Electrocatalytic oxidation of L-tryptophan using copper hexacyanoferrate film modified gold nanoparticle graphite-wax electrode

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Electrocatalytic oxidation of L-tryptophan using copper hexacyanoferrate film modified gold nanoparticle graphite-wax electrode

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ABSTRACT

A novel copper hexacyanoferrate (CuHCF) film modification on cysteamine (Cys)-gold nanoparticle (AuNp) graphite-wax (GW) composite electrode was achieved for the quantitative determination of L-Tryptophan (L-Trp) at a reduced overpotential of 400 mV in comparison with the bare Cys-AuNp-GW composite electrode. This modified electrode exhibited a well resolved pair of redox peaks corresponding to the hexacyanoferrate (II/III) reactions of CuHCF film at a formal potential of 0.65 V at a scan rate of $20\,mV\,s^{-1}$. Electrochemical impedance spectroscopy (EIS) studies with the modified electrode showed a very low charge transfer resistance to the electron transfer kinetics of Fe^{II}/Fe^{III} reactions. A linear range of 8.5×10^{-7} M to 1.2×10^{-4} M with a detection limit of 1.85×10^{-8} M was achieved for the determination of L-Trp with a sensitivity of $0.1198\,\mu A/\mu M$. The influence of ultrasonication on the stability of the CuHCF film modified electrode was investigated. In addition, the CuHCF film modified electrode displayed an excellent reproducibility towards the real time analysis of L-Trp in commercial milk samples.

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1. Introduction

L-Tryptophan (L-Trp) is one of the essential amino acids in protein biosynthesis of living organisms. L-Trp concentration is a sensitive parameter to monitor the biochemical balance of the brain. It is very essential for people with sleep deprivation, anxiety and mood enhancement due to its ability to increase brain levels of serotonin and melatonin. L-Trp functions as a biochemical precursor for serotonin (a neurotransmitter) and melatonin (a neurohormone) [1,2]. It is a routine constituent of most protein-based dietary foods. The common side effects in high dosage of L-Trp are drowsiness, nausea, dizziness, and loss of appetite. Therefore a rapid, simple, sensitive and low cost detection method for L-Trp is of great interest. To date, various methods have been reported on determination of L-Trp such as chemiluminescence [3], fluorometry [4], capillary electrophoresis [5], high-performance liquid chromatography [6] and spectrometry [7]. Electrochemical techniques have gained much attention for its high sensitivity, high accuracy and simple operation mode. The poor electron transfer behavior with high overpotential for L-Trp oxidation at the conventional electrodes has resulted in efforts to develop novel chemically modified electrodes (CMEs) to improve the sensitivity and reduce the overpotential for the electrochemical oxidation of L-Trp.

Self assembled monolayer (SAM) using organo-sulfur compounds to develop prussian blue (PB) analogues modified electrodes has attracted researchers to develop a tailored mono or multilayer modifications on bare electrodes either by electrodeposition or by anchoring redox mediators and dyes to improve the sensitivity and selectively of detection. Recently, few papers have reported SAM of metal hexacyanoferrates (MHCFs) using cysteamine such as cobalt hexacyanoferrate (CoHCF) immobilized on Au-colloid modified electrode for determination of thiosulphate [8] and nickel hexacyanoferrate (NiHCF)–polyamidoamine (PAMAM) dentrimer gold electrode [9] for potentiometric detection of K^+ ion. Cysteamine (2-aminoethanethiol), a short chain organic linker with a thiol (-SH) terminal at one end, which attaches itself to the gold nanoparticle (AuNp) present at the electrode and the amino ($-NH_2$) terminal that binds to the Cu^{2+} ion which was subsequently derivatized to give an insoluble inorganic ferrocyanide film on the electrode surface.

In the present work, a surface modified copper hexacyanoferrate (CuHCF) film on cysteamine (Cys) capped AuNp graphite wax (GW) composite surface (herein after called as CuHCF film modified electrode) was achieved using paraffin wax as the binder. The CuHCF film modified electrode fabricated by the above approach was found to exhibit an excellent electrocatalytic activity towards the oxidation of L-Trp. From the earlier reports [10,11], it is evident that the paraffin wax matrix exhibits a good stability, reproducibility

