International Journal of Pharmaceutical Research and Development (IJPRD)
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# AMPEROMETRIC DETERMINATION OF L-CYSTEINE USING RUTHENIUM HEXACYANOFERRATE FILM MODIFIED ELECTRODE

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***ABSTRACT***

*A unique ruthenium hexacyanoferrate (RuHCF) film modified on the surface of cysteamine functionalised gold nanoparticles (GNP) graphite – wax composite electrode was fabricated by a simple technique. The modified electrode was characterised by cyclic voltammetry and applied for the electrocatalytic oxidation of L-cystiene which is a biologically important. The modified electrode showed a good electrocatalytic activity towards the oxidation of L-cystiene at a reduced over potential. The electrocatalytic oxidation of L-cystiene at the modified electrode was investigated by cyclic voltammetry, hydrodynamic voltammetry and chronoamperometric techniques. The determination range for the oxidation of L-cystiene was found to be $5.9\times10^{-6}$ – $1.02 \times 10^{-3}$ M with the detection limit of 1.96 µM. The sensor showed a constant and reproducible response for more than two months when stored at 4º C.*

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***KEYWORDS :*** *Ruthenium hexacyanoferrate; Modified electrode; L-cystiene; amperometric determination; Electrocatalytic oxidation*

## INTRODUCTION

L-Cysteine, (2-amino-3-mercaptopropionic acid) a sulfur-containing amino acid, is a conditional essential amino acid under certain circumstances, for example, in the case of premature neonates [1]. It is found in most food with high protein content such as yogurt, pork, sausage, duck, wheat germ, oat flakes as well as garlic, onions, broccoli and red peppers [2,3]. It has been used as a radio-protective agent, cancer indicator, and implicated in a number of pathological conditions, including Alzheimer's and Parkinson's diseases [4]. Low levels of L-Cysteine (L-cys) causes certain diseases including slow growth in children, depigmentation of hair, edema, lethargy, liver damage, loss of muscle and fat, skin lesions and weakness [5]. Therefore, measuring L-cys in body fluids is very important from biological and

pharmacological stand points, where much effort has been made to develop sensitive methods for its detection. Several methods for its determination have been reported including chemiluminescence [6], high-performance liquid chromatography [7], fluorimetry [8] and electrochemistry [9,10]. Electrochemical techniques which are cost effective, sensitive and reproducible have been used to study the electrochemical oxidation, detection and determination of l-cysand its derivatives [14].

The chemically modified electrodes (CME) have been developed to overcome the drawbacks such as poor electron transfer, high potential oxidation of L-cystiene at the conventional electrodes. The Prussian blue (PB) analogues modified electrodes has attracted researchers to develop a tailored mono or multilayer modifications on bare electrodes either by electro deposition or by anchoring redox mediators and dyes to improve the sensitivity and selectivity of detection. Recently, few papers have reported self assembled monolayer (SAM) of metal hexacyanoferrates (MHCFs) using cysteamine such as cobalt hexacyanoferrate (CoHCF) immobilized on Au-colloid modified electrode for determination of thiosulphate [12] and nickel hexacyanoferrate (NiHCF)–polyamidoamine (PAMAM) dendrimer gold electrode [13] for potentiometric detection of $K^+$ ion and copper hexacyanoferrate for the determination of L-tryptophan [14]. In the present investigation, cysteamine (2-aminoethanethiol), a short chain organic linker with a thiol (-SH) terminal at one end and amino group at the other end was used as a cross linker. The thiol group attaches itself to the gold nanoparticle (GNP) which is present at the electrode and the amino ($–NH_2$) terminal is made to bind with the $Ru^{3+}$ ion which was subsequently derivatized to give an insoluble inorganic ferrocyanide film on the electrode surface. The proposed modified electrode shows excellent electrocatalytic activity towards the determination of L-cys with good selectivity, sensitivity and reproducibility.

## EXPERIMENTAL

### Chemicals and reagents

Graphite powder (1 – 2 µm) and ruthenium chloride ($RuCl_3.3H_2O$) were purchased from Aldrich chemicals, Germany. L- cysteine hydrochloride monohydrate and potassium ferrocyanide were purchased from Himedia Laboratories and Merck, respectively. All other reagents were of analytical grade and all the aqueous solutions were prepared using double distilled water. The adjustment of the solution pH was done using 0.01 M of HCl and NaOH.

**Instruments**

All electrochemical measurements were performed with CHI 660B electrochemical workstation (CH Instruments, USA) coupled with data acquisition and potential control. Electrochemical experiments were carried out using a conventional three-electrode system with the RuHCF film modified electrode as the working electrode, a Pt wire as the auxillary electrode and saturated calomel (SCE) as the reference electrode. All the experiments were performed at room temperature in a conventional electrochemical cell.

