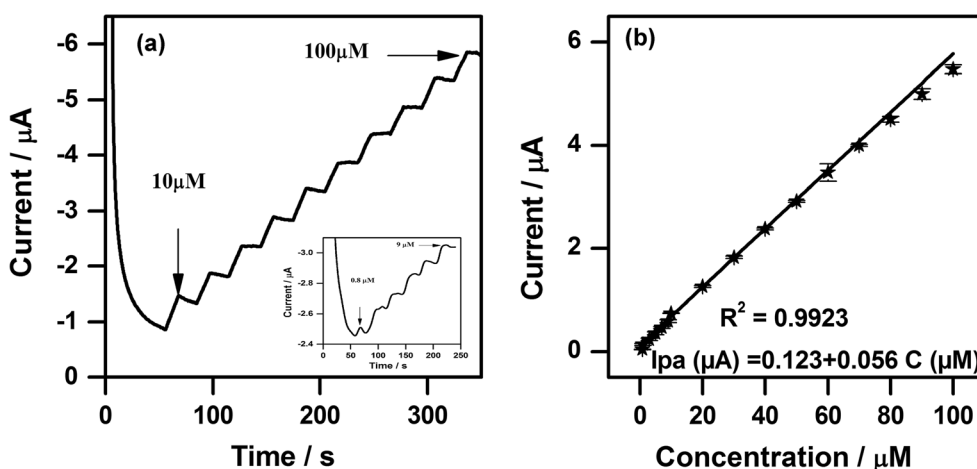


Fig. 6 a Amperometric current response of Tyr in the concentration range 10.0 to 100.0 μM Tyr at MCPE/COOH-MWCNTs in 0.1 M PBS of pH 7.0 under hydrodynamic conditions. Applied potential 640.0 mV. *Inset* shows the amperometric current response of Tyr in concentration

range of 0.8 to 9.0 μM . **b** Calibration plot of I_{pa} versus concentration of Tyr from 0.8 to 100.0 μM at MCPE/COOH-MWCNTs in 0.1 M PBS of pH 7.0. *Inset* shows the calibration plot of I_{pa} versus concentration of Tyr from 10.0 to 100.0 μM

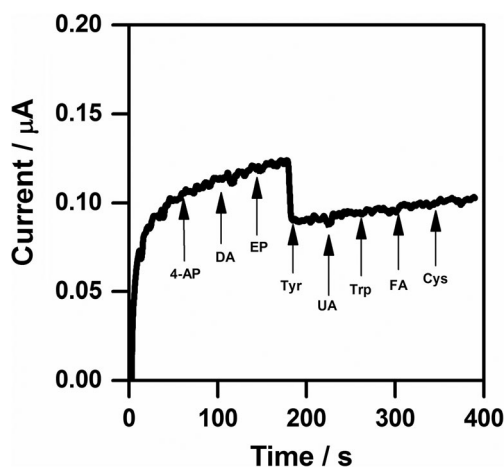


Fig. 7 Amperometric response for the addition of Tyr in the presence of various interferents at MCPE/COOH-MWCNTs in 0.1 M PBS of pH 7.0. Applied potential 640.0 mV

square treatment of the calibration graph yielded the following regression equation: $I_{pa} (\mu A) = 0.123 + 0.056 C (\mu M)$ with coefficient of regression 0.9923 and a sensitivity of 0.056 AM^{-1} for the linear dynamic range of 0.8–100.0 μM . The detection limit for Tyr was found to be $(14.0 \pm 1.36) \times 10^{-9} \text{ M}$ at a signal-to-noise ratio of 3.

To assess its application in the biomedical field, the response of MCPE/COOH-MWCNTs in the presence of many interferents such as 4-AP, DA, UA, epinephrine (EP), L-tryptophan (Trp), folic acid (FA) and L-cysteine (Cys) was investigated. Figure 7 shows the amperometric response obtained for Tyr at MCPE/COOH-MWCNTs in the presence of several interferents in 0.1 M PBS of pH 7.0. The results indicate that the determination of 4.5 μM of Tyr is possible in the presence of 15-fold excess of Trp, FA and Cys, 6-fold excess of UA, 2-fold excess of 4-AP while EP and DA were not detected up to 6.5 and 8.0 μM of concentrations. This suggests MCPE/

Table 2 Determination of Tyr in real samples using MCPE/COOH-MWCNTs

Real sample	Tyr added (μM)	Tyr found (μM)	Recovery (%)
Blood serum	3.0	2.9 ± 0.13	99.9
	5.0	5.2 ± 0.09	104.6
	7.0	7.02 ± 0.06	100.4
Milk	4.0	4.1 ± 0.02	101.6
	6.0	5.7 ± 0.80	95.5
	7.0	6.8 ± 0.31	97.1

COOH-MWCNTs are highly selective towards Tyr and completely free from interference of all the abovementioned molecules.

Stability and reproducibility of the sensor

The amperometric response of $3.0 \times 10^{-6} \text{ M}$ Tyr at MCPE/COOH-MWCNTs is shown in Fig. S4. A constant amperometric response was achieved for 20 min. This suggests that the MCPE/COOH-MWCNTs are stable and the electrode is free from the antifouling effects. The long-term stability of the electrode was analysed by storing it in a dry place for 1 week. The cyclic voltammetric response of $1.0 \times 10^{-5} \text{ M}$ Tyr at MCPE/COOH-MWCNTs retained 93.1 % of its initial activity.