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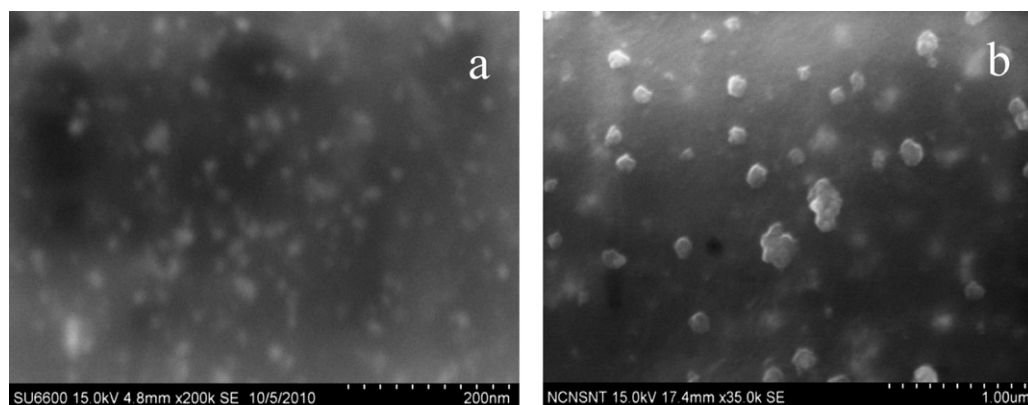*E-mail address:* sriman55@yahoo.com (S.S. Narayanan).

Fig. 1. FESEM images of (a) Cys–AuNp–GW electrode and (b) CuHCF film modified electrode.

and reliability for the MHCFs film electrodes and the ratio of wax to graphite determines the hardness and electron transfer properties of the modified electrode. Among the various reported methods for the electrocatalytic determination of L-Trp on Carbon nanofibers-carbon paste electrode (CNF-CPE) [12], Butyrylcholine/GCE [13], 4-aminobenzoic acid (ABA) polymer film modified GCE [14], Hybrid Cu-CoHCF modified electrode [15], Hemin/GCE [16], MWCNT-CoSal electrode [17] and AuNp deposited GCE [18], the present CuHCF film modified electrode displayed a better performance towards oxidation of L-Trp with a lower detection limit. The CuHCF film modified electrode was characterized using field emission scanning electron microscopy (FESEM). The influence of AuNp to the electrocatalytic behavior of CuHCF film modified electrode for the oxidation of L-Trp was also investigated. Ultrasonication gives rise to specific effects like acoustic streaming, shockwaves and cavitation which in turn leads to mechanical and chemical effects in any heterogeneous processes [19,20]. Hence an attempt was also made to study the influence of ultrasonication on the stability of CuHCF film modified electrode.

2. Experimental

2.1. Instruments and chemicals

All the electrochemical experiments were carried out with CHI 660 B Electrochemical Analyzer, using a cell volume of 60 mL with standard three electrode configuration. CuHCF film modified electrode was used as the working electrode, the platinum wire served as the auxiliary electrode and standard calomel electrode acted as the reference electrode. The experiments were carried out after purging the electrolyte solutions with pure nitrogen to ensure the solutions are free from oxygen. Spectroscopic grade graphite powder ($\approx$1–2 μm) purchased from Aldrich Chemicals, Germany was used for the construction of modified electrode. L-Tryptophan (Himedia), chloroauric acid, supporting electrolytes and all other reagents were of analytical grade. L-Tryptophan (0.01 M) was dissolved in double distilled (DD) water for 10–15 min using cyclomixer. Sonoelectrochemical studies were investigated with a cell containing 60 mL solution kept in the ultrasonic bath of frequency of 25 kHz. UV–vis characterization for the synthesized AuNp was carried out by a fiber optic spectrometer (Ocean optics, Inc. Florida, USA) with deuterium tungsten source lamp. Field emission scanning electron microscope (FESEM) images of the electrode surfaces were obtained using FESEM (Hitachi SU6600) at an accelerating voltage of 15 kV.

2.2. Construction of CuHCF film modified electrode

The CuHCF film modified electrode was constructed using the following procedure: Citrate capped AuNp was synthesized by sodium borohydride reduction method in accordance with earlier report [21]. Two grams of spectroscopic graphite powder was stirred with an optimized volume of 60 mL of AuNp suspension (0.012 mM) for 2 h. After centrifugation the graphite–AuNp composite powder was dried at room temperature. The above AuNp adsorbed graphite powder was further stirred with an optimized volume of 60 mL of 20 mM ethanolic solution of cysteamine for 2 h and the final residue was centrifuged, filtered and dried at room temperature. The cysteamine–AuNp–graphite composite was mixed with paraffin wax in the ratio of 4:1 in warm condition. Using a glass tube of 3 mm diameter, the cysteamine–AuNp–graphite composite powder was packed tightly into the glass tube and the composite electrode was gently pushed out of the tube. One end of composite electrode surface thus constructed was dipped in ethanolic solution of CuCl$_2$ (0.01 M) for 25 min and then derivatized using potassium ferrocyanide (0.02 M) in KNO$_3$ solution (0.1 M) by cycling 20 times at the scan rate of 50 mV s^{-1} in the potential range between −0.2 V to 1.0 V. After derivatization, the electrode was washed thoroughly with DD water. Similar surface modification of CuHCF film was carried out without AuNp to investigate the role of AuNp in the electrochemical behavior of CuHCF film modified electrode.

