

COPPER HEXACYANOFERRATE-BASED SENSOR FOR ANODIC DETERMINATION OF PARACETAMOL IN PURE FORM AND IN PHARMACEUTICAL TABLETS**R.A. KALAIVANI^{*,1,2} AND S. SRIMAN NARAYANAN^{2,3}**¹Department of Chemistry, School of Basic Sciences, VELS University, Pallavaram, 17, India²Department of Analytical Chemistry, School of Chemical Sciences, University of Madras, Guindy campus, Chennai – 600 025, India³National Centre for Nanoscience and Nanotechnology, University of Madras, Guindy campus, Chennai – 600 025, India**R.A. KALAIVANI****Department of Chemistry, School of Basic Sciences, VELS University,
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ABSTRACT

Paracetamol (Acetaminophen) is one of the most frequently used analgesic drugs. At therapeutic dosage levels the drug is relatively non-toxic. Because of its increasing therapeutic use, its assay and quality control are of vital importance. A number of methods, including instrumental and titrimetric are available for the quantitative determination of paracetamol in literature. A simple and accurate sol-gel method has been developed for the assay of paracetamol in pure form and in tablets using Copper hexacyanoferrate (CuHCF) modified carbon ceramic composite electrode. The electrode was characterized using voltammetric techniques and its electrocatalytic activity for the oxidation of paracetamol was also studied. The carbon composite electrode has the advantage of sensing paracetamol at a lower potential and with a higher sensitivity. The results obtained using the modified electrode was applied for the determination of paracetamol in commercially available tablets. The results obtained reveal that the electrode under study could be used as an effective sensor for online monitoring of paracetamol.

KEYWORDS

Sol-gel, CuHCF modified electrode, CCEs, Amperometry, Paracetamol

INTRODUCTION

There has been considerable interest in developing methods to measure compounds of medicinal and biological interest among which electrochemical technique has proven to be significantly advantageous. Paracetamol (*N*-acetyl-4-amino-phenol) is a popular analgesic and antipyretic medication that is readily absorbed after administration and has few side effects and little toxicity when used in recommended dose. After ingestion of an overdose quantity of paracetamol, the accumulation of toxic metabolites may cause severe and sometimes fatal hepatotoxicity and nephrotoxicity¹. So, the accurate determination of paracetamol in pharmaceutical preparations and biological fluids has appeared especially attractive. Therefore, development of a simple, economical and accurate analytical method for the determination of paracetamol would be useful for many commercial applications and also to investigate the stability of paracetamol in pharmaceutical preparations for quality control. For its measurement, many methods have been developed, such as fluorometry², chemiluminescence³, nuclear magnetic resonance-mass spectrometry^{4,5}, gas chromatography⁶, liquid chromatography^{7,8} and capillary electrophoresis⁹. Though these methods have been proven to be effective methods of analysis, they usually involve steps like preconcentration and take a longtime to perform. With the growing realization of the challenges in the determination of compounds of chemical and biological interest, there is tremendous demand to develop high performance catalytic surfaces that are reliable, efficient, and stable to long time operation under a variety of operating conditions and the possibility for miniaturization and also for on-line applications.

Chemically modified electrodes which are of recent interest as chemical sensors have attracted immense attention because of their sensitivity and selectivity in analysis of compounds of trace level concentrations. The slow electron transfer kinetics at the bare/unmodified electrode surface is substantially changed on modifying the bare electrode which in turn speeds up the electron transfer kinetics of the reaction at the surface and enables the reaction to occur at a faster rate and also at an appreciable reduction in over potential/voltage. Recently there has been renewed interest in the preparation and characterization of metal hexacyanoferrate as electro active material^{10,11}, among which copper hexacyanoferrate (CuHCF) is an important member of mixed-valence transition metal hexacyanoferrate, as it has proven to have excellent properties in electrocatalysis and electrochromicity¹²⁻¹⁴, its capacity to store counter cations^{15,16} and ion exchange selectivity¹⁷. CuHCF exhibits well defined and reproducible electrochemical responses because both the oxidized and reduced form of CuHCF seem to be fairly open and permit transport of alkali metal cations providing charge balance during redox reactions. All of the above traits make it an obvious candidate for electroanalytical applications such as electron transfer mediator, electrochromic devices and ion-selective devices since they show good electrocatalytic activity toward a variety of substrates including sulfite, glucose, *N*-acetylcysteine, and *L*-tryptophan, ascorbic acid, nitrite, hydrazine, NADH and hydroxylamine¹⁷.

