Voltammetric Determination of Tryptophan Using a Carbon Paste Electrode Modified with Magnesium Core Shell Nanocomposite and Ionic Liquids

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A novel carbon paste electrode modified with ionic liquid (n-hexyl-3-methylimidazolium hexafluoro phosphate) and magnetic core-shell manganese ferrite nanoparticles (MCSILCPE) was fabricated. The electrochemical study of the modified electrode, as well as its efficiency for electro-oxidation of tryptophan, is described. Cyclic voltammetry (CV), choronoamperometry (CHA) and square wave voltammetry (SWV) were used to investigate oxidation of tryptophan in aqueous solution. The diffusion coefficient ($D = 3.53 \times 10^{-6}$ cm^2 s^{-1}), was determined using electrochemical approaches. It has been found that under an optimum condition (pH 7.0), the oxidation of tryptophan at the surface of such an electrode occurs at a potential about 150 mV less positive than that of an unmodified carbon paste electrode. SWV exhibits a linear dynamic range from 5.0×10^{-6}-4.0×10^{-4} M tryptophan. The morphology of the modified electrode was studied using SEM. The limit of detection was equal to 1.1×10^{-6} M. The electrode was also employed for determination of tryptophan in urine samples.

INTRODUCTION

Since the discovery of the carbon paste electrode (CPE) by Ralph N. Adams in 1958 and its introduction as an alternative to the dropping mercury electrode, particularly in anodic polarography [1], this type of electrode (and sensors based on it) has undergone considerable development. The scope for the development is due to several advantages, such as low cost, low background current, wide potential window, high sensitivity, and renewable surface [2]. However, since the fabrication process involves simply mixing graphite with suitable binders to form a paste, modification of this material has branched out across several lines of innovation. Among the more relevant ones are: research into alternative binding components such as

teflon [3], sol-gel [4], and ionic liquid [5], modification of the paste with polymers, redox mediators and recognition elements to construct electrochemical sensors and biosensors, and the doping of the electrode with different materials such as clay, carbon nanotubes and metal nanoparticles to enhance the analytical performance of the associated electrochemical devices [6-13].

Electrochemical methods offer the practical advantages including operation simplicity, satisfactory sensitivity, wide linear concentration range, low expense of instrument, possibility of miniaturization, suitability for real-time detection and less sensitivity to matrix effects in comparison with separation and spectral methods [14-24]. Modification of the electrode surface can grant some remarkable advantages in the electrochemical responses and electrochemical research. Some special properties can be considered in modification of the electrodes using

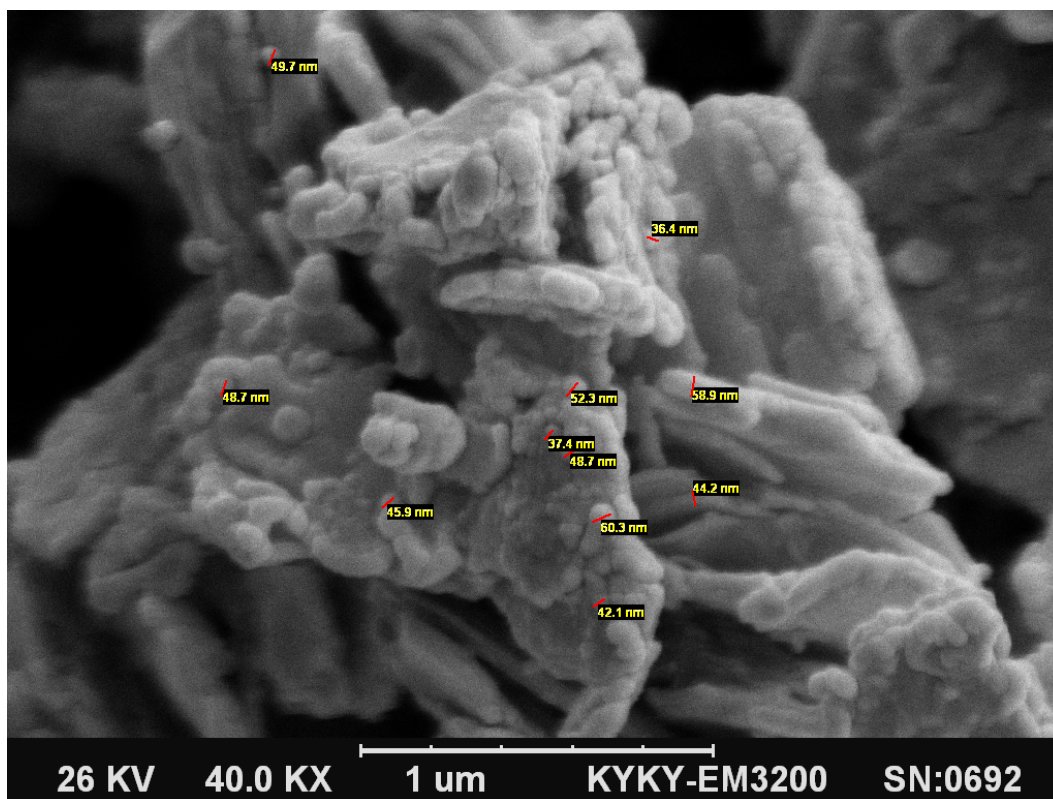*Corresponding author. E-mail: szmohammadi@yahoo.com

