Talanta 85 (2011) 1056–1062

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

In situ copper oxide modified molecularly imprinted polypyrrole film based voltammetric sensor for selective recognition of tyrosine

Varghese Saumya, Krishnapillai P. Prathish, Talasila P. Rao*

Chemical Sciences and Technology Division (CSTD), National Institute for Interdisciplinary Science and Technology (NIIST), CSIR, Trivandrum 695019, India

ARTICLE INFO

Article history:
Received 4 April 2011
Received in revised form 6 May 2011
Accepted 12 May 2011
Available online 31 May 2011

Keywords:
Molecularly imprinted polypyrrole film
In situ metal oxide modification
Tyrosine sensor
Differential pulse voltammetry
Electrocatalytic studies
Urine analysis

ABSTRACT

Organic–inorganic hybrids are promising functional materials as they combine the special characteristics of both organic (polymer) and inorganic phases. Among different existing approaches for the preparation of such polymer–inorganic hybrid coatings, in situ electrochemical methods are very advantageous because of their high sensitivity and simplicity. In the present study, voltammetric sensors for tyrosine are designed and developed via various modifications on glassy carbon electrode such as polypyrrole coated GCE, molecularly imprinted polypyrrole coated GCE (MIPPy) and in situ copper oxide modified MIPPy coated GCE. Of these, in situ copper oxide modified MIPPy coated GCE sensor responds to tyrosine concentrations in the range 1×10^{-8} to 1×10^{-6} and 2×10^{-6} to 8×10^{-6} M with a very low detection limit of 4.0×10^{-9} M and by far the most sensitive one. Detailed linear sweep voltammetric and chronoamperometric experiments were undertaken to investigate the electrocatalytic behavior of tyrosine. The electron transfer coefficient, diffusion coefficient and charge transfer rate constants involved in the sensing process using in situ copper oxide modified MIPPy film coated GCE are 0.47, 1.88×10^{-6} cm^2 s^{-1}, 4.7×10^6 L mol^{-1} s^{-1}, respectively. Furthermore, the designed sensor is highly selective and has been applied successfully for the analysis of synthetic and real samples of human urine.

1. Introduction

Tyrosine is a well-known essential amino acid in human and herbivores bodies and takes part in synthesis of proteins and catecholamines. It is a vital constituent of proteins, which are indispensable in human nutrition for establishing and maintaining positive nitrogen balance [1] and itself a precursor for dopa, dopamine, catecholamine, melanin, lyothyronine and epinephrine [2,3] that are necessary for human body. Imbalances of tyrosine in body causes metabolic disorders like tyrosenemia, Parkinson's disease [4,5], atherosclerosis [6,7], lung diseases, liver diseases [8,9] and mental illness [10,11]. Moreover, tyrosine is an oral cancer biomarker. In this scenario, rapid and sensitive determination methods for tyrosine are necessary, and are of vital interest in pharmacology. Currently, several methods have been reported for tyrosine determination including spectrophotometry [12], fluorimetry [13], high performance liquid chromatography [14], capillary electrophoresis [15], gas chromatography [16] and ion exchange chromatography [17]. Even though these methods are quite accurate, they need multistep sample clean-up procedures. Moreover, these laboratory based instruments are expensive, time consuming and are not portable. On the other-hand, since tyrosine is an electroactive compound it can be easily determined by electrochemical methods. Electrochemical methods have additional advantages such as simplicity, low cost, convenience and sensitivity. Of various electrochemical methodologies, voltammetric approaches are preferred in view of its superior sensitivity and selectivity. Salient features of different voltammetric/amperometric transducer based approaches for detection and quantification of tyrosine are summarized in Table 1 [1,18–30]. Among these, differential pulse voltammetric procedure employing MWCNT-ionic liquid composite coated GCE in presence of cupric ion offers better sensitivity in the calibration range 1×10^{-8} to 5×10^{-6} M with a limit of detection of 8×10^{-9} M [29]. However, detailed selectivity studies were not undertaken in this report.

Molecular imprinting technique is becoming a more commonly accepted and useful method for the selective recognition and isolation of key biological target molecules. The preparation of polymeric materials with tailor made molecular recognition sites owes the capability to distinguish target molecules through their size, shape and functional group distribution. Molecular imprinting is a field of fast growing interest and has been applied in variety of applications, especially in sensing [31].

Conducting polymers offer a wide platform for chemical sensing and can be used as solid contact electrodes. They are more

* Corresponding author. Tel.: +91 471 2515317; fax: +91 471 2491712.
E-mail addresses: tprasadarao@rediffmail.com, tprasadarao07@gmail.com (T.P. Rao).

0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.05.025

Table 1
Features of chemically modified electrodes for voltammetric sensing of tyrosine.