**Fabrication of RuHCF film modified electrode**

The fabrication procedure adopted is similar to that of CuHCF modified electrode [14]. An optimum volume of 100 mL of 6 µM GNP suspension (synthesized as reported by Brown *et al* [15]) was added to graphite powder of 2 g and the mixture was stirred for two and half hours at room temperature. The stirred mixture was centrifuged and dried. The above GNP adsorbed graphite powder was again stirred with 60 mL of 20 mM ethanolic solution of cysteamine followed by centrifugation. The resulting mixture was dried and thoroughly mixed with paraffin wax in the ratio of 4:1 on a water bath. Then this composite mixture was tightly packed into a small glass tube of 3 mm diameter and gently removed under warm condition. One surface of the electrode was polished and dipped in ethanol-water (1:1) solution of ruthenium chloride of 0.01 M followed by derivatising with potassium ferrocyanide of 0.001 M in 0.1 M $KNO_3$ in the potential window of -0.4 to 1.2 V at a scan rate of 50 $mVs^{-1}$ (15 cycles). Finally the electrode was washed with distilled water to remove the weakly adsorbed ferrocyanide and then dried.

## RESULTS AND DISCUSSION

### Electrochemical characterization of RuHCF film modified electrode:

The modified electrode showed well-defined two redox peaks when scanned between the potential of -0.4 to 1.2 V in 0.1 M $KNO_3$ at a scan rate of 50 $mVs^{-1}$ using cyclic voltammetry technique. The two peaks with the formal potentials of 0.15 and 0.81 V can be assigned to Fe (II) / Fe (III) and Ru(III)-O / Ru(IV)-O redox species, respectively as proposed by Chen *et al* at pH 7 [16]. The modified electrode was also characterized by varying the scan rate which showed that the peak current increased with increase in scan

rate. The **Fig.1** shows the linearity between peak current and square root of scan rate when plotted between them which imply that the redox process is a diffusion controlled process.

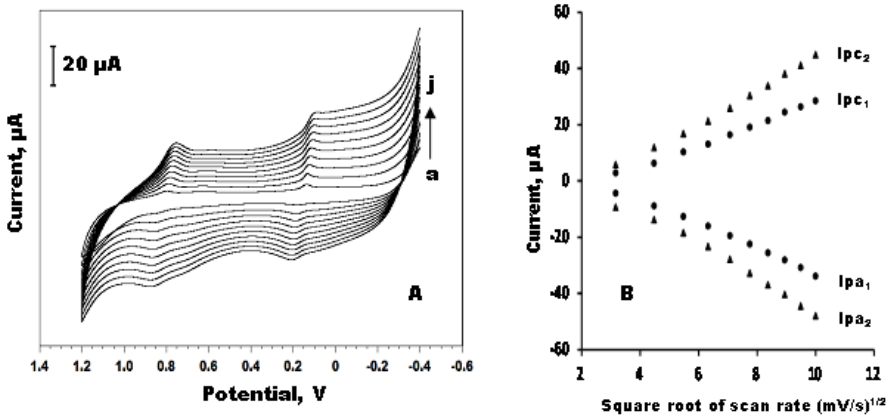


**Fig.1 (A)** Cyclic voltammograms of RuHCF film modified electrode at different scan rates, from a to j [10-100 $mVs^{-1}$] with increments of 10 $mVs^{-1}$ in 0.1 M $KNO_3$. (B) shows the dependence of peak currents on square root of scan rate (ν)

**Electrocatalytic activity of RuHCF modified electrode for the oxidation of L-cystiene.**

The electrocatalytic activity of the surface modified RuHCF film modified electrode was investigated for the oxidation of L-cys by cyclic voltammetry. The curve **a** in the **Fig.2 (A)** represents the cyclic voltammograms of the bare electrode and curve **b** shows the oxidation of 1.15 × $10^{-4}$ M L-cys at the bare electrode in 0.1 M $KNO_3$ as supporting electrolyte. The curve **c** corresponds to the CV of the modified electrode and curve **d** shows the oxidation of 1.15 × $10^{-4}$ M L-cys at the modified electrode. The CV of the modified electrode showed a prominent increase in the anodic peak current in the presence of L-cys. The increase in the current occurs at both the reaction centres viz, Fe(II) /Fe(III) and Ru(III)-O /Ru(IV)-O. But it was greater for Fe(II) /Fe(III) ie, at the potential of 0.24 V. Thus in the electrocatalysis of the present system, Fe(II) /Fe(III) was responsible for the oxidation of L-cys which is in agreement with literature [17]. The response obtained for the oxidation of L-cys at the bare electrode was very poor and occurs only at 0.9 V. As seen from the figure the over potential for the oxidation of L-cys is reduced when the modified electrode was used and the current response is also increased three fold which makes the modified electrode as an effective sensor.