Fig. 1. SEM image of the ionic liquid and magnetic core-shell manganese ferrite nanoparticles carbon paste electrode.

nanomaterials, *e.g.*, catalysis, the large specific surface area and more adsorption sites [25-35].

Nanotechnology has recently become one of the most exciting fields in sciences. Nanostructured materials including nanoparticles, nanowires and nanotubes have attracted a considerable interest and have become a vast area of research owing to their unique physical and chemical properties which can provide an important and feasible platform for electroanalysis particularly in the design of modified electrodes for electrochemical sensing. Nanostructured materials can be used to construct novel and improved sensing devices, such as electrochemical sensors and biosensors. Many types of nanoparticles of different sizes and compositions are now available, which facilitate their application in electroanalysis [36-44].

Recently room temperature ionic liquid [RTIL] has been used as a new kind of modifier for a chemically modified electrode. RTIL is composed entirely of ions and exists as a liquid at room temperature with the characteristic of negligible vapor pressure and good solubility and chemical stability. As a new green media, RTIL has many unique electrochemical properties, such as higher ionic conductivity and wider electrochemical windows [45-50].

Tryptophan (TRP) is one of the essential amino acids which cannot be synthesized by the organism. Therefore, it must be supplied in the diet. This compound is a precursor for serotonin (a neurotransmitter), melatonin (a neurohormone) and niacin. It has been implicated as a possible cause of schizophrenia in people who cannot metabolize it properly. Faulty metabolization of TRP creates a toxic waste product in the brain that causes hallucinations and delusions [51]. The direct electrochemical oxidation of TRP is known to be kinetically sluggish, and a relatively high overpotential is required for
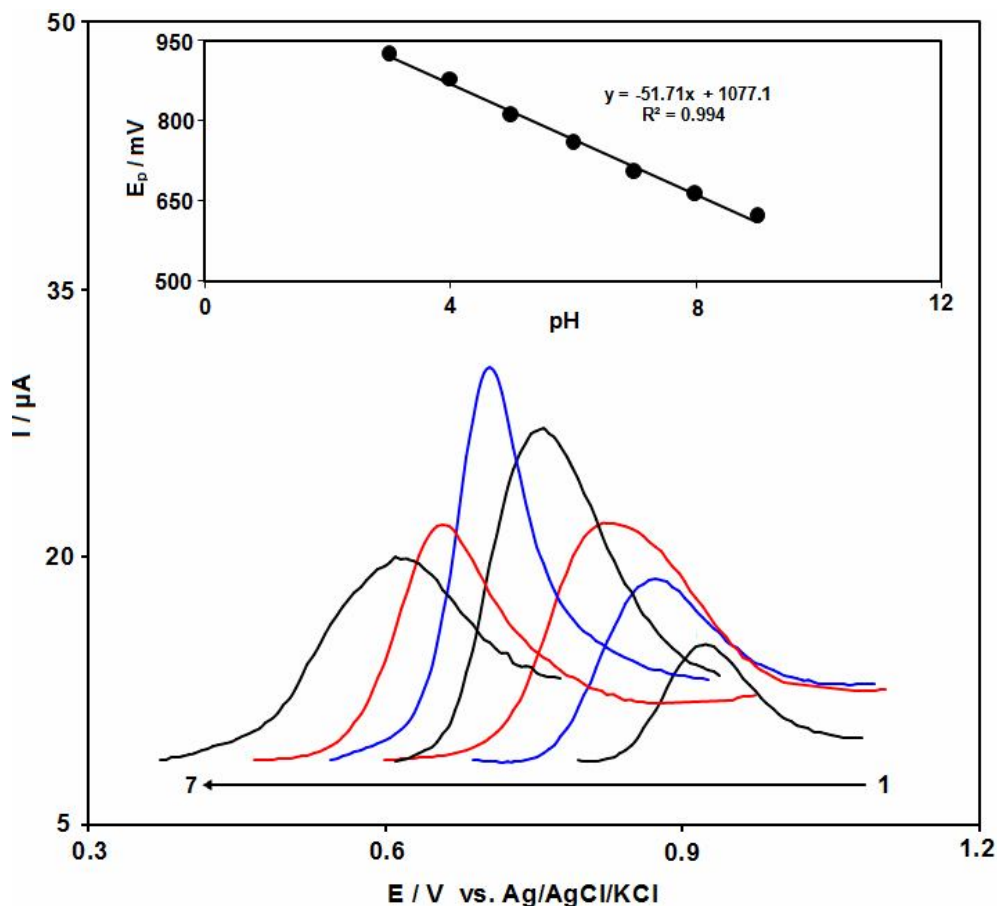
Fig. 2. DPVs (at 50 mV s^{-1}) of the MCSILCPE in the presence of 120.0 μM tryptophan at various buffered pHs. The numbers 1-7 correspond to pHs 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0, respectively. Inset: plot of E_p *vs*. pH.

its oxidation at bare electrodes [52]. Electrochemical detection of TRP has been shown to be facilitated by chemically modified electrodes [53-61].

In the present work, we describe the preparation of a new carbon paste electrode modified with ionic liquid and magnetic core-shell manganese ferrite nanoparticles (MCSILCPE) and investigate its performance for the determination of tryptophan.

EXPERIMENTAL

Apparatus and Chemicals

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System (GPES) software. A conventional three electrode cell was used at 25 ± 1 °C. An Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and MCSILCPE were used as the reference, auxiliary and working electrodes, respectively. A Metrohm 710 pH meter was used for pH measurements.