Sl. no.	Electrode construction	Technique	Sample medium and pH	Linear calibration range, M	LOD (M)	Reference
1	MWCNT/4-amino benzene sulfonic acid, GCE	DPV	Phosphate buffer, 7	1×10^{-7} to 5×10^{-5}	8×10^{-8}	[18]
2	MWCNT, GCE	CV	Citric acid buffer, 6.5	9×10^{-7} to 3.5×10^{-4}	3.5×10^{-7}	[19]
3	Gold nanoparticles, GCE	DPV	Phosphate buffer, 7	1×10^{-7} to 3×10^{-4}	4×10^{-8}	[20]
4	Butyl choline, GCE	DPV		4×10^{-6} to 1×10^{-4}	4×10^{-7}	[1]
5	MWCNT, GCE	SWSV	Phosphate buffer, 7	2×10^{-6} to 5×10^{-4}	4×10^{-7}	[21]
6	SWCNT, GCE			5×10^{-6} to 2×10^{-5}	9×10^{-8}	[22]
7	Boron doped diamond electrode	DPV	Phosphate buffer, 11.2	2×10^{-5} to 1×10^{-3}	1×10^{-6}	[23]
8	Zeolite, carbon paste	DPV	Phosphate buffer, 3	1.26×10^{-6} to 9×10^{5}	3.2×10^{-7}	[24]
9	Ag/Rutin-WGE			3×10^{-7} to 1×10^{-5}	7×10^{-8}	[25]
10	Screen printed electro-chemical sensor			5×10^{-5} to 5×10^{-4}	–	[26]
11	Cu(II), HMDE	LSASV	Phosphate buffer, 9.6	1×10^{-7} to 5×10^{-5}	5×10^{-8}	[27]
12	Polypyrrole, Ni electrode MIP	Coulometry	Phosphate buffer, 2	5×10^{-3} to 4.5×10^{-2}	–	[28]
13	MWCNT, ionic liquid, copper (II), GCE	DPV	Phosphate buffer, 5.5	1×10^{-8} to 5×10^{-6}	8×10^{-9}	[29]
14	L-Serine			3×10^{-7} to 1×10^{-4}	1×10^{-7}	[30]
15	Copper (II), MICP, MIP	DPV	Phosphate buffer, 5.5	1×10^{-8} to 1×10^{-6} & 2×10^{-6} to 8×10^{-6}	4×10^{-9}	Present method

advantageous with good mechanical stability, simplicity and possibility of miniaturization. Among different conducting polymers, polypyrrole have many attractive features like ease of preparation, high stability and amenability to use in neutral pH region. Again, polypyrrole exhibits electric conductivity and electrochemical redox activity even in neutral pH solutions, which allows the entrapment of a wide range of biomolecules [32,33]. Thus Ozcan et al. [32] and Ozkorucuklu et al. [33] have reported molecularly imprinted polypyrrole and over oxidized polypyrrole on pencil graphite electrodes for sensing of ascorbic acid and sulfamethaxazole, respectively. Pardieu et al. [34] designed molecularly imprinted conducting polymer (MICP) sensor for atrazine with poly (3,4-ethylene dioxy thiophene, co-thiophene acetic acid) film coated on platinum electrode. Another study by Wang et al. [27] reported the incorporation of copper (II) ions during adsorptive stripping voltammetric determination of tyrosine from pH 9.6 solutions. The only tyrosine sensor that utilizes metal ion incorporation is the MWCNT-ionic liquid composite coated glassy carbon by Liu et al. [29]. In the present investigation we describe, the design and development of copper oxide modified molecularly imprinted polypyrrole films, for the electrochemical sensing of tyrosine. The developed sensor has been successfully tested for the analysis of synthetic and real samples of human urine.

2. Experiments

2.1. Materials

L-Tyrosine, pyrrole and copper (II) chloride were purchased from Aldrich, Milwauke, WI, USA and tetrabutyl ammonium perchlorate from Fluka Analytical. All of the working solutions were prepared using deionized double distilled water. All other chemicals were of Analytical Reagent grade (E-Merck, Mumbai, India) and were used as received without further purification. Supporting electrolyte used was 0.1 M phosphate ($NaH_2PO_4 \cdot H_2O$) or 0.1 M sodium acetate buffer (CH_3COONa) of pH of 5.5 and 7.

2.2. Apparatus

All electrochemical experiments were performed at room temperature in a three electrode cell using a potentiostat/galvanostat μ-Autolab system (Ecochemie, Netherlands). The system was run on a PC using GPES 4.9 software. Working electrode was glassy carbon disc of surface area 0.07065 cm^2. Reference electrode and counter electrode used were Ag/AgCl (in saturated KCl solution) and platinum sheet, respectively. The surface morphology of molecularly imprinted polypyrrole coated GCE were studied using Scanning Electron Microscopy (SEM) (JEOL, Model JSM 5600LV). The pHs of all samples were adjusted using a digital pH meter (ELICO-LI 120) with accuracy of 0.01 pH unit.

2.3. Sensor construction

Details regarding construction of polypyrrole (PPy), molecularly imprinted PPy(MIPPy), non-imprinted PPy(NIPPy) and in situ copper oxide modified MIPPy films on glassy carbon electrode(GCE) substrate are shown in Figs. 1 and 2, respectively.

2.3.1. Molecularly imprinted polypyrrole (MIPPy) film sensor

The MIPPy film was prepared by electropolymerizing 2×10^{-3} to 4×10^{-3} M (0.134–0.268 μg/ml) of pyrrole in 20 ml of acetonitrile as supporting electrolyte containing 0.01 M of tetrabutyl ammonium perchlorate and 35 μl trifluoroacetic acid at a deposition potential in the range 0.8–0.9 V vs. Ag/AgCl for a duration of 60 ± 5 s in presence 10^{-5} to 10^{-3} M of L-tyrosine (Template) onto glassy carbon electrode. The electrode was then taken out and template was ejected out by electrochemical over oxidation in sodium hydroxide (>0.1 M) by repeated cycling in the potential range 0.8–1.2 V for about 100 times at scan rate of 50 mV/s. The template leached MIPPy film is then washed by cycling 75 times in phosphate buffer (pH 2) in the potential range 0–0.9 V at scan rate of 100 mV/s. Subsequently, the above film electrodes were stabilized in phosphate buffer (pH 5.5) by cycling in the potential range 0–0.9 V for 20 times to obtain molecularly imprinted polypyrrole (MIPPy) sensor.

2.3.2. NIPPy film sensor

The non-imprinted polypyrrole film based sensor was constructed on similar lines to MIPPy sensor by omitting tyrosine template.