3. Results and discussion

3.1. FESEM images of the electrodes

The FESEM image presented in Fig. 1a confirms the presence of AuNp incorporated in Cys–AuNp–GW electrode and the size of AuNp ranges between 15–30 nm. This is in confirmation with the results obtained from the UV–vis absorption studies. Fig. 1b shows the FESEM image of CuHCF particles on CuHCF film modified electrode and the size ranges from 150 to 300 nm.

3.2. Electrochemical characterization of CuHCF film modified electrode

Prussian blue (PB) and its analogues exhibit an intrinsic zeolite structure which results in a selective permeability of various alkali and alkaline earth cations from the solution. The counter cations flow through the channels and holes of the zeolite structure is accompanied during the redox reactions at the CuHCF film. Hence the electrochemical response of CuHCF film modified elec-
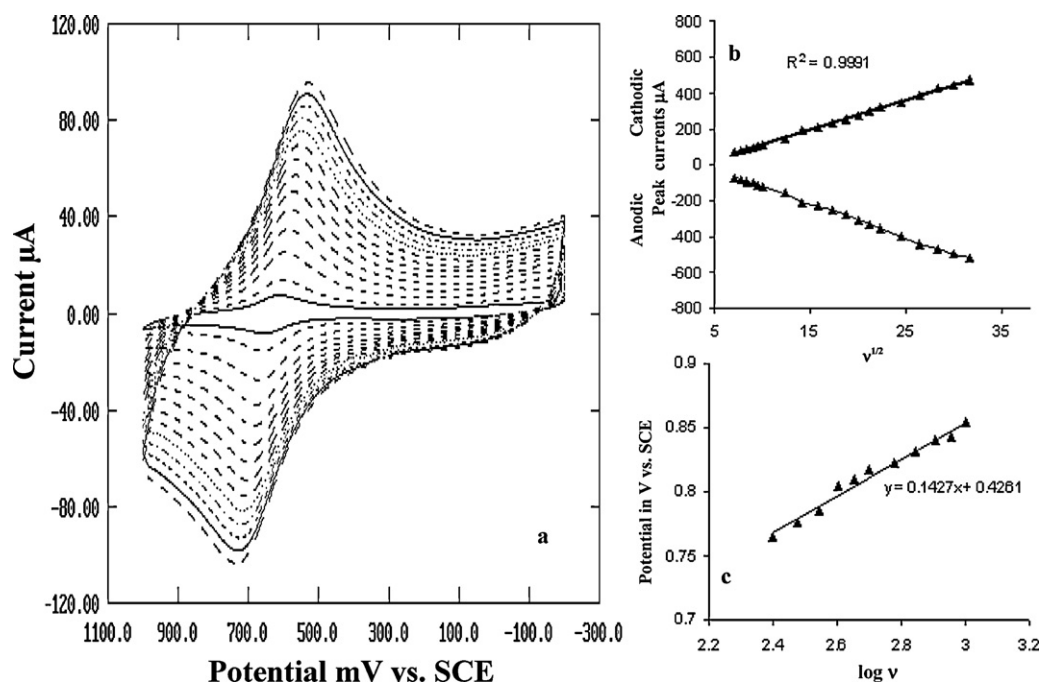
Fig. 2. (a) Cyclic voltammograms of CuHCF film modified electrode at different scan rates, from inside to outer are 5, 10–100 mV s^{-1} with increments of 10 mV s^{-1} in 0.1 M KCl. (b) Dependence of peak current I_{pa} and I_{pc} on square root of scan rate (ν). (c) Variation of peak potential vs. logarithm of scan rates (log ν).