Tryptophan and all of the other reagents were of analytical grade and were obtained from Merck (Darmstadt, Germany). The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 2.0-9.0. Ionic liquid (n-hexyl-3-methylimidazolium hexafluoro
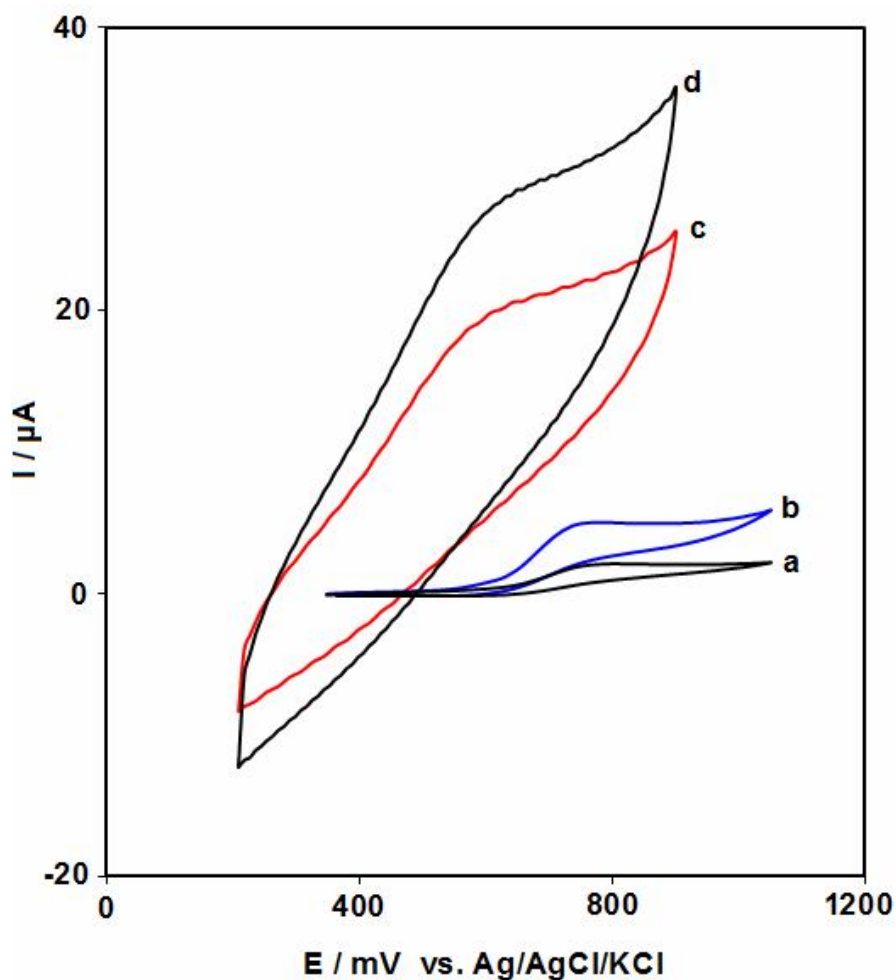
Fig. 3. CVs of a) CPE, b) MCSCPE, c) ILCPE and d) MCSILCPE in the presence of 100.0 µM tryptophanat