2.3.3. Copper oxide modified MIPPy sensor

In situ copper oxide modified MIPPy was prepared by depositing Cu (II) as Cu (0) at −0.3 V for 5 s from $CuCl_2$ solutions (in phosphate buffer) and scanning anodically to 0.9 V in presence of tyrosine

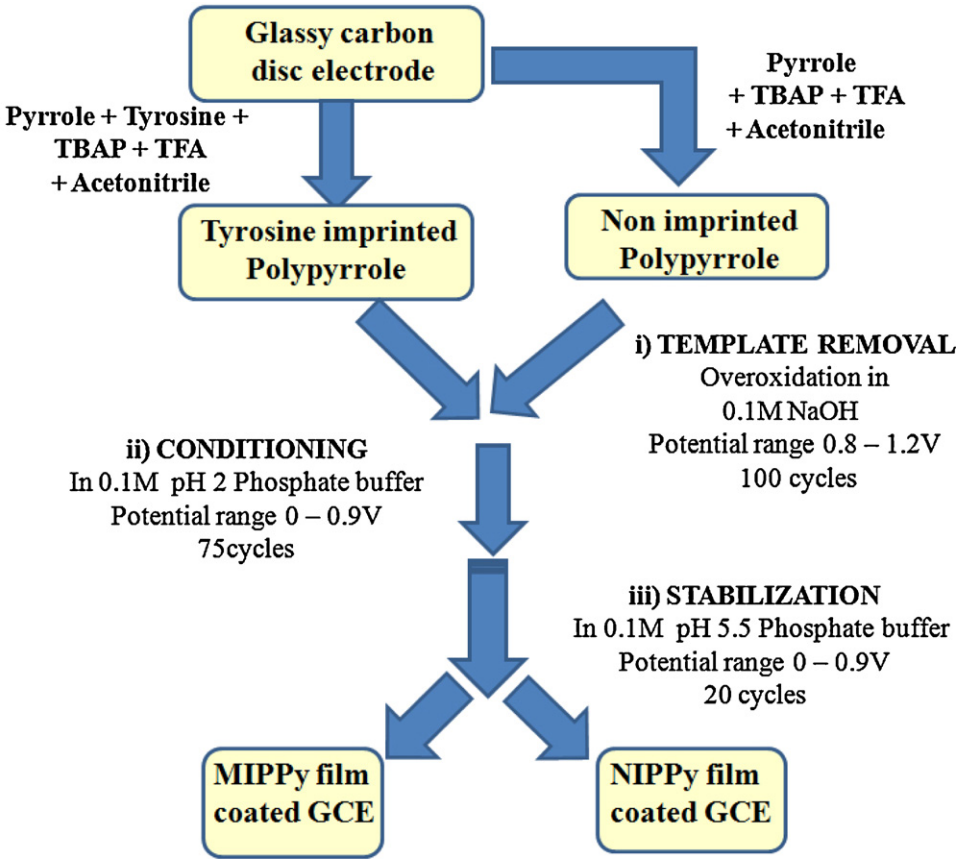

Fig. 1. Detailed construction of polypyrrole (PPy), molecularly imprinted polypyrrole (MIPPy) and non-imprinted PPy (NIPPy) on to GCE.

without medium exchange. Copper oxide modification of MIPPy coated GCE was also done sequentially by conducting the copper deposition (from $CuCl_2$ solutions) over MIPPy modified GCE and anodic scanning in phosphate buffer media. The copper oxide deposition on MIPPy film coated GCE is the modified version of the procedure reported by Wen-Zhi and You-Qin [35] where in copper is first deposited on to GCE from 1.66×10^{-6} M $CuCl_2$ + 0.1 M KCl solutions and then scanning anodically in 0.1 M NaOH medium with medium exchange. Fig. 2 depicts schematic representation of in situ copper oxide modified molecularly imprinted polypyrrole film based voltammetric sensor for tyrosine.

1 mmol L^{-1} K_3 $Fe(CN)_6$ is used as a probe to measure the "real" area of the copper oxide modified molecularly imprinted polypyrrole coated GCE and bare GCE by cyclic voltammetry at different scan rates. As per Randles–Sevcik equation,

$$I_{pa} = 2.69 \times 10^5 \, n^{3/2} \, Ac_o \, D_R^{1/2} \, \nu^{1/2}$$

where I_{pa} refers to anodic peak current, n is the electron transfer number, A is the surface area of the electrode, D_R is diffusion coefficient, c_o is the concentration of $K_3Fe(CN)_6$, ν is the scan rate. For $K_3Fe(CN)_6$, $n = 1$, $D_R = 7.6 \times 10^{-6}$ cm s^{-1} (0.01 mol L^{-1} KCl). The microscopic areas can be calculated from the slope of the I_{pa}–$\nu^{1/2}$ relation, yields 0.0015 cm^2 for bare GCE, 0.0025 cm^2 for PPy coated GCE and 0.003 cm^2 for in situ copper oxide modified molecularly imprinted polypyrrole coated GCE.

2.3.4. Regeneration of MIPPy and copper oxide modified MIPPy film

After each determination, the MIPPy film is regenerated by stirring in 0.1 M phosphate buffer for 5 min for removing tyrosine bound in cavities. Metal oxide formed on the electrode is removed and the molecularly imprinted polypyrrole surface is replenished for next cycle of quantification by holding the electrode at 0 V for ~10 s (in case of metal oxide modified MIPPy) followed by stirring in pH 5.5 phosphate buffer (for all modified electrodes) for removal of oxidized tyrosine.