In this paper, we report the fabrication, electrochemical characterizations, and electrocatalytic application of CuHCF surface modified composite electrode prepared by sol-

gel method towards paracetamol. Cu^{2+} ions which possess high affinity for the nitrogen of amine were coordinated to the amine of amino propyl trimethoxy silane (APT MOS). The coordinated transition metal was then derivatised to its stable hexacyanoferrate form by cycling in potassium ferrocyanide solution of suitable concentration to form a thin layer of CuHCF on the electrode surface. The CuHCF modified electrode exhibited excellent electrocatalytic activity towards the oxidation of paracetamol. This study has led to the development of a voltammetric method with good characteristics, such as simplicity of electrode preparation, high selectivity, wide linear dynamic range, and low detection limit. Finally the analytical performance of this electrode was applied for determination of paracetamol in commercial tablet samples too.

EXPERIMENTAL

Chemicals and reagents

All chemicals and solvents were of analytical grade. Distilled water was used throughout. Paracetamol was purchased from Himedia Laboratories (P) Ltd, Mumbai, India and Graphite powder was from Aldrich Chemicals, USA. Paracetamol tablets were purchased from a local drug store. Copper solution (0.01 M) was prepared by dissolving appropriate quantity in ethanol, respectively. KNO_3 (0.1M) was used as the background electrolyte. All measurements were done after carefully degassing the solutions with pure nitrogen for 10 –15 minutes.

A stock solution of paracetamol was prepared by dissolving appropriate quantity of the solid product in distilled water using an ultrasonic water bath to ensure a complete dissolution. This solution was stored in a dark bottle, and was highly stable for long time. Working standard solutions were prepared daily from the stock solution by diluting the stock solution. Studies on effect of pH were carried out using 0.1M solutions of HCl and NaOH.

Apparatus

All electrochemical experiments were carried out in a 100 mL electrochemical cell at 25 °C. A three - electrode assembly incorporating CuHCF modified CCE as working electrode, a calomel reference and a platinum auxiliary electrode were used in all measurements. Cyclic voltammetric measurements were performed with an Electrochemical workstation, (CH Instruments, 400A), USA controlled by an IBM personal computer equipped with a data acquisition and treatment software to record the signal generated in the electrochemical cell.

Tablet sample preparation

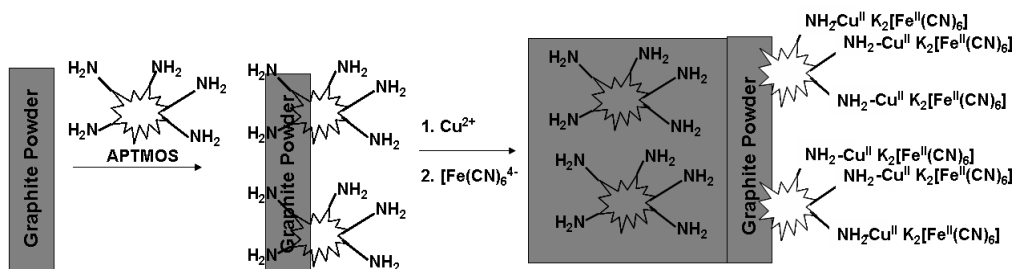
Six pharmaceutical tablets (500 mg paracetamol per tablet) were finely pulverized and dispersed in a 25mL of 0.5M acetic acid in a 100mL standard flask. The flask was shaken vigorously using a cyclomixer for about 10 min until most of the sample got dissolved and the mixture was centrifuged to obtain a clear solution which was filtered through a Whatmann 41 filter paper and finally diluted to the required concentration. The supernatant was adjusted to pH 7.0.