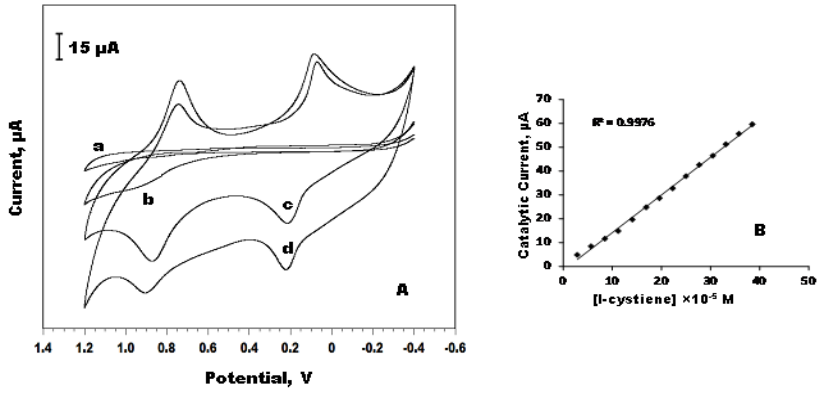


**Fig.2 (A)** Cyclic voltammograms of (a) bare electrode (b) bare electrode in presence of 1.15 × $10^{-4}$ M L-cys (c) RuHCF film modified electrode and (d) RuHCF film modified electrode in presence of 1.15 × $10^{-4}$ M L-cys in 0.1 M $KNO_3$; Scan rate: 50 mV/s; (B) Calibration plot for the catalytic oxidation of L-cys using the modified electrode

The increase in the current when an analyte was added indicates that the current response is directly related to the concentration of L-cys. A plot of catalytic current Vs concentration of L-cys gives a linear relationship which is shown in **Fig. 2 (B)**.The linearity was observed for the successive addition of $2.85\times10^{-5}$ M of L-cys with a sensitivity of 0.157 µA/µM. A linear relationship for determination of L-cys by the surface modified RuHCF GWCE was found in the range $5.9\times10^{-6}$ – $1.02\times10^{-3}$ M with the detection limit of 1.96 µM.

*Effect of pH*

The effect of pH on the catalytic activity of the modified electrode was tested in the presence of $5.7 \times 10^{-5}$M of L-cys by varying the pH of the medium which is shown in the **Fig.3**. According to the acid dissociation constants for cysteine, the major species of cysteine was in its zwitterion form ($H_2A$) in pH 2.0–8.0. The distribution fractions ranged from 54.5% (pH 2), 92.3% (pH 3), 99.0% (pH 4–6), 95.9% (pH 7) and 70.1% (pH 8). It was reasonable to suggest that the $H_2A$ species predominated in the irreversible oxidation reaction which occurred at in pH 2.0–8.0 [18]. The modified electrode showed maximum current response at pH 6. Hence, pH 6 was chosen for subsequent experiments.

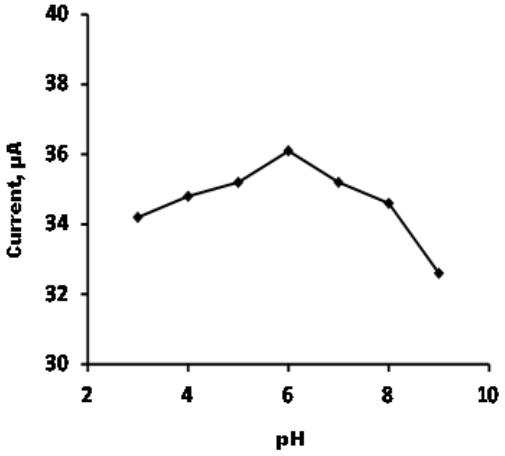


**Fig.3** Effect of pH on peak current of RuHCF GWCE in the presence of $5.7\times10^{-5}$ M of L-cys in 0.1 M $KNO_3$ at 50 $mVs^{-1}$

*Hydrodynamic Voltammetric Studies*

The electrocatalytic response of the RuHCF film modified electrode under dynamic condition was appraised by hydrodynamic voltammetric studies. The HDV curve demonstrates the variation of the current with the applied potential for the oxidation of L-cys with the modified electrode in the range 0 to 0.6 V and is shown in the **Fig.4**. The curve **a** in the figure represents the hydrodynamic voltammograms obtained for the modified electrode and curve **b** shows the response of the modified electrode with$2.3\times10^{-4}$ M of L-cys. The current of the modified electrode for the oxidation of L-cys started at a potential of 0.1 V and offered a higher sensitivity with the maximum response at 0.25 V and then finally reaches a plateau. Hence an operating potential of 0.3 V was chosen for amperometric studies.