2.4. Experimental procedure

Appropriate amounts of L-tyrosine (1×10^{-8} to 8×10^{-6} M) in 0.1 M phosphate buffer solution (pH 5.5) were taken in 20 ml electrochemical cell, and then 3 electrode system was installed on it where in the working electrodes are NIPPy, MIPPy and in situ metal oxide modified MIPPy film coated electrodes. Differential pulse voltammograms were recorded by scanning in the potential range 0–0.9 V at modulation amplitude of 100 mV. The unknown concentrations of tyrosine were determined by referring to the calibration graph. Above procedure is equally applicable to bioassays as there is only slight diminution in magnitude of analytical signal at physiological pH of 7.

3. Results and discussions

3.1. SEM characterization

The SEM micrograph obtained for the polypyrrole modified electrode surface shows a uniform formation of polymer film (see Fig. 3). Also one can observe uniformly arranged flakes of polymer layers on the electrode surface which substantially increases the surface area of the modified electrode and is evident from the surface area calculation as described in Section 2.3.3.

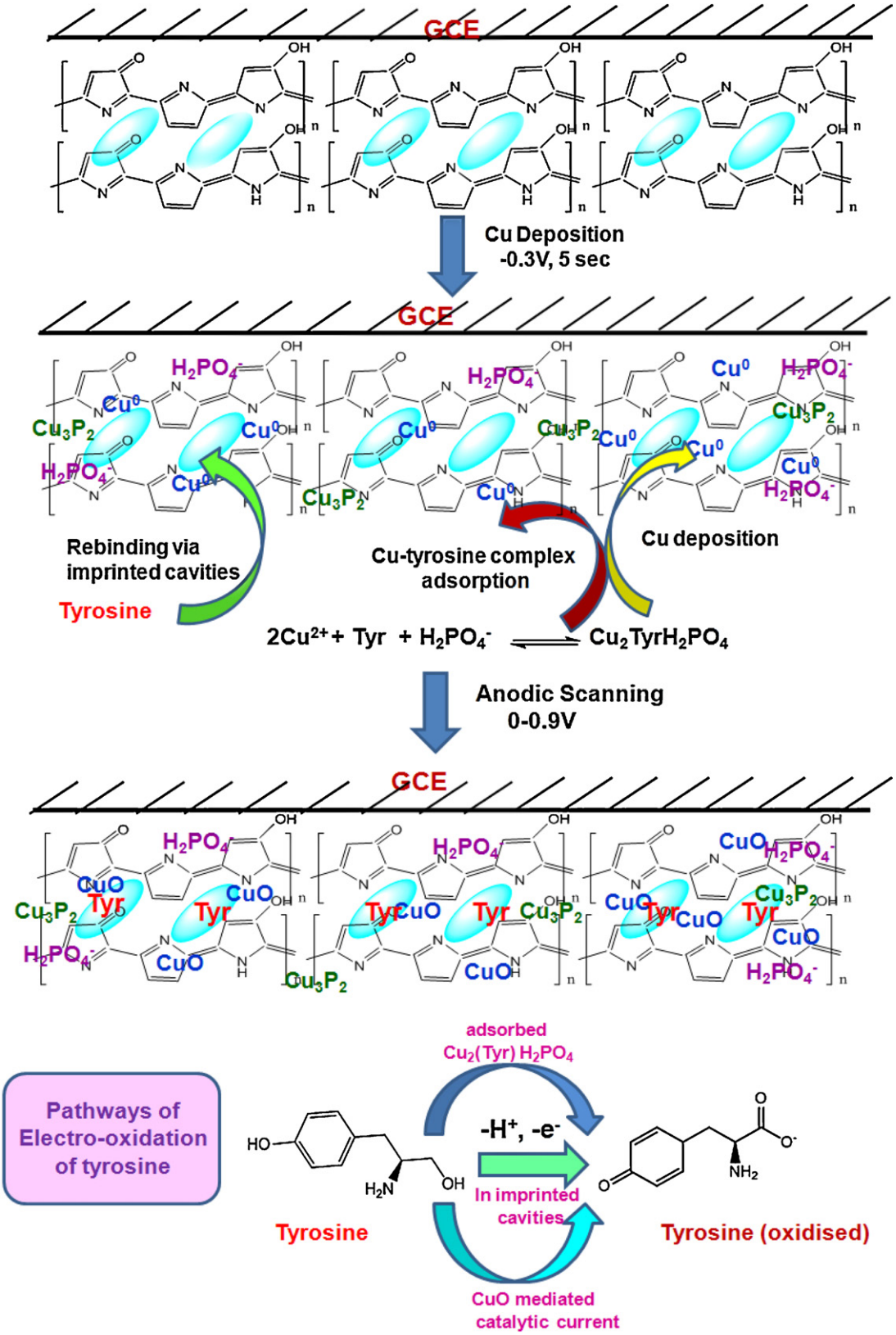

Fig. 2. Schematic representation of in situ copper oxide modified molecularly imprinted polypyrrole coated GCE and corresponding electrode-solution interfacial processes.

3.2. Cyclic voltammetric studies

The electrochemical behavior of unmodified and various modified electrodes were studied by cyclic voltammetry in presence of phosphate buffer (pH 5.5) media in the potential range 0–0.9 V (see Fig. 4). Curves A, B and C show the cyclic voltammograms of molecularly imprinted polypyrrole (MIPPy) film coated GCE, copper oxide modified MIPPy coated GCE and in situ copper oxide modified MIPPy coated GCE, respectively. It can be observed that the base current for MIPPy modified electrode as well as copper oxide