a pH 7.0, respectively. In all cases the scan rate was 50 mV s^{-1}.

Table 1. Cyclic Voltammetric Data Obtained for Electro Oxidation of Tryptophan at the Surface of Various Prepared Electrodes

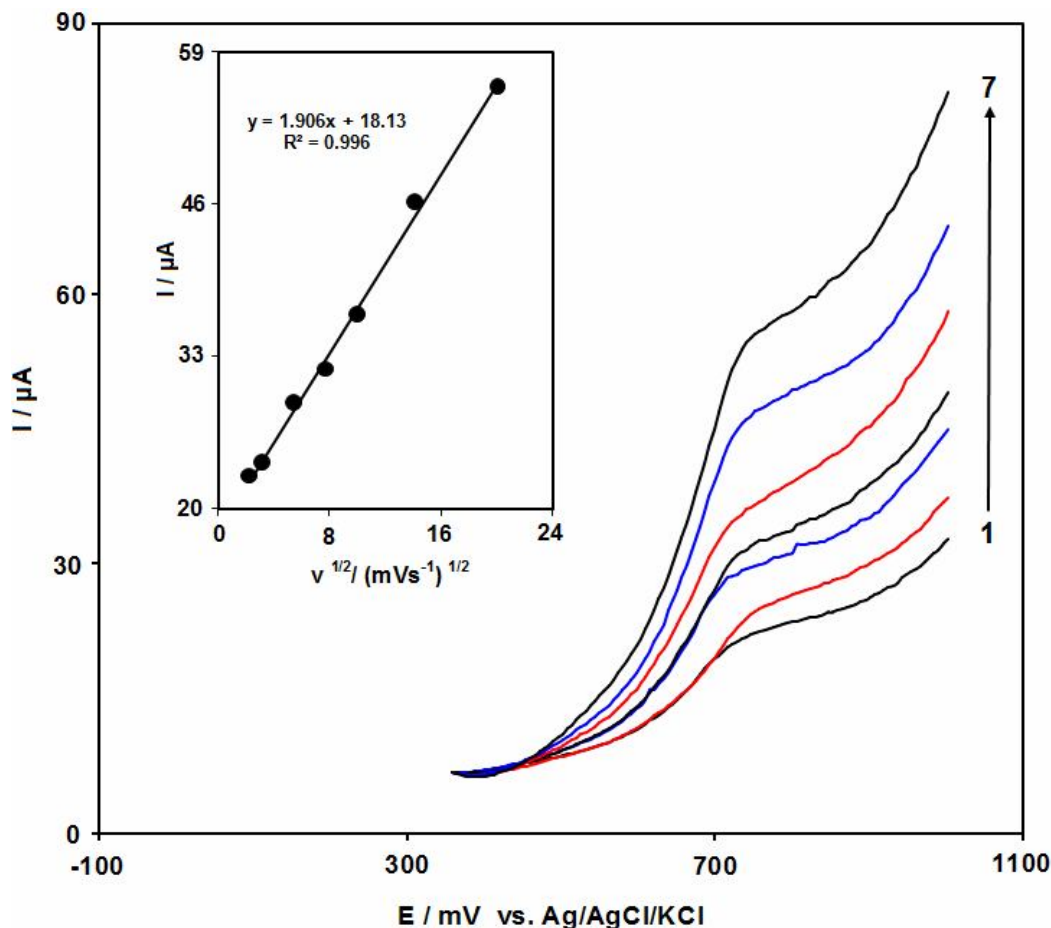| Electrode	Anodic peak potential (mV)	Anodic peak current (µA)
Bare CPE	795	2.04
MCSCPE	760	5.0
ILCPE	660	20.6
MCSILCPE	660	28.6