The reproducibility of the results is an important parameter to assess analytical performance of a sensor. The MCPE/COOH-MWCNTs were prepared separately five times following the same procedure and the cyclic voltammetric responses of $1.0 \times 10^{-5} \text{ M}$ Tyr were recorded. All the electrodes exhibited similar responses and the relative standard deviation (RSD) was 3.86 %. Therefore, MCPE/COOH-MWCNTs possess good reproducibility.

Table 1 Comparison of major characteristics at different electrodes for determination of Tyr

Electrode	pH	Linear dynamic range (M)	Detection limit (M)	Technique used	Reference
CPE/MWCNT	3.0	$4.0 \times 10^{-7} - 1.0 \times 10^{-4}$	1.0×10^{-7}	DPV	[43]
GNS/GCE	3.0	$5.0 \times 10^{-6} - 1.2 \times 10^{-4}$	2.0×10^{-8}	DPV	[44]
Nano-Au/CA/GCE	7.0	$1.0 \times 10^{-7} - 3.0 \times 10^{-4}$	4.0×10^{-8}	Amperometry	[45]
MWCNTs-modified GCE	7.0	$2.0 \times 10^{-6} - 5.0 \times 10^{-4}$	4.0×10^{-7}	SWSV	[46]
Fe-HA/tyrosinase/GCE	7.0	$1.0 \times 10^{-7} - 1.0 \times 10^{-5}$	2.4×10^{-7}	DPV	[47]
Nafion/TiO ₂ -GR/GCE	6.0	$1.0 \times 10^{-5} - 1.6 \times 10^{-4}$	2.3×10^{-6}	DPV	[48]
SWCNH modified GCE	2.0	$2.0 \times 10^{-6} - 3.0 \times 10^{-5}$	4.0×10^{-7}	LSV	[49]
AuNPs/poly(trisamine)/GCE	7.0	$3.9 \times 10^{-6} - 6.2 \times 10^{-5}$	9.0×10^{-7}	DPV	[50]
MCPE/COOH-MWCNTs	7.0	$3.0 \times 10^{-7} - 1.0 \times 10^{-3}$	1.4×10^{-8}	Amperometry	Present work

GNS graphene nanosheets, CA electrochemically pretreated cysteamine and functionalised with gold nanoparticles, Tyrosinase Fe-doped hydroxyapatite nanoparticles, Nafion/TiO₂-GR Nafion and TiO₂-graphene nanocomposite, SWCNH single-walled carbon nanohorns, AuNPs/poly(trisamine) Au-nanoparticles/poly-(2-amino-2-hydroxymethyl-propane-1,3-diol) film, DPV differential pulse voltammetry, LSV linear sweep voltammetry, SWSV square wave stripping voltammetry

Comparison of analytical performance of MCPE/COOH-MWCNTs with other electrodes

The analytical performance of MCPE/COOH-MWCNTs has been compared with other reported electrodes and the results are displayed in Table 1. An acidic medium of pH 3.0 is used to carry out electrochemical analysis of Tyr in biological samples at CPE-MWCNT and graphene nanosheet (GNS)/GCE [43, 44]. Moreover, GNS/GCE suffers high Tyr oxidation overpotential of 213.0 mV compared to MCPE/COOH-MWCNTs. Pretreatment of glassy carbon electrode required in the case of nano-Au/CA/GCE and MWCNTs-modified GCE [45, 46], and the latter requires time-consuming preparation. No analytical applications were performed at Fe-HA/tyrosinase modified GCE and Nafion/TiO₂-GR/GCE [47, 48]. MCPE/COOH-MWCNTs show better detection limit and wider linear dynamic range for Tyr determination compared to SWCNH modified GCE and AuNPs/poly (trisamine)/GCE [49, 50]. Compared with most of the aforementioned modified electrodes, a much better current sensitivity and wide linear dynamic range is achieved at MCPE/COOH-MWCNTs without involving complicated and time-consuming methods of preparation. Hence, MCPE/COOH-MWCNTs can easily extend its applications to biological fields and can be used as a potential electrochemical sensor for the determination of Tyr for various applications.

Analytical application in real sample analysis

The practical utility of MCPE/COOH-MWCNTs was evaluated by employing it for the determination of Tyr in cow's milk and in human blood serum. The cow's milk and human blood samples were analysed at MCPE/COOH-MWCNTs using standard addition method in order to prevent any matrix effect. Both milk and blood serum were diluted with 0.1 M PBS of pH 7.0 without any pretreatment. The samples were spiked with known quantities of Tyr and its amperometric responses were recorded at MCPE/COOH-MWCNTs. Good quantitative recoveries were obtained as shown in Table 2 providing further evidence that this is a reliable method to determine Tyr in real samples such as milk and blood serum. This also suggests the accuracy and selectivity of the developed sensor.

Conclusions

An electrochemical sensor based on bulk modification of CPE with COOH-MWCNTs has been developed for Tyr determination at physiological pH. The remarkable electrocatalytic properties of COOH-MWCNTs qualify them as modifiers for the construction of MCPE/COOH-MWCNTs which

resulted in the enhanced current sensitivity and reduced oxidation overpotential for Tyr. The sensor was found to be sensitive and selective towards the determination of Tyr in the presence of various interfering biomolecules. MCPE/COOH-MWCNTs showed a better detection limit and a wider linear dynamic range for the determination of Tyr. The proposed sensor was used for the determination of Tyr in cow's milk and human blood serum with a good recovery and without the necessity of subjecting it to sample treatment. The modified electrode carries excellent stability and reproducibility. Because of the low cost, simple preparation, fast response and freedom from the constraint of pretreatment, it can be conveniently employed for the routine analysis of different samples.

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