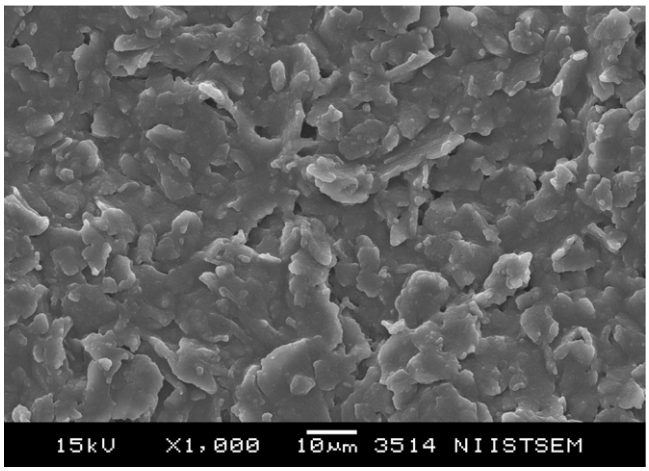

Fig. 3. SEM image of molecularly imprinted polypyrrole coated GCE surface.

modified MIPPys has increased compared to that of bare GCE due to the increase in surface area of the modified electrodes. CVs of MIPPy film coated GCE, copper oxide modified MIPPy coated GCE and in situ copper oxide modified MIPPy coated GCE in presence of 10^{-5} M of tyrosine (0 to +0.9 V) were shown in curves D, E and F, respectively. The bare GCE gives a featureless voltammogram (not shown in Fig. 4) where as a predominant oxidation peak was observed in presence of MIPPy modified electrode (D). The signal corresponding to tyrosine oxidation was found to increase further in presence of copper oxide modified MIPPy (E) while a significant enhancement of peak current was observed in the case of in situ copper oxide modified MIPPy film (F). This clearly indicates that not only the islands of copper oxide deposited on MIPPy film during anodic scan but also the metal ions present in the solution in complexed and uncomplexed states are playing a major role in deriving the enhanced analytical signal. However, in situ copper oxide modification results in two fold enhancement in sensitivity without compromising on selectivity. Further studies were undertaken with in situ copper oxide modified MIPPy film coated GCE as an indicator electrode for voltammetric sensing of tyrosine.

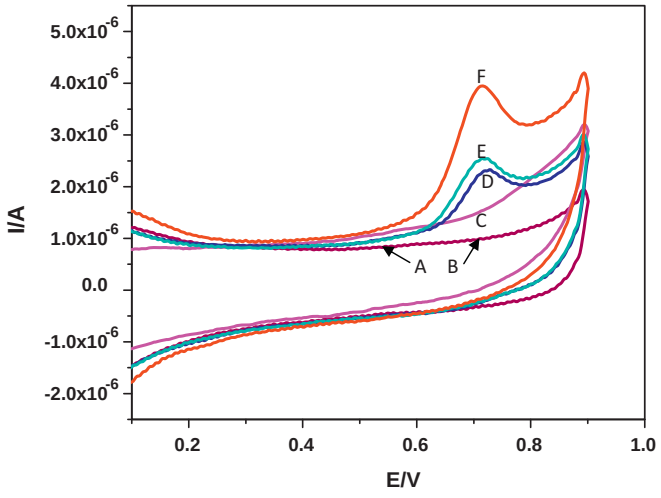

Fig. 4. Cyclic voltammetric studies of MIPPY coated GCE, copper oxide modified MIPPy coated GCE and in situ copper oxide modified MIPPy coated GCE with 0 (curves A, B and C, respectively) and 1×10^{-5} M of L-tyrosine (curves D, E and F, respectively).

Table 2
Effect of metal ion incorporation on tyrosine determination with MIPPY coated GCE.

Sl. no.	Metal ion	I_p (μA)	Stability constants
1	Copper (II)	0.58	14.5
2	Palladium (II)	0.43	–
3	Gold (III)	0.5	–
4	Platinum (IV)	0.43	–
5	Mercury (II)	0.12	19.5
6	Zinc (II)	0.30	8.31
7	Nickel (II)	0.20	8.61
8	Lead (II)	0.02	8.56
9	Manganese (II)	0.01	2.4
10	Cadmium (II)	0	6.4

3.3. Differential pulse voltammetric studies

3.3.1. Influence of metal ion

Effect of metal ions incorporation (by in situ methods as described in Section 2.3.3) on the electro oxidation of tyrosine was investigated. Enhanced analytical signals were observed in upon addition of different metal ions as shown in Table 2. The tyrosine responses were in the order Cu (II) > Au (III) > Pd (II) ~ Pt (IV) > Zn (II) > Ni (II) > Hg (II) < Pb (II) < Mn (II) < Cd (II) and are not according to the stability constants of metal–tyrosine complexes (see Table 2). Furthermore, the tyrosine response in case of copper ions, is maximum in pH 5.5 phosphate buffer (I_p = 0.58 μA). The effect of concentrations of copper in pH 5.5 phosphate buffered solution indicate that the tyrosine response is maximum when copper (II) concentration is in the range 3×10^5 to 10^{-4} M. Hence, 10^{-4} M of copper (II) was added in subsequent investigations. The higher analytical signal obtained in presence of copper ions may be attributed to the formation of copper tyrosine phosphate complexes in solution which resemble the microenvironment of tyrosinase enzyme where a binuclear copper centre complexed with histidine ligands act as catalytic centre [31]. In the present case a similar scenario can be envisaged where the copper complexes present in solution as well as on the electrode surface enhances the catalytic oxidation of tyrosine.

3.3.2. Effect of pH and buffered medium

The effect of pH on differential pulse voltammetric signal of 10^{-6} M tyrosine was studied in the range pH 2–9 in steps of 0.5 employing in situ copper oxide modified MIPPy film coated GCE. From the results obtained, it is observed that the highest tyrosine analytical signal is obtained in pH 5.5 solutions, the isoelectric point of tyrosine. Of the various buffers investigated, pH 5.5 phosphate (0.58 μA) gave highest analytical signal compared to pH 5.5 acetate buffer media (0.54 μA), pH 5.5 sodium acetate–NaCl (0.38 μA), pH 7 ammonium acetate (0.34 μA) and pH 9 tris buffer (0.05 μA) during sensing of 10^{-6} M of tyrosine. However, analogous response profiles were obtained for different concentrations of tyrosine in pH 7 phosphate buffered media albeit with slight diminution in sensitivity (Fig. S_1).