Electrode preparation

The surface modified CuHCF sol-gel composite electrode was prepared by a new approach using amino propyl trimethoxy silane (APT MOS) and methyl trimethoxy silane (MT MOS). The silica-gel based approach has demonstrated some benefits by using ceramic as binder in the fabrication of carbon ceramic composite electrodes (CCE). The hydrophobicity, polarity and rigidity can be controlled by chemical modification of the gel precursors. These advantages make it attractive choice in the preparation of electrodes with good conductivity, high stability and porosity. Graphite powder (550 mg), APT MOS (0.4 mL) and MT MOS (0.6 mL) were used for the fabrication of the sol-gel composite electrode. The surface of the electrode was modified with a monolayer of CuHCF. The modification followed a simple route, by just dipping the

electrode in ethanolic solution of Cu^{2+} followed by derivatising the metal ion to its hexacyanoferrate form. After cycling, the electrode was rinsed well with double distilled water. The modification procedure was

schematically represented in Scheme 1. The CuHCF layer thus formed is effectively attached to the electrode surface. The modified electrode displayed an excellent stability



Scheme 1.

Schematic representation of the entire fabrication approach of CuHCF modified electrode

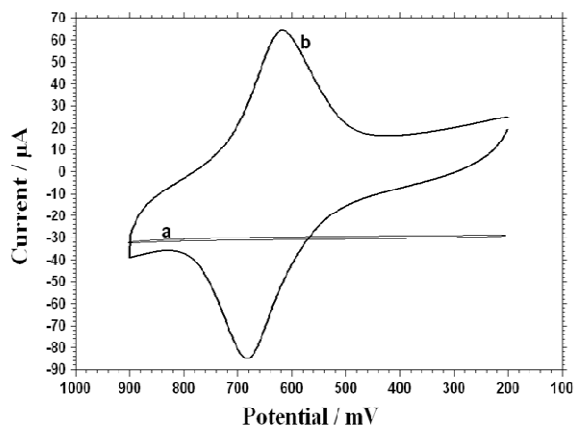
RESULT AND DISCUSSION

Electrochemical characterization of the modified electrode

A typical cyclic voltammograms of the CuHCF modified electrode recorded in 0.1M KNO_3 at 20mVs^{-1} is shown in figure 1. When the potential was scanned in the range of 0.2 to 0.9V, a cathodic peak at 0.615V and an anodic peak at 0.682V were observed for the modified electrode corresponding to the reduction and oxidation of the CuHCF. The formal potential, E_0

$= (E_{p_a} + E_{p_c})/2$ was found to be 0.651 V, where E_{p_a} and E_{p_c} are the anodic and cathodic peak potentials respectively. An E_0 of 0.651V with peak-to-peak separation (ΔE_p) of 0.067 V for the $\text{Fe}(\text{CN})_6^{4-}/\text{Fe}(\text{CN})_6^{3-}$ transition is observed which is analogous to those reported to the low spin iron transition in Prussian blue¹⁸. These typical characteristics of surface wave are well expected for this surface modified system

Figure 1.
Cyclic voltammograms of (a) Bare CCE and (b) CuHCF-CCE at a scan rate of 20 mV/s in 0.1M KNO_3



The cyclic voltammograms of the CuHCF modified CCE under different electrolytes were

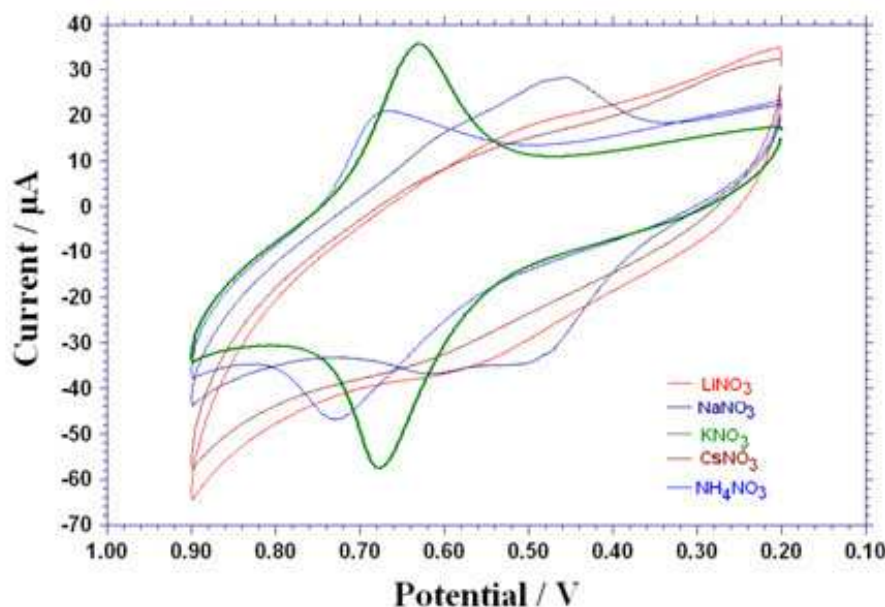
also studied. Figure 2 shows the voltammetric behavior of the CuHCF modified CCE in a