trode was investigated in presence of K$^+$, Na$^+$, NH$_4^+$, Li$^+$, Ca^{2+} and Ba^{2+} ions in the solution. CuHCF film modified electrode showed a better electrochemical behavior in the solution containing K$^+$ ions. Redox peaks with a formal potential of 0.65 V corresponding to Cu^{II}/Fe^{II} to Cu^{II}/Fe^{III} transition of CuHCF film was observed. The redox reaction can be written as follows [22]:

$$KCu^{II}[Fe^{III}(CN)_6] + K^+ + e^- \leftrightarrow K_2Cu^{II}[Fe^{II}(CN)_6] \tag{1}$$
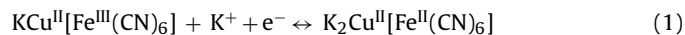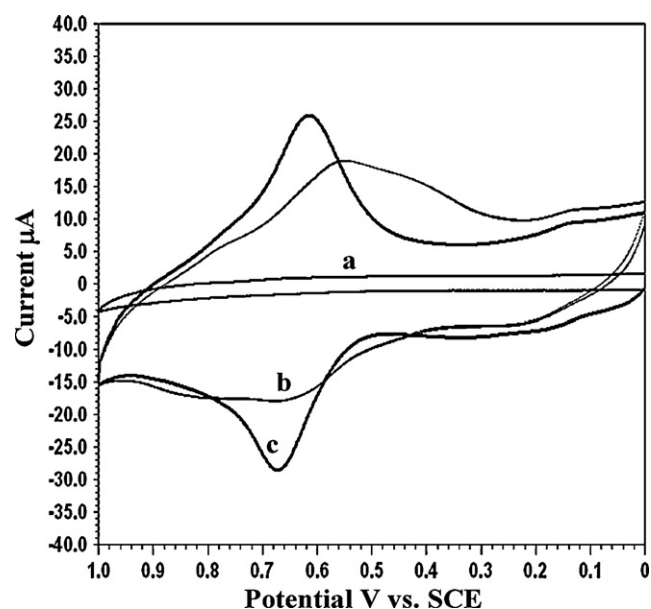
The CuHCF film modified electrode was subjected to different scan rates and it was observed that both the anodic and cathodic peak currents increased linearly with increase in scan rate and also with a corresponding shift in potential (Fig. 2a and b) which confirms that the redox reaction at this modified electrode is confined to diffusion controlled process. Based on the Laviron theory [23], the charge transfer coefficient (α) as well as the electron transfer rate constant (k_s) can be calculated from the slope obtained from the plot of potential vs. log ν (Fig. 2c) and they were found to be 0.76 and 0.99 s^{-1} respectively. The surface coverage of the CuHCF film modified electrode (Γ) was calculated to be 8.94 × 10^{-8} mol cm^{-2}. In order to investigate the role of AuNp, the cyclic voltammograms of CuHCF film modified electrodes with and without AuNp were recorded and are shown in (Fig. 3). It was observed that the CuHCF film modified electrode with AuNp has a higher peak current with a well resolved nerstian behavior (ΔE_p = 57 mV) as compared to the modified electrode without AuNp.

Electrochemical Impedance Spectroscopy (EIS) was used to investigate the surface bound features of the CuHCF film modified electrode at the electrode-electrolyte interface. Nyquist plots of the EIS for the bare Cys–AuNp–GW and CuHCF film modified electrode at varied potentials (0.62–0.70 V) were studied in the frequency range of 0.1 Hz to 1.0 MHz. EIS parameters such as charge transfer resistance (R_{ct}), double-layer capacitance (C_{dl}), solution resistance (R_s) and Warburg impedance (Z_w) for the electron transfer characteristics of the CuHCF film modified electrode at the formal potential of 0.65 V was found to be 28.35 kΩ cm^{-2}, 12.29 nF cm^{-2}, 303.6 kΩ cm^{-2} and 8.778 × 10^{-4} Ω cm^{-2} s$^{1/2}$ respectively and that of Cys–AuNp–GW electrode are respectively 21.76 kΩ cm^{-2}, 9.49 nF cm^{-2}, 382.5 kΩ cm^{-2} and 6.356 × 10^{-4} Ω cm^{-2} s$^{1/2}$. The

schematic representation of the modified Randle's equivalent circuit for this CuHCF film modified electrode is shown in Fig. 4a. The impedance data obtained for CuHCF film modified electrode at 0.65 V offer a lower resistance to the charge transfer kinetics as shown in Fig. 4b which confirms that this modified electrode is a very attractive for the electron transfer reactions.

3.3. Electrocatalytic oxidation of L-Trp at CuHCF film modified electrode

Cyclic voltammograms of the CuHCF film modified electrode, mechanically immobilized CuHCF modified electrode and


Fig. 3. Cyclic voltammograms of (a) Cys–AuNp–GW electrode, (b) CuHCF film modified electrode without AuNp and (c) CuHCF film modified electrode with AuNp in 0.1 M KCl solution; scan rate: 20 mV s^{-1}.

Table 1
Electroanalysis of L-Trp at the different electrodes and its results.