3.4. Sensitivity and precision

Electroanalytical characteristics of in situ copper oxide modified MIPPy coated GCE is shown in Table 3. Under these optimal conditions, the variation in anodic peak current with tyrosine concentration employing in situ copper oxide modified MIPPy coated GCE is given in Fig. 5. They show linear relationship with tyrosine concentration in the range 1×10^{-8} to 8×10^{-6} with a change of slope at 1×10^{-6} M. The limit of detection corresponding to three times the signal to noise ratio (where anodic peak is still discernible) is 4×10^{-9} M and limit of quantification of 6.3×10^{-9} M. In addition, under experimental conditions the in situ copper oxide

Table 3
Electroanalytical and electrocatalytic characteristics of in situ copper oxide modified MIPPy film coated GCE.

pH	5.5
$[Cu^{2+}]$M	3×10^{-5} to 10^{-4}
Sample medium	Phosphate buffer
Scanning mode	Differential pulse
Scan range (V vs. Ag/AgCl)	0–0.9
Modulation amplitude (mV)	100
Linear calibration range (M)	10^{-8} to 10^{-6} and 2×10^{-6} to 8×10^{-6}
LOD (M)	4×10^{-9}
LOQ (M)	6×10^{-9}
RSD (10^{-6} M) tyrosine	2.47%
Diffusion coefficient (D)	1.88×10^{-6} cm^2 s^{-1}
Electron transfer coefficient (α)	0.47
Charge transfer rate constant (k)	4.7×10^6 L mol^{-1} s^{-1}

modified MIPPy coated GCE shows good reproducibility and stability (up to 30 scans). When 1 μM of tyrosine is determined three times, the relative standard deviation (RSD) of peak current for in situ copper oxide modified MIPPy electrode is 2.47%. Fig.S$_1$ shows analogous calibration graph obtained with response profiles drawn at pH 7.0 phosphate buffered solutions instead of pH 5.5.

3.5. Selectivity studies

Molecular recognition of biomolecules especially demands high selectivities in view of the complexity of the biological fluids and coexistence of several structurally and functionally similar compounds. Detailed selectivity studies were undertaken with in situ copper oxide modified MIPPy and NIPPy coated GCE for tyrosine and other amino acids. The results obtained are shown in Fig. 6. The selectivity factors for tyrosine in admixtures show about 50–60 fold selectivities for tyrosine over serine, cysteine, valine, phenylalanine and leucine, 1.2 fold over D-tyrosine and 1.5 fold for tryptophan, respectively. Molecular imprinting resulted in better selectivity factors with both individual and in admixtures (mainly undertaken to simulate real sample solution) showing significant imprinting effect. Thus, the developed biomimetic inorganic–organic hybrid copper oxide modified polypyrrole film based sensor has shown excellent selectivities for tyrosine with respect to other amino acids.

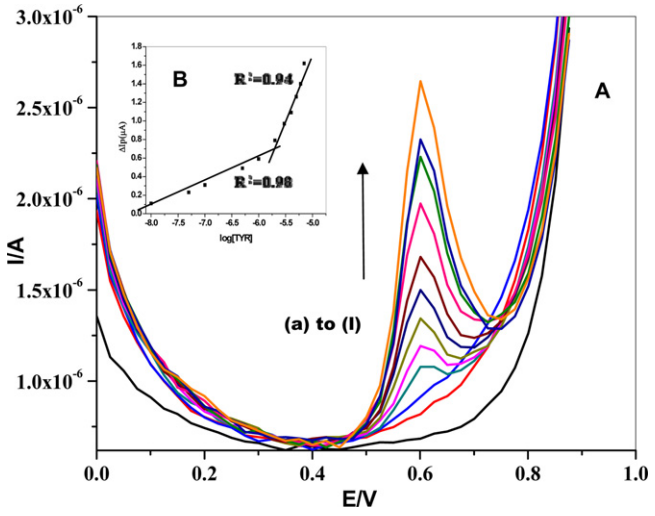

Fig. 5. (A) Differential pulse voltammograms of 0, 1×10^{-8}, 5×10^{-8}, 1×10^{-7}, 5×10^{-7}, 1×10^{-6}, 2×10^{-6}, 3×10^{-6}, 4×10^{-6}, 5×10^{-6}, 6×10^{-6} and 7×10^{-6} M (a to l) of tyrosine at pH 5.5. (B) Inset: calibration graph ($y_1 = 0.24x_1 + 2$ and $y_2 = 1.45x_2 + 9$).

Table 4
Analysis of human urine sample.

Sl. no.	Sample	Tyrosine added (M)	Tyrosine found (M)[a]	Recovery (%)
1	Human urine	–	3.3×10^{-7}	–
2		1×10^{-6}	1×10^{-6}	103.0 ± 6
3		2×10^{-6}	2.1×10^{-6}	99.6 ± 3.1%

[a] Average of three determinations.