supporting electrolyte containing 0.1 M solutions of alkali metal cations such as Li^+ , Na^+ , K^+ and Cs^+ . The anodic and cathodic peaks observed with the modified electrode in the electrolyte solution containing the alkali cations decrease with the increase of the ionic radius, demonstrating that the counter-ions influences in the voltammetric behavior of the electrode. Redox potential shifts induced by the replacements of alkali metal cations in the electrolytes were also evident in other metal hexacyanoferrates¹⁹. CuHCF has a zeolitic structure, which allows only certain ions to penetrate into and to pass out of the channels

of CuHCF^{20} . The voltammetric response of the CuHCF modified CCE was also affected by the concentration of K^+ ions in supporting electrolyte. With the increase of the concentration of K^+ ions, the redox peak potentials were displaced to more anodic regions. It was suggested that the counter-ion is slowly exchanged between the CuHCF structure and solution. Not much appreciable changes were observed when the anions were switched between Cl^- and NO_3^- . Thus potassium nitrate solution was used in subsequent experiments.

Figure2.

Cyclic voltammograms of the CuHCF-CCE in the presence of different alkali metal nitrate supporting electrolytes (0.1 M), (A) Li^+ , Na^+ , K^+ Cs^+ as well as NH_4^+ , Scan rate: 20 mV s^{-1}

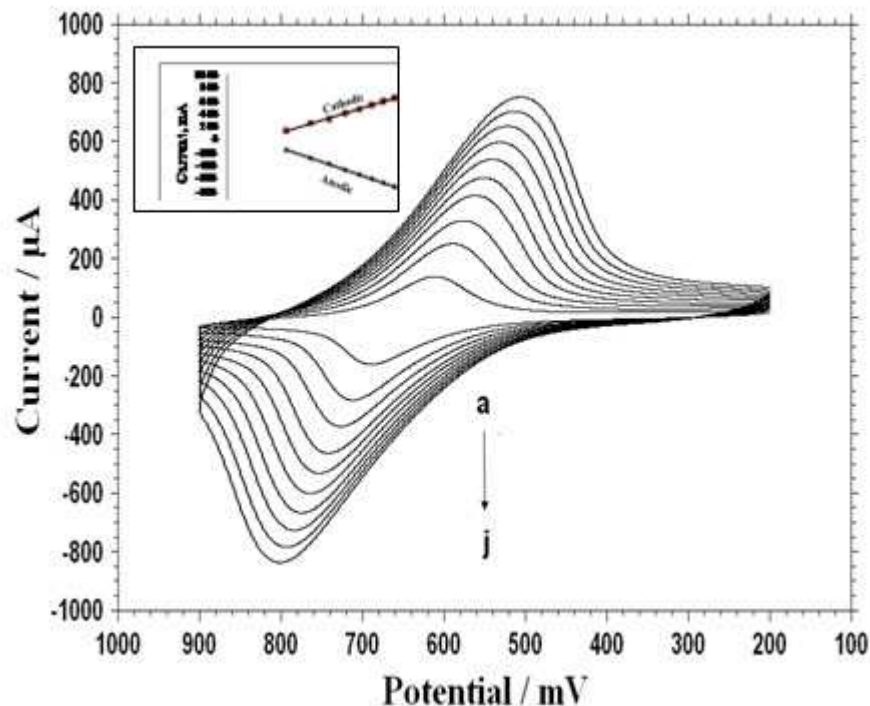


The effect of the scan rate (figure 3) on the voltammetric response of the carbon ceramic composite electrode modified with CuHCF in a range of 25 to 250 mVs^{-1} was studied. The anodic currents are dependent on the square root of the scan rates, indicating that the

electrochemical process is controlled by diffusion²¹. The anodic and cathodic peak currents were linearly dependent on the logarithm of scan rates (shown as inset of figure 3).