Electrode	Range of detection (μM)	Detection limit (μM)	Oxidation potential (V)	Sensitivity (μA/μM)	Ref
4-ABA polymer GCE	1.0–100	0.20	0.94	0.2825	[14]
MWCNT–CoSal-DPV	0.5–50	0.10	0.93	0.8969	[17]
AuNp/GCE	0.09–0.5	0.08	0.90	0.0766	[18]
Hemin/GCE	0.1–100	0.025	0.88	–	[16]
CNF–CPE	0.1–119	0.10	0.78	0.0124	[12]
Cu-CoHCF graphite	10.0–900	6.00	0.70	0.0460	[15]
Butyrylcholine-GCE	2.0–60	0.60	0.67	–	[13]
CuHCF film modified	0.85–120	0.018	0.65	0.1198	This work
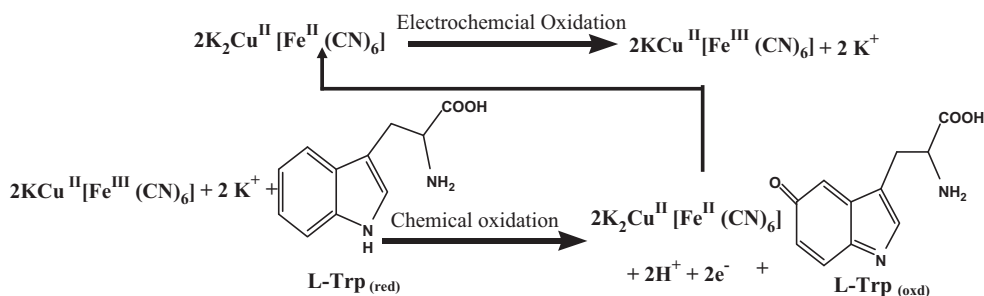
Scheme 1. Mechanism of electrocatalytic oxidation of L-Trp at CuHCF film modified electrode.

Cys–AuNp–GW electrode towards the oxidation of L-Trp were recorded. It was observed that the CuHCF film modified electrode exhibited a reduction in overpotential of about 240 mV and 400 mV for the L-Trp oxidation as compared to the mechanically immobilized CuHCF modified electrode and Cys–AuNp–GW electrode respectively (Fig. 5). When compared with recent literature reports on modified electrodes for the determination of L-Trp with 4-ABA/GCE [14], MWCNT-CoSal [17] and AuNp/GCE [18], it was found that the proposed CuHCF film modified electrode was able to oxidize L-Trp at very low potential of 650 mV which is about 300 mV less as compared to the 4-ABA/GCE method. The limit of detection and sensitivity of CuHCF film modified electrode is comparable with the reported methods as given in Table 1. A very good electrocatalytic activity towards L-Trp oxidation was achieved by the CuHCF film modified electrode with a linear increase in catalytic current

($R^2 = 0.9988$) with increase in the concentration of L-Trp. The determination range was 8.5×10^{-7} M to 1.2×10^{-4} M, with a limit of detection of 1.85×10^{-8} M and with a sensitivity of 0.1198 μA/μM. The presence of AuNp in the composite matrix has offered a stable platform to the immobilization of CuHCF film and in addition it has improved the electrocatalytic activity of CuHCF film modified electrode towards the oxidation of L-Trp. The oxidation mechanism of L-Trp at CuHCF film modified electrode is shown in Scheme 1.