3.6. Electrocatalytic studies

3.6.1. Sweep rate variation studies

The influence of sweep rate on linear sweep voltammetry of 1×10^{-5} M of tyrosine in phosphate buffer (pH 5.5) solution containing 10^{-4} M of copper (II) is shown in Fig. S$_2$. At lower sweep rates, I_p vs. ν gives better linearity compared to I_p vs. $\nu^{1/2}$ indicating surface adsorption controlled processes are operative. However, at high sweep rates, I_p vs. $\nu^{1/2}$ gives better linearity compared to I_p vs. ν indicating the diffusion controlled process. Utilizing Randle–Sevick's equations and slope of I_p vs. $\nu^{1/2}$ plot the diffusion coefficient was calculated to be 1.88×10^{-6} cm^2 s^{-1}. From E_p vs. log ν plot (see Fig. S$_3$) and from $(E_p - E_{p1/2})$ values, the $n\alpha$ values were calculated to be 0.47. In general α values are likely to be in the range $0.2 > \alpha < 0.7$, from which the electron transfer rate constants and number of electrons involved in tyrosine oxidation are 0.47 and 1, respectively.

3.6.2. Single step chrono amperommetric studies

Single step chrono amperommetric curves (A, B and C) and I_C/I_L vs. $t^{1/2}$ plots (see insets) for bare GCE, MIPPY coated GCE and in situ copper oxide modified MIPPY coated GCE are shown in Fig. S$_4$ where I_C is the catalytic current and I_L denotes limiting current. From the slope of I_C/I_L vs. $t^{1/2}$ plot and using Cottrell's equation, the charge transfer rate constants calculated for bare GCE, MIPPY coated GCE and in situ copper oxide modified MIPPY coated GCE are 0.26×10^6 L mol^{-1} s^{-1}, 1.25×10^6 L mol^{-1} s^{-1} and 4.7×10^6 L mol^{-1} s^{-1}, respectively.

3.7. Stability and reusability of the sensor

The developed sensor was found to be stable for three months. The sensing substrate can be regenerated in the case of MIPPy modified and copper oxide modified MIPPy film by following the procedure as given in Section 2.3.4. The regenerated sensor can be used about 25–30 repetitive determinations with negligible deviations from the standard values.

3.8. Analytical application

Since the developed voltammetric sensor has shown excellent selectivity for tyrosine when compared to other coexisting amino acids due to the imprinting effect, we have applied the developed sensor for the analysis of synthetic and real urine samples. Analysis of urine samples spiked with 1×10^{-7}, 2×10^{-6}, 3×10^{-6} and 4×10^{-6} M amounts of tyrosine in presence of admixtures of serine, alanine, valine, phenyl alanine and leucine in the proportion corresponding to human urine sample resulted in recoveries of 106.0, 97.5, 103.0 and 97.5%, respectively (for details see Table S$_1$ of supporting information). The applicability of the developed sensing strategy for analyzing tyrosine in human urine sample was carried out (see Table 4). Table 4 also shows quantitative recoveries upon spiking known amounts of tyrosine to human urine samples.

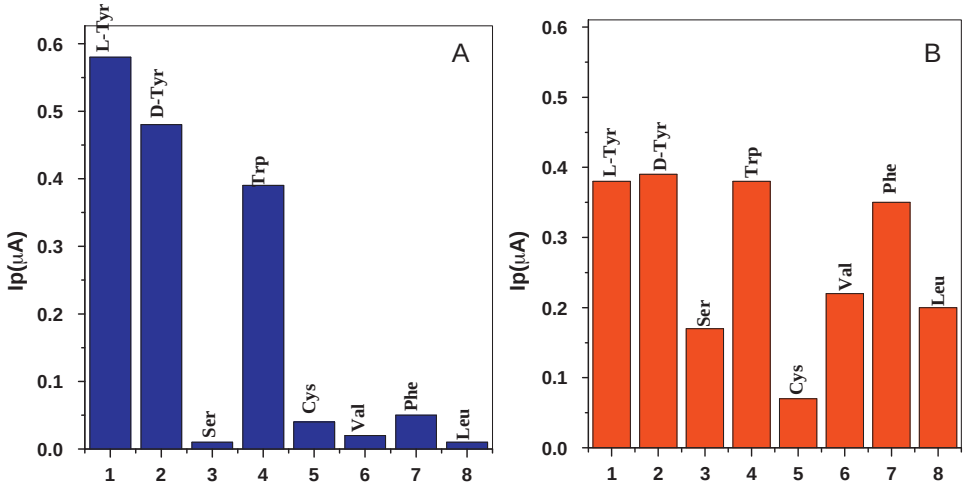

Fig. 6. Voltammetric sensing signals for molecularly imprinted (A) and corresponding non imprinted (B) in situ copper oxide modified polypyrrole films with various amino acids [Cu (II)] = 1×10^{-4}, [aminoacid] = 1×10^{-6} M, [pyrrole] = 4×10^{-3} M, E_p = 0.6 V.

4. Conclusions

In conclusion, we have introduced a new strategy for the design and development of hybrid biomimetic amino acids (L-tyrosine) sensing film. The in situ metal oxide modified molecularly imprinted polypyrrole (MIPPy) coated GCE based sensors were designed by combining molecular imprinting (enables better selectivity) and conducting polymer-polypyrrole (results in mechanically stable, electrically conducting film from neutral solutions) for detection and quantification of tyrosine in aqueous media. In addition the studies shows judicious choice of such bioinspired approaches can generate excellent sensing platforms. The results indicated that incorporation of copper gave better sensitivity over other metal ions during metal oxide modification of MIPPy film coated GCE. Again, detailed selectivity studies indicated that in situ copper oxide modified MIPPy film coated sensor resulted in significant improvement in selectivity over corresponding non-imprinted film. Electrokinetic studies revealed the changeover from adsorption controlled to diffusion controlled processes as well as better electron transfer and charge transfer rate constants for the developed inorganic–organic hybrid metal oxide modified MIPPy film coated sensors. In addition, the application potential of the developed sensor for analysis of synthetic and real urine samples have been demonstrated. Further studies are envisaged to design such sensing surfaces for biomacromolecules like peptides, nucleic acids, biotoxins which are extremely important in early stages diagnosis and treatment.