Figure 3.

Cyclic voltammograms of CuHCF–CCE in 0.1M KNO₃ at different scan rates (increasing from inner to outer trace (a - j)) 25 –250 mV/s at increments of 25 mV/s. Inset is the plot of peak current (*I_{pa}* and *I_{pc}*) versus square root of scan rate for Fe(CN)₆⁴⁻/Fe(CN)₆³⁻ redox couple respectively

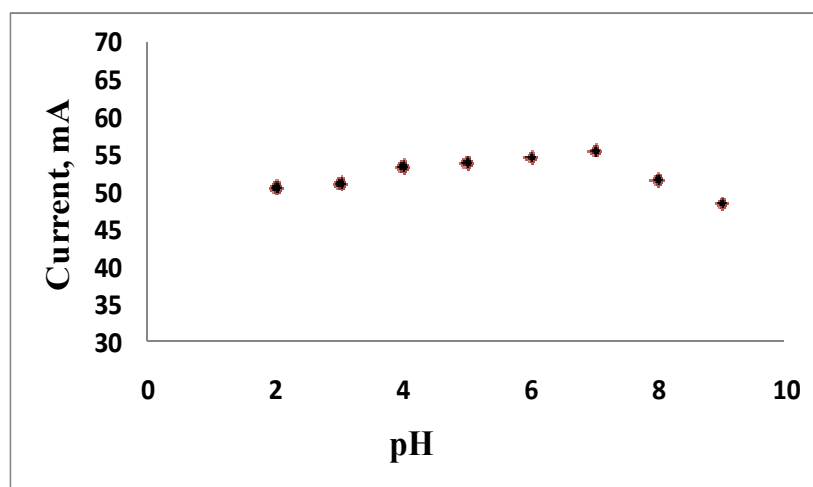


The effect of pH on the electrochemical behavior of the surface modified CuHCF-CCE was studied in the range 2-9 (figure 4). The maximum current response was observed at pH

7.0. At higher pH, a decrease in current response was observed which could possibly be due to the hydroxylation of mediator.

Figure 4.