Fig. 4. LSVs of MCSILCPE in 0.1 M PBS (pH 7.0) containing 120.0 μM tryptophanat various scan rates; numbers 1-7 correspond to 5, 10, 30, 50, 100, 200 and 400 mV s^{-1}, respectively. Inset: Variation of anodic peak current *vs*. square root of scan rate.

phosphate) was purchased from Sigma Aldrich Co. Magnetic core-shell manganese ferrite nanoparticles were synthesized in our laboratory as reported previously [62].

Preparation of the Electrode

MCSILCPEs were prepared by mixing 0.04 g of magnetic core-shell manganese ferrite nanoparticles with 0.96 g graphite powder and approximately, ~0.8 ml of ionic liquids with a mortar and pestle. The paste was then packed into the end of a glass tube (*ca*. 3.4 mm i.d. and 15 cm long). A copper wire inserted into the carbon paste

provided the electrical contact.

For comparison, ionic liquid/carbon paste electrode in the absence of magnetic core-shell manganese ferrite nanoparticles (ILCPE), magnetic core-shell manganese ferrite nanoparticles carbon paste electrode (MCSCPE) consistent of magnetic core-shell manganese ferrite nanoparticles, graphite powder and paraffin oil, and bare carbon paste electrode (CPE) consisiting of graphite powder and