The effect of H⁺ ions (pH) towards the electrocatalytic oxidation of L-Trp using CuHCF film modified electrode is shown in the plot of pH versus peak current in presence and absence of L-Trp (Fig. 6). The CuHCF surface modified electrode exhibited a very stable current in a wide pH range of 2–10 and a maximum current response
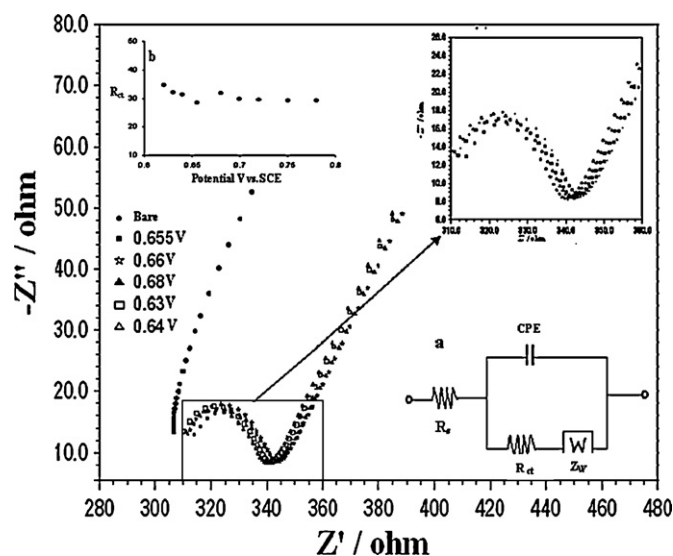
Fig. 4. Nyquist plots of the CuHCF film modified electrode and Cys–AuNp–GW electrode obtained at various potentials from scanning over a range of 1 Hz to 0.1 MHz: (a) Modified Randels equivalent circuit diagram (b) Plot of potential (V) versus charge transfer resistance (R_{ct}).
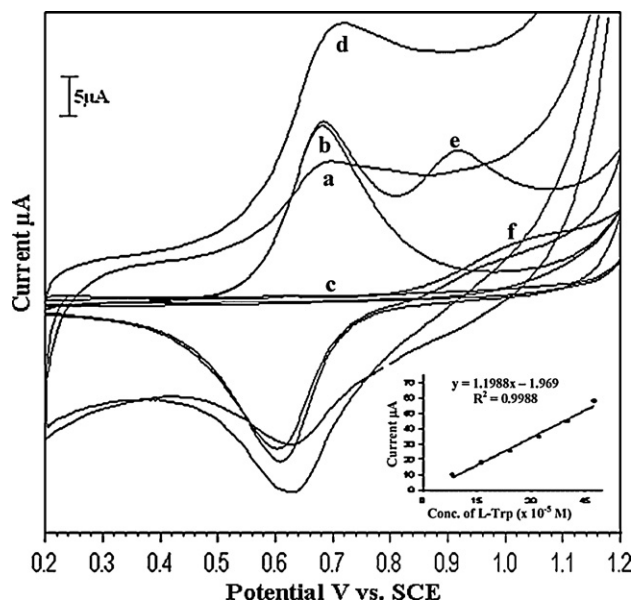
Fig. 5. Cyclic voltammograms of CuHCF film modified electrode, mechanically immobilized CuHCF modified electrode and Cys–AuNp–GW electrode respectively in absence (curves a, b, c) of L-Trp and in presence (d, e, f) of 1.0×10^{-4} M of L-Trp respectively in 0.1 M KCl solution; scan rate: 20 mV s⁻¹.

Table 2
Real sample analysis of L-Trp from milk samples ($n = 5$).

Sample	Concentration of L-Trp			RSD	Recovery
	Added (10^{-5} M)	Found (10^{-5} M)	Milk sample (mean ± SD) (10^{-5} M)	(%)	(mg/500 mL)
MS-1	25.0	29.15 ± 0.14	4.15 ± 0.14		
	50.0	54.23 ± 0.10	4.23 ± 0.10	1.36	42.0
	75.0	79.07 ± 0.22	4.07 ± 0.22		
MS-2	25.0	29.01 ± 0.28	4.01 ± 0.28		
	50.0	54.14 ± 0.17	4.14 ± 0.17	1.69	41.2
	75.0	78.97 ± 0.05	3.97 ± 0.05		

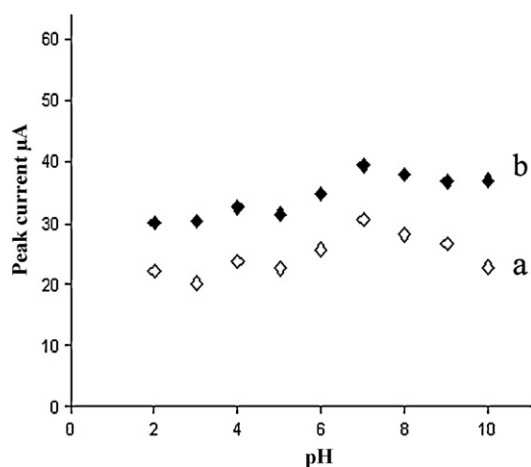* L-Trp was determined from two different branded milk samples (MS-1 and MS-2).

was observed at the neutral (pH 7) which makes this CuHCF film modified electrode highly suitable for use in real time applications.

3.4. Hydrodynamic study

The applicability of CuHCF film modified electrode in flow systems was studied for the determination of L-Trp at dynamic condition. Fig. 7 shows the plot of potential versus anodic current for the oxidation of 9.75×10^{-5} M of L-Trp at different electrodes. It is evident from the plot that the mechanically immobilized CuHCF modified electrode and Cys–AuNp–GW electrode oxidize L-Trp at higher potentials of 900 mV and 1000 mV respectively. But at the CuHCF film modified electrode, the L-Trp oxidation starts well before 585 mV and it attains a maximum current at 650 mV and drops down.