Effect of pH on the CuHCF surface modified carbon ceramic composite electrode



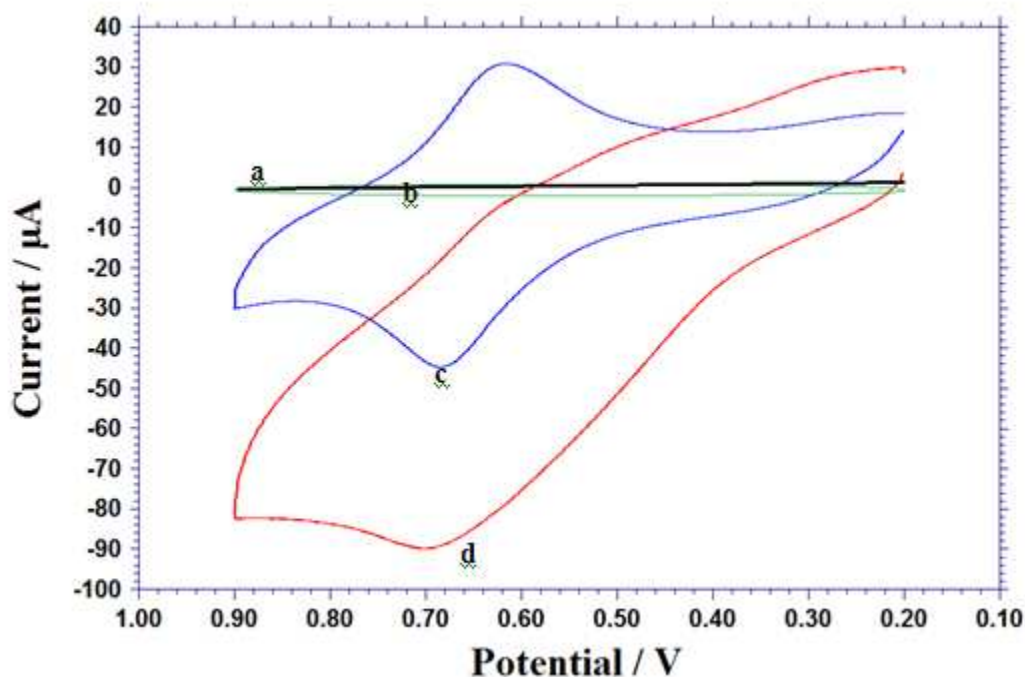
ANALYTICAL UTILITY

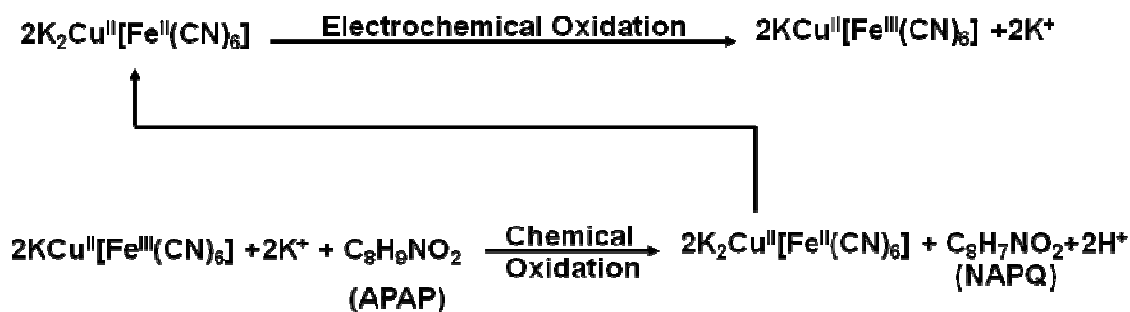
Electrocatalytic oxidation of paracetamol

The catalytic oxidation of paracetamol at the surface modified CuHCF-CCEs has been examined to evaluate the feasibility of using the electrodes in electrocatalysis and electroanalysis. In order to test the electrocatalytic activity of the CuHCF modified CCE, the cyclic voltammograms were obtained in the absence and presence of paracetamol. Figure 5 shows cyclic voltammograms for the electrocatalytic oxidation of paracetamol at the bare and modified CCEs in 0.1 M phosphate buffer (pH = 7.0). Upon the addition of 2.0 mM paracetamol, there is a dramatic enhancement of the anodic

peak current and the cathodic peak current disappeared completely (figure 5d), which indicate a strong catalytic effect. The anodic peak potential for the oxidation of paracetamol at CuHCF modified CCE is about 699 mV while at the bare CCE the paracetamol is not oxidized until 1000 mV (figure 5b). Thus, a decrease in over potential and enhancement peak current for paracetamol oxidation is achieved with the modified electrode. The mechanism of electrocatalytic oxidation of paracetamol on CuHCF-CCE electrode is given in Scheme 2.

Figure 5.
Cyclic voltammograms of (a) In the absence and (b) in the presence of 5.4×10^{-6} M of paracetamol for bare carbon ceramic composite electrode; (c) in the absence and (d) in the presence of 5.4×10^{-6} M of paracetamol for CuHCF-carbon composite electrode.
 Supporting electrolyte 0.1 M KNO_3 ; scan rate 20mV/s.




Scheme 2:
The mechanism of electrocatalytic oxidation of paracetamol on CuHCF-CCE electrode

The mediator, which is in the reduced form at the electrode surface, gets oxidized at the applied potential. This oxidized form of the mediator in turn oxidizes paracetamol in solution and gets reduced. The regenerated reduced form of mediator at the surface of the electrode again gets oxidized producing the oxidation current at the same time. The process of analyte oxidation and catalyst regeneration proceeds in a cyclic manner enhancing the oxidation current of the mediator. A calibration graph was constructed by plotting the catalytic current against concentration of paracetamol (figure 5). This accomplishment led to a linear working range of $3.82 \times 10^{-7} \text{ M}$ to $4.07 \times 10^{-3} \text{ M}$ with a correlation coefficient of 0.9951. The detection limit was calculated as $1.53 \times 10^{-7} \text{ M}$.