Acknowledgments

Ms. Varghese Saumya and Mr. K.P. Prathish, thank CSIR, New Delhi for awarding senior research fellowships. The authors thank CSIR, New Delhi for funding to procure Electrochemical analyzer through Network project on “Functional organic materials for energy efficient devices” (NWP0023).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.05.025.

References

[1] J.P. Jin, X.Q. Lin, Electrochem. Commun. 6 (2004) 454–460.
[2] J.P. Grienstein, M. Winitz, Chemistry of Amino Acids, vols. 1–3, Wiley, New York, Passim, 1961.
[3] C.R. Sciver, A.L. Beaudet, V.S. Sly, D. Valle, The Metabolic and Molecular Bases of Inherited Diseases, vols. 1–3, Mc Graw Hill, New York, 1995.
[4] D. Offen, A. Hochmaan, S. Gorodin, I. Ziv, A. Shirvan, A. Barzilai, E. Melamed, Adv. Neurol. 80 (1999) 265–269.
[5] J.A. Molina, F.J. Jimenenez-Jimenenez, P. Gomez, C. Vargas, J.A. Navarro, M. Ortí-Pareja, T. Gasalla, J. Benito-León, F. Bermejo, J. Arenas, J. Neurol. Sci. 150 (1997) 123–127.
[6] T. Heitzer, T. Schlizig, K. Krohn, T. Meinertx, T. Munzel, Circulation 104 (2001) 2673–2678.
[7] O. Meilhac, S. Ramachandran, K. Chiang, N. Santanam, S. Parthasarathy, Arterioscl. Throm. Vas. Biol. 21 (2001) 1681–1688.
[8] H. Ischriopoulos, A.B. al-Mehdi, A.B. Risher, Am. J. Physiol. 269 (1995) L158–L164.
[9] S. Sherlock, Diseases of the Liver and Biliary System, 8th ed., Blackwell Scientific Publications, Oxford, London, 1989.
[10] J.F. Leckman, W.K. Goodman, G.M. Anderson, M.A. Riddle, P.B. Chapppel, P.B. Mcwiggan Hardin, C.J. McDougle, L.D. Scahill, S.I. Ert, D.L. Pauls, D.J. Cohen, L.H. Price, Neurophysiol. Pharmocol. 12 (1995) 73–86.
[11] J.C. Garbutt, D.P. van Kammen, R.A. Lercine, J. Huijmans, J. Chromatogr. B 744 (2000) 183–188.
[12] Y. Azuma, M. Mackawa, Y. Kuwbara, Clin. Chem. 35 (1989) 1399–1403.
[13] F. Wang, K.Z. Wu, Y. Qing, Anal. Lett. 25 (1992) 1469–1478.
[14] L. Sabine, P.G. Jean, S. Joelle, B. Bernard, J. Chromatgr. B 696 (1997) 9–17.
[15] J. Wang, S. Mannino, C. Camera, M.P. Chatrathi, M. Scampicchio, J. Zima, J. Chromatogr A 1091 (2005) 177–182.
[16] M.J. Nozal, J.L. Bernal, M.L. Toribio, J.C. Diego, A. Ruiz, J. Chromatogr. A 1047 (2004) 137–146.
[17] G. Ravindran, W.L. Bryden, Food Chem. 89 (2005) 309–314.
[18] K. Huang, D. Luo, W. Xie, Y. Yu, Colloid Surf. B 61 (2008) 176–181.
[19] J. Xu, Y. Wang, Y. Xian, L. Jin, K. Tanaka, Talanta 60 (2003) 1123–1130.
[20] Z. Chen, K. Okamura, M. Hanaki, T. Nagaoka, Anal. Sci. 18 (2002) 427–432.
[21] Q. Xu, S. Wang, Microchim. Acta 151 (2005) 47–52.
[22] X. Yu, Z. Mai, Y. Xiao, X. Zou, Electroanalysis 20 (2008) 1246–1251.
[23] G. Zhao, Y. Qi, Y. Tian, Electroanalysis 18 (2006) 830–834.
[24] A. Babaei, S. Mirzakhani, B. Khalilzadeh, J. Braz. Chem. Soc. 20 (2009) 1862–1869.
[25] G. Jin, X. Peng, Q. Chen, Electroanalysis 20 (2008) 907–915.
[26] M. Vasjari, A. Merkoçi, J.P. Hart, S. Alegret, Microchim. Acta 150 (2005) 233–238.
[27] L. Wang, C. Ma, X. Zang, Y. Ren, Y. Yu, Fresen. J. Anal. Chem. 351 (1995) 689–691.
[28] H. Liang, T. Ling, J.-F. Rick, T. Chou, Anal. Chim. Acta 542 (2005) 83–89.
[29] L. Liu, F. Zhao, F. Xio, B. Zeng, Electroanalysis 20 (2008) 2148–2152.
[30] C. Li, Colloid Surf. B 50 (2006) 147–151.
[31] C. Alexander, H.S. Andersson, L.I. Andersson, R.J. Ansell, N. Kirsch, I.A. Nicholls, J. O'Mahony, M.J. Whitcombe, J. Mol. Recogn. 19 (2006) 106–180.
[32] L. Ozcan, M. Sahin, Y. Sahin, Sensors 8 (2008) 5792–5805.
[33] S. Ozkorucuklu, Y. Sahin, G. Alsancak, Sensors 8 (2008) 8463–8478.
[34] E. Pardieu, H. Cheap, C. Vedrine, M. Lazerges, Y. Lattach, F. Garnier, S. Remita, C. Pernelle, Anal. Chim. Acta 649 (2009) 236–245.
[35] L. Wen-Zhi, L. You-Qin, Sens. Actuators B: Chem. 141 (2009) 147–153.