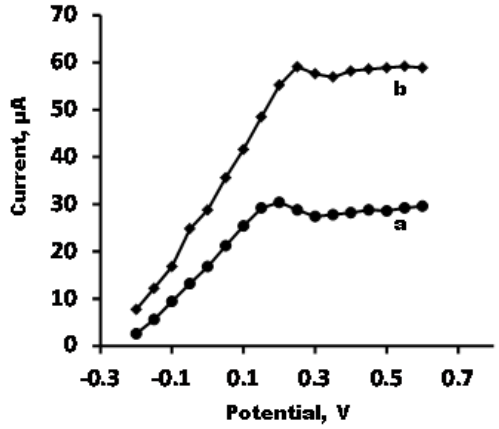


**Fig.4** Hydrodynamic voltammograms of (a) surface modified RuHCF film electrode and (b) with $2.3\times10^{-4}$ M L-cys in 0.1 M $KNO_3$ ; Stirring rate 300rpm

*Chronoamperometric studies*

The amperometric response of the surface modified RuHCF GWCE for the electrocatalytic oxidation of L-cys was studied by fixing the potential at 0.3 V. The current-time response of the modified electrode in a stirred solution (300rpm) for successive increments of $7.14\times10^{-5}$ M L-cys at pH 6 is shown in **Fig.5**. The current response increases with the increase in the concentration of L-cys . The inset of **Fig.5** represents the plot of catalytic current verses concentration of L-cys . A good linear response was obtained with a correlation coefficient of 0.9986. Such a good response of the modified electrode for oxidation of L-cys under dynamic conditions justifies its viable application in flow systems.

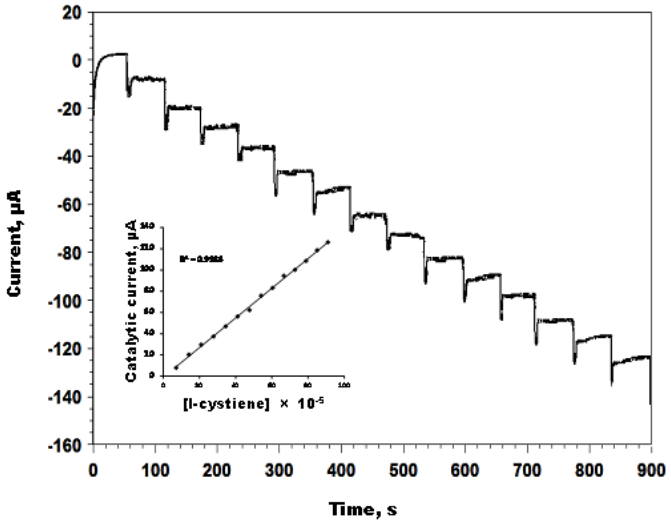


**Fig.5** Amperometric response of the modified electrode for the successive addition of 0.5 mL of 0.01M L-cys in 70 mL of 0.1 M $KNO_3$; Stirring rate: 300 rpm; applied potential 0.3 V; Inset: Corresponding calibration plot

*Interference studies*

The interference effects of various amino acids such as glycine, tyrosine, lysine and proline were investigated due to their coexistence in the biological samples. Tyrosine, proline and lysine were oxidised at higher potentials of 0.58, 0.9 and 1.2 V respectively at the modified electrode. Thus, it seems that tyrosine, proline and lysine didn't interfere in the determination of L-cys . It was found that a higher concentration of glycine interferes the determination of L-cys .

## CONCLUSION

The long term stability and the shelf life of the modified electrode were investigated and found that it showed good stability and suffered a minimal current loss of 4-5% for the determination of L-cys when stored at 4ºC for 60 days. Thus a novel surface modified RuHCF film electrode was fabricated and characterised by cyclic voltammetric technique. The proposed sensor was found to have excellent electrocatalytic activity towards the oxidation of L-cys. The electrode showed a stable amperometric response in the hydrodynamic systems, which makes it suitable for the electrochemical detection of L-cys in flow systems. While the present investigation has focused on the sensing of L-cys, the RuHCF film modified electrode should also benefit the detection of other clinically and environmentally important compounds.

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