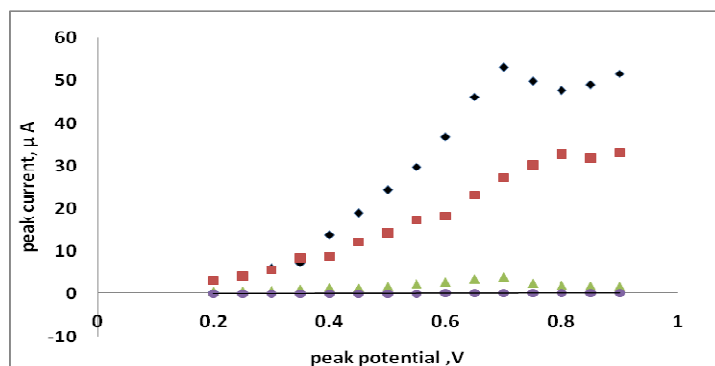
The linear range observed with this sensor is better than previous reports^{22, 23}.

Hydrodynamic studies

Hydrodynamic voltammograms were recorded in order to study the effect of potential on the electrocatalytic behavior of the modified electrode. Figure 6 shows the hydrodynamic response of the bare and modified electrode of $5.4 \times 10^{-6} \text{ M}$ concentration of paracetamol at pH 7.0 when compared to bare electrode response to paracetamol, an increased response was observed at the modified electrode. The catalytic current for oxidation of paracetamol is maximum at 0.69 V and constant beyond that potential. The potential can be fixed at any value in the constant current region and the determination of the paracetamol in flow systems can be carried out easily.

Figure 6.

Hydrodynamic voltammograms of (a) bare CCE in the absence (b) bare CCE in the presence of $5.4 \times 10^{-6} \text{ M}$ paracetamol (c) CuHCF- CCE in the absence and (d) CuHCF-modified electrode in the presence of $5.4 \times 10^{-6} \text{ M}$ p in 0.1 M KNO_3 at 300 rpm