3.5. Interference study

Among the various amino acids, L-Tyrosine (L-Tyr) is found to coexist with L-Trp. The interference of L-Tyr in the determination of L-Trp under different concentrations was investigated. The results showed that L-Trp determination was interference free even at sixty fold concentrations of L-Tyr. Differential pulse voltammograms (DPVs) of CuHCF film modified electrode for the oxidation of 8.0×10^{-5} M L-Trp in presence of 2.5×10^{-3} M L-Tyr showed negligible interference. In addition, the effect of high concentrations of mono and divalent ions like Na^+, K^+, NH_4^+, Ca^{2+}, Ba^{2+}, Mn^{2+}, Zn^{2+}, Cl^-, SO_4^{2-} and NO_3^- did not interfere in L-Trp determination.

3.6. Analysis of L-Trp in milk sample

Real sample analysis of L-Trp content in two different brands of milk bought from commercial market was investigated with the CuHCF film modified electrode using similar procedure as detailed below: 5 mL of the boiled milk was filtered and diluted to ten times. To 2 mL of diluted milk sample, standard L-Trp solution was added and the standard L-Trp spiked milk sample was subjected to analysis. The recovery of L-Trp from the milk sample is satisfactory for the proposed method and the results are shown in Table 2.

3.7. Reproducibility of the CuHCF film modified electrode

Chronoamperometric response for CuHCF film modified electrode under sonication was investigated for 1000 s at a fixed potential of 0.65 V. A stable peak current response revealed the high reproducibility and reliability of the CuHCF film modified electrode prepared as above. The plot of peak current versus time measured at intra days for a period of 25 days in presence of 7.0×10^{-5} M of L-Trp showed a reliable and reproducible CuHCF film modified electrode even at ultrasonic dynamic conditions (Fig. 8).
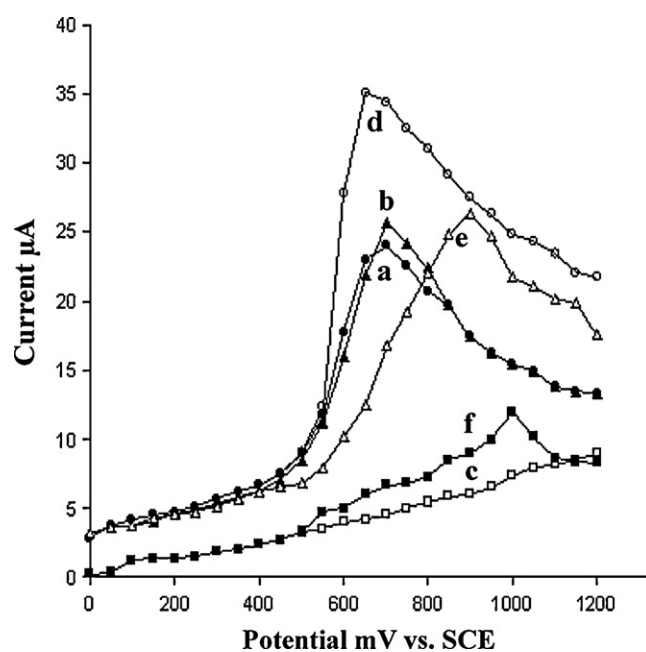

Fig. 6. Effect of pH for the peak current response of CuHCF film modified electrode (a) in absence and presence of (b) 7.5×10^{-5} M of L-Trp in 0.1 M KCl solution; scan rate: 20 mV s^{-1}.

Fig. 7. Hydrodynamic voltammograms of CuHCF film modified electrode, mechanically immobilized CuHCF modified graphite electrode and Cys–AuNp–GW electrode respectively in absence (a, b, c) of L-Trp and in presence (d, e, f) of 9.75×10^{-5} M of L-Trp respectively in 0.1 M KCl, scan rate: 20 mV s^{-1}, stirring rate 300 rpm.
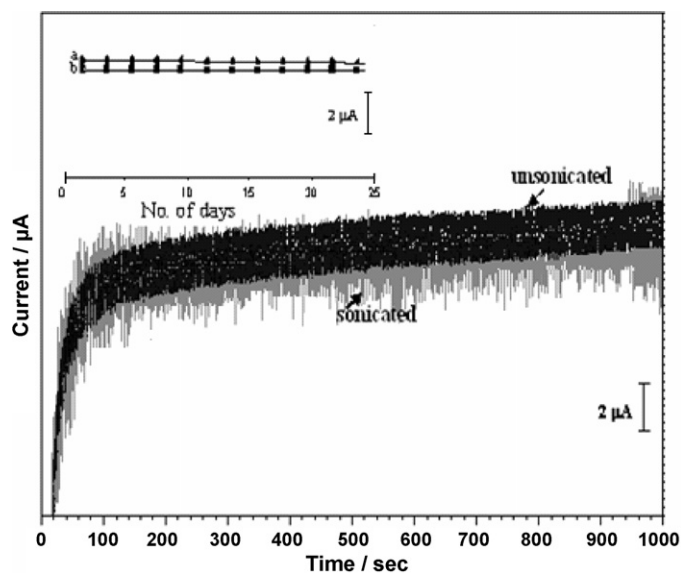
Fig. 8. Amperometric response of CuHCF film modified electrode in presence 7.0×10^{-5} M of L-Trp at an applied potential of 0.65 V in absence and presence of sonication in 60 mL of 0.1 M KCl at the scan rate of 20 mV s^{-1}, stirring rate 300 rpm. Inset: shows the plot of amperometric current versus no. of days (a) in absence and (b) in presence of sonication.