paraffin oil were also prepared in the same way. A typical SEM of MCSILCPE is shown in Fig. 1. As can be seen, magnetic core shell nanoparticle and n-hexyl-3-methylimidazolium hexafluoro phosphate are dispersed in
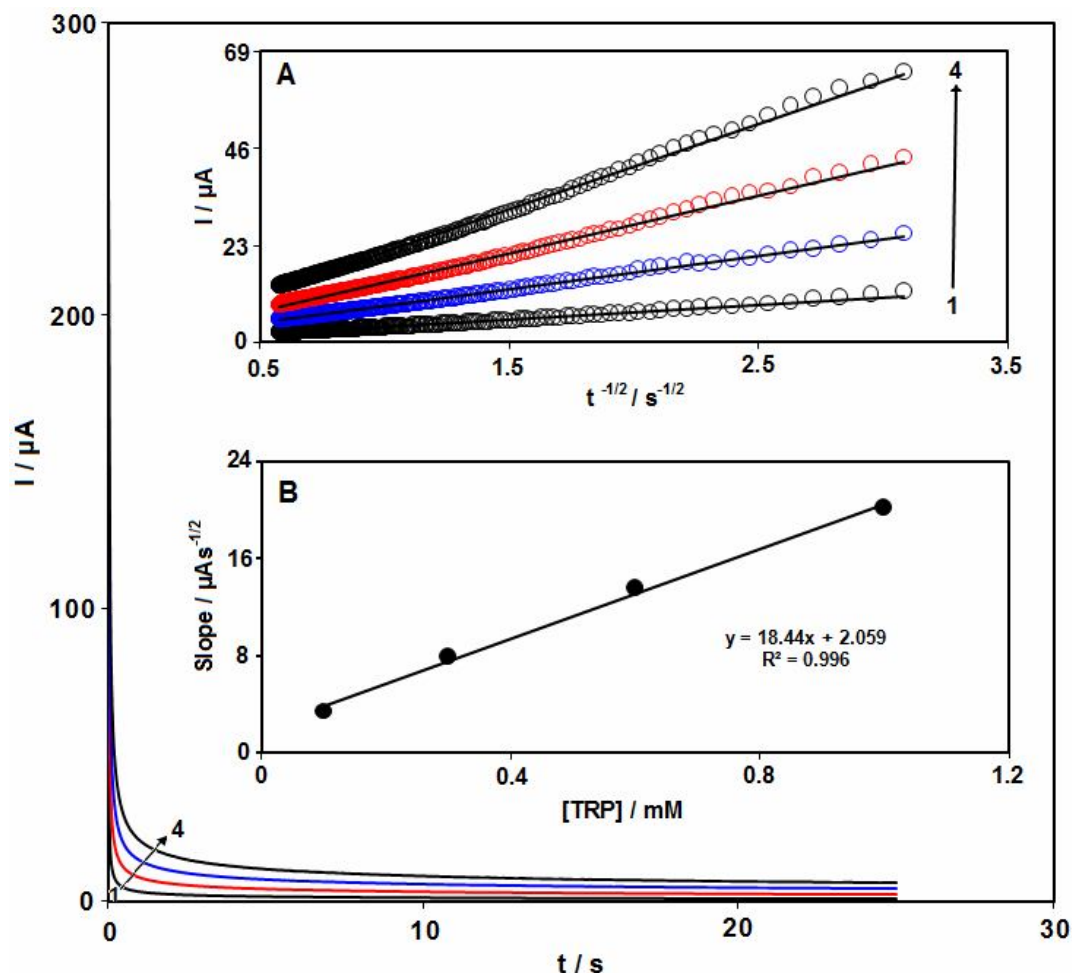
Fig. 5. Chronoamperograms obtained at MCSILCPE in 0.1 M PBS (pH 7.0) for different concentration of tryptophan. The numbers 1-4 correspond to 0.1, 0.3, 0.6 and 1.0 mM of tryptophan. Insets: (A) Plots of I *vs.* $t^{-1/2}$ obtained from chronoamperograms 1-4. (B) Plot of the slope of the straight lines against tryptophan concentration.

whole of the material.

RESULTS AND DISCUSSION

Influence of pH

The electrochemistry of tryptophan is generally pH dependent. Thus, the electrochemical behavior of tryptophan was studied at different pHs using DPV (Fig. 2). It was noticeable that the anodic peak potentials of the tryptophan shift to less positive values with increasing pH. The inset of Fig. 2 shows potential-pH diagrams constructed by plotting the peak potential values as functions of pH. As can be seen, the slope is 51.71 mV/pH indicating that the system obeys the Nernst equation for an equal electron and proton transfer reaction [63].