4. Conclusion

The present study has resulted in a simple fabrication approach for a novel surface modified CuHCF electrode. The electrochemical impedance studies showed that this CuHCF film modified electrode exhibited a low resistance to transfer of electrons. It also exhibited an excellent electrocatalytic activity towards L-Trp oxidation with a reduction in overpotential of 400 mV. The limit of detection was 0.018 μM with a good sensitivity of 0.1198 μA/μM. A successful determination of L-Trp from spiked milk samples was also achieved using CuHCF film modified electrode.

References

[1] A.A. Ensafi, R. Hajian, Anal. Chim. Acta 580 (2006) 236.
[2] A.R. Fiorucci, E.T.G. Cavalheiro, J. Pharm. Biomed. Anal. 28 (2002) 909.
[3] Z.J. Lin, X.M. Chen, Z.M. Cai, P.W. Li, X. Chen, X.R. Wang, Talanta 75 (2008) 544.
[4] E. Sikorska, A.G. ska-Swigło, M. Insinska-Rak, I. Khmelinskii, D. Keukeleire, M. Sikorska, Anal. Chim. Acta 613 (2008) 207.
[5] I. Ilisz, G. Fodor, R. Berkecz, R. Iványi, L. Szente, A. Pétera, J. Chromatogr. A 1216 (2009) 3360.
[6] Y. Dale, V. Mackey, R. Mushi, A. Nyanda, M. Maleque, J. Ike, J. Chromatogr. B 788 (2003) 1.
[7] C.J. Lee, J. Yang, Anal. Biochem. 359 (2006) 124.
[8] W. Tao, D. Pan, Y. Liu, L. Nie, S. Yao, J. Electroanal. Chem. 572 (2004) 109.
[9] J.A. Lopez, J. Manrıoquez, S. Mendoza, L.A. Godıonez, Electrochem. Commun. 9 (2007) 2133.
[10] S.J. Richard Prabakar, S. Sriman Narayanan, Talanta 72 (2007) 1818.
[11] D. Jayasri, S. Sriman Narayanan, Food Chem. 101 (2007) 607.
[12] X. Tang, Y. Liu, H. Hou, T. You, Talanta 80 (2010) 2182.
[13] G.P. Jin, X.Q. Lin, Electrochem. Commun. 6 (2004) 454.
[14] K.J. Huang, C.X. Xu, W.Z. Xie, W. Wang, Colloids Surf. B: Biointerfaces 74 (2009) 167.
[15] Y. Liu, L. Xu, Sensors 7 (2007) 2446.
[16] C.G. Nan, Z.Z. Feng, W.X. Li, D.J. Ping, C.H. Qin, Anal. Chim. Acta 452 (2002) 245.
[17] S. Shahrokhian, S.L. Fotouhi, Sens. Actuators B: Chem. 123 (2007) 942.
[18] C. Li, Y.G. Zhan, Colloids Surf. B: Biointerfaces 76 (2010) 340.
[19] D.J. Walton, S.S. Phull, A. Chyla, J.P. Lorimer, T.J. Mason, L. Burke, M. Murphy, R.G. Compton, J.C. Eklund, S.D. Page, J. Appl. Electrochem. 25 (1995) 1083.
[20] K.B. Holt, J.D. Campo, J.S. Foord, R.G. Compton, F. Marken, J. Electroanal. Chem. 513 (2001) 94.
[21] K.R. Brown, D.G. Walter, M.J. Natan, Chem. Mater. 12 (2000) 306.
[22] P. Baioni, M. Vidotti, P.A. Fiorito, E.A. Porizio, S.I. Cordoba de Torresi, Langmuir 23 (2007) 6796.
[23] E. Laviron, J. Electroanal Chem. 101 (1979) 19.