Electrochemical Behavior of Tryptophan at the Surface of Various Electrodes

Figure 3 displays cyclic voltammetric responses from the electrochemical oxidation of 100.0 μM tryptophan at the surface of MCSILCPE (curve d), ILCPE (curve c), MCSCPE (curve b) and bare CPE (curve a). The results
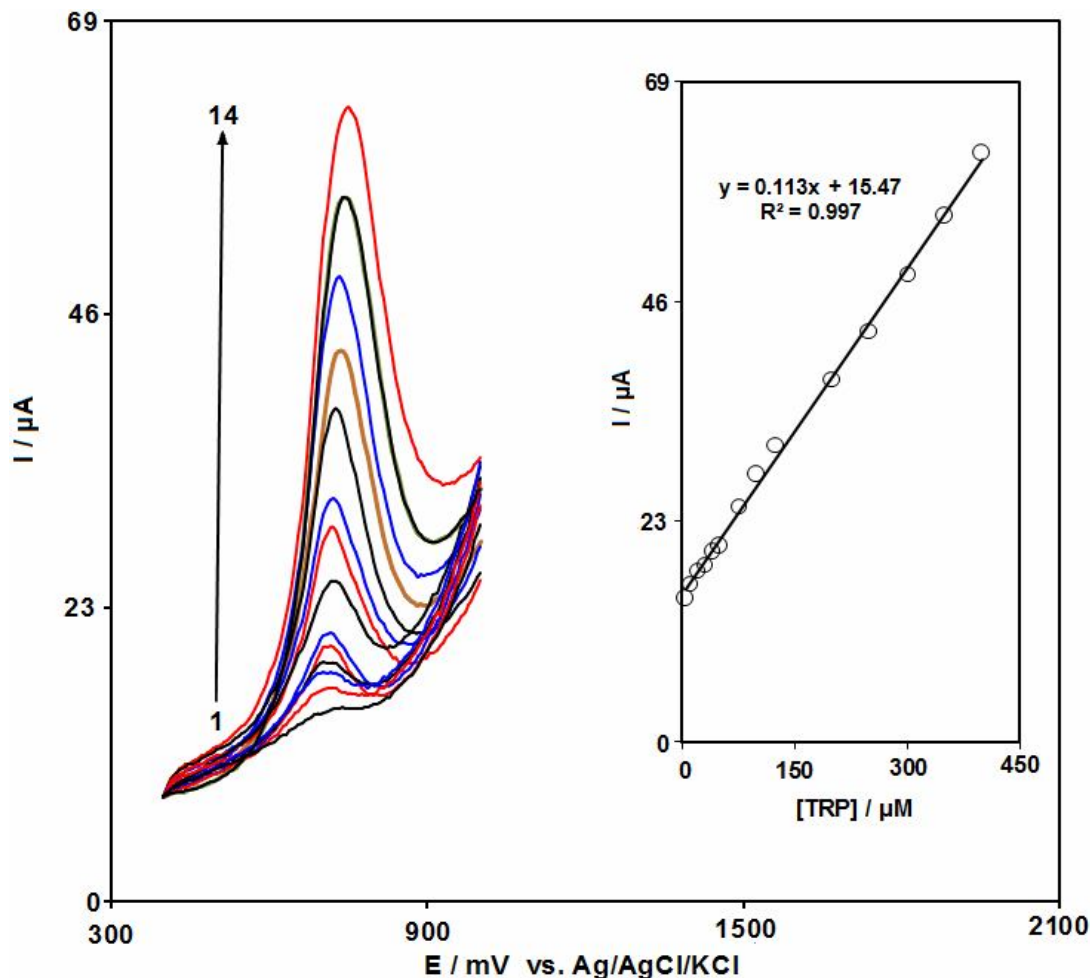
Fig. 6. SWVs of MCSILCPE in 0.1 M PBS (pH 7.0) containing different concentrations of tryptophan (5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 75.0, 100.0, 125.0, 200.0, 250.0, 300.0, 350.0 and 400.0 μM). Inset shows the plots of the peak current as a function of tryptophan concentration in the range of 5.0-400.0 μM.

showed that the oxidation of tryptophan is very weak at the surface of the bare CPE, but in the presence of ILs in CPE could enhance the peak current and decrease the oxidation potential (decreasing the overpotential). A substantial negative shift of the currents starting from oxidation potential for tryptophan and dramatic increase of the current indicate the catalytic ability of MCSILCPE (curve d) and ILCPE (curve c) to tryptophan oxidation. The results showed that the combination of magnetic core-shell manganese ferrite nanoparticles and the ionic liquid (curve d) definitely improved the characteristics of tryptophan

oxidation. However, MCSILCPE shows much higher anodic peak current for the oxidation of tryptophan compared to ILCPE, indicating that the combination of magnetic core-shell manganese ferrite nanoparticles and IL has significantly improved the performance of the electrode toward tryptophan oxidation. The results are summarized in Table 1.

Effect of Scan Rate

The effect of potential scan rates on the oxidation current of tryptophan has been studied (Fig. 4). The results

Table 2. Comparison of Some Characteristics of the MCSILCPE for the Determination of Tryptophan

Electrode	Modifier	Dynamic range (μM)	Detection limit (μM)	Ref.
CPE	Graphene and ethyl 2-(4-ferrocenyl-I[1,2,3]triazol-1-yl) acetate	20-350	15	[64]
CPE	Ionic liquid	8-1000	4.8	[65]
CPE	Ta wire coated with graphene sheet layers	0.4-100	0.4	[66]
MWCNPE[a]	8,9-Dihydroxy-7-methyl-12H-benzothiazolo[2,3-b]quinazolin-12-one	10-800	4.0	[67]
GCE[b]	Gold/multi-walled carbon nanotubes	5-100	3	[68]
GCE	Nafion/TiO$_2$-graphene	5-140	0.7	[69]
GCE	Graphitic carbon nitride-multi-walled carbon nanotubes	65-1815	1	[70]
CPE	Magnetic core shell nanoparticle and n-hexyl-3-methylimidazolium hexafluoro phosphate	5.0- 400.0	1.1	This work

[a]Glassy carbon electrode. [b]Multi wall carbon nanotubes paste electrode.

Table 3. The Application of MCSILCPE for Determination of Tryptophan in Urine Sample (n = 5)

Sample	Spiked	Found	Recovery (%)	R.S.D. (%)
Urine	0	ND	-	-
	7.5	7.4	98.7	3.3
	17.5	18.1	103.4	1.9
	27.5	27.9	101.4	2.8
	37.5	37.3	99.5	2.4

showed that increasing the potential scan rate induces an increase in the peak current. In addition, the oxidation process is diffusion controlled as deduced from the linear dependence of the anodic peak current (I_p) on the square root of the potential scan rate ($v^{1/2}$) over a wide range from 5-400 mV s^{-1}.

Chronoamperometric Measurements

Chronoamperometric measurements of tryptophan at MCSILCPE were carried out by setting the working electrode potential at 0.7 V *vs*. Ag/AgCl/KCl (3.0 M) for the various concentrations of tryptophan in PBS (pH 7.0) (Fig. 5). For an electroactive material (tryptophan in this case) with a diffusion coefficient of D, the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation [63].

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2} \tag{1}$$

where D and C_b are the diffusion coefficient (cm^2 s^{-1}) and the bulk concentration (mol cm^{-3}), respectively. Experimental plots of I *vs*. t$^{-1/2}$ were employed, with the best fits for different concentrations of tryptophan (Fig. 5A). The slopes of the resulting straight lines were then plotted vs. tryptophan concentration (Fig. 5B). From the resulting slope and Cottrell equation the mean value of the D was found to be 3.53×10^{-6} cm^2 s^{-1}.

Calibration Plot and Limit of Detection

The peak current of tryptophan oxidation at the surface of the modified electrode can be used for determination of tryptophan in solution. Therefore, DPV experiments were performed for different concentrations of tryptophan (Fig. 6). The oxidation peak currents of tryptophanat at the surface of a modified electrode were proportional to the concentration of the tryptophan within the range of 5.0×10^{-6}-4.0×10^{-4} M with detection limit (3σ) of 1.1×10^{-6} M. These values are comparable with those obtained by the other modified electrodes (Table 2).

Interference Study

The influence of various foreign species on the determination of 100 µM tryptophan was investigated. The tolerance limit was set as the concentration of foreign substances causing ±5% error in the determination. On the basis of the results, Na$^+$, Cl$^-$, K$^+$, Mg^{2+}, Ca^{2+}, l-lysine, glucose, l-asparagines, glutamic acid, glycine, acetaminophen, ascorbic acid, uric acid, dopamine, methyldopa, levodopa, histidine, phenylalanine, glucose, urea, starch and NADH, do not show interference in determination of tryptophan.

Real Sample Analysis

In order to evaluate the analytical applicability of the proposed method, it was also applied for the determination of tryptophan in urine sample. The results for determination of the tryptophan in urine sample are given in Table 3. Satisfactory recovery of the experimental results was found for tryptophan. The reproducibility of the method was demonstrated by the mean relative standard deviation (R.S.D.).

CONCLUSIONS

Electrochemical behavior of tryptophan was studied at the surface of a magnetic core-shell manganese ferrite nanoparticles/ionic liquid carbon paste electrode. The proposed modified electrode presented a low detection limit, good linear range and reproducibility which make it a suitable tryptophan sensor for practical applications.

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