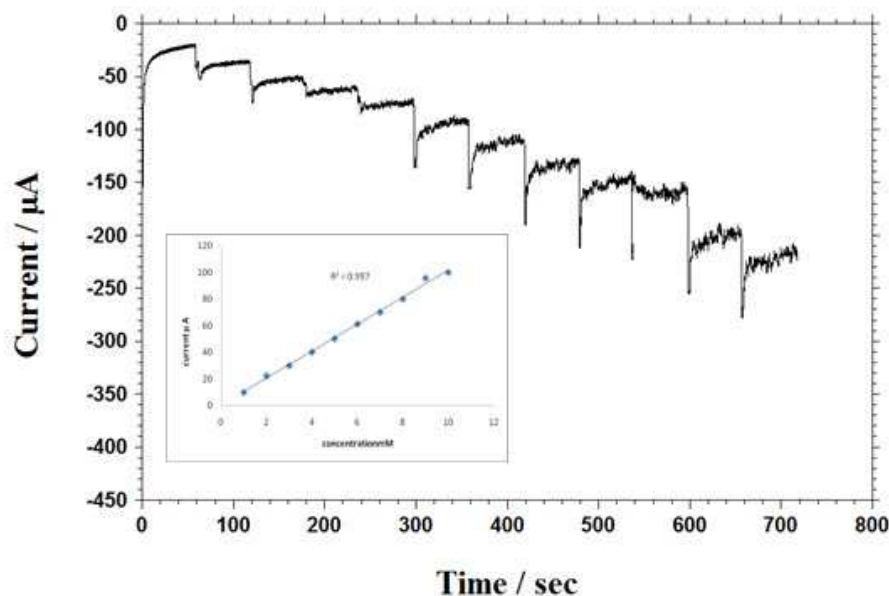
Amperometry

Since the amperometry under stirred conditions has a much higher current sensitivity than cyclic voltammetry, it was used to estimate the lower limit of detection of paracetamol at surface modified CuHCF- CCE. Figure 7 shows, a typical amperometric response was obtained by successively adding paracetamol to continuously stirred modified electrode (rotation speed 300 rpm) in phosphate buffer (pH =7.0). As shown in Figure 7(a), during the successive addition of 2 μM , a well defined response is observed. Under conditions in which the potential of CuHCF modified electrode was kept at 699 mV, the electrode response time was < 2 s. As shown in Figure 7(b) and (d), the measured currents increase by

increasing paracetamol concentration in solution while for a high concentration of substrate, the plot of I vs .paracetamol concentration deviates from linearity. The calibration plots for paracetamol oxidation current were linear for a wide range of concentrations, 1 to 10 μM . correlation coefficient of 0.9972. The detection limit was 0.65 μM when the signal to noise was 3. Since the modified CCE has several major advantages such as high sensitivity, low detection limit, good and long term stability at broader pH range and fast response to oxidation paracetamol, it can be used as an amperometric detector in chromatography instruments.

Figure 7.

Current versus time curve of the CuHCF-modified electrode for the successive addition of 2 μM Hydrazine. Inset is the plot of catalytic current versus increase in hydrazine concentration. Supporting electrolyte 0.1 M KNO_3 , potential 0.69 V, stirring rate 300 rpm



Stability and reproducibility

The long term stability of the modified electrode was checked by recording the anodic current response for the oxidation of $4.6 \times 10^{-6} \text{M}$ for 3 hour period and almost a near constant response was obtained .The shelf life of the electrode for substrate oxidation was monitored

by recording the anodic current response at an interval of 5 days for $4.6 \times 10^{-6} \text{M}$ over a period of 60 days. The electrode offered a very stable response with retention in response of nearly 95% for paracetamol at the end of 60 days. The electrode-to-electrode reproducibility was also examined between eight different electrodes in

4.6×10^{-6} M paracetamol and the relative standard deviation was calculated to be 3.6%. This clearly demonstrates that the fabrication procedure of the sensor was reliable, thereby allowing reproducible electro analytical responses to be obtained with different sensors constructed in the same manner.

Interference study

The influences of foreign species were investigated by analyzing the interference of a series of typical active species (acetylsalicylic acid, codeine, caffeine, chloropheniramine and ascorbic acid) that are currently present in pharmaceutical preparations of paracetamol to 8.0×10^{-5} M paracetamol. The results indicated

that they did not interfere with the determination of paracetamol.

Sample analysis

Several commercial paracetamol tablets were grounded to powder and a portion of the powder was weighted and dissolved in water. After filtering, aliquots of the filtrate were further diluted with water in order that the concentration of paracetamol was in the working range. The proposed method was applied to the determination of paracetamol concentration in pharmaceutical preparations. The contents of paracetamol in three kinds of pharmaceutical preparations were determined. The results (shown in table 1) agree with the manufacturers' stated contents of paracetamol.

Table 1
Determination of Paracetamol in different tablets

Sl. No.	Tablet	Manufacturers specification	Paracetamol found	R.S.D %
		Mg/Tablet	Mg/Tablet	
1.	Dolo 650	650	648	0.6
2.	Saridon	500	497	0.9
3.	Metacin	500	493	0.7
4.	Crocic	500	498	0.8
5.	Calpol	500	496	0.9
6.	Fepanil	500	497	0.5

CONCLUSIONS

The proposed method offers several advantages over previously reported procedure. The proposed method is rapid and simple, and has been applied satisfactorily to the analysis of paracetamol in pharmaceutical preparations. The CuHCF modified wax composite electrode shows very good catalytic activity with good linearity for paracetamol determination over a range of 3.82×10^{-7} to 4.07×10^{-3} M with a detection limit of 1.53×10^{-7} M and a correlation coefficient of 0.9951. The modified electrode can overcome the kinetic limitation of

paracetamol oxidation at a bare electrode by catalytic process and can lower the over potential for the oxidation reaction. The real sample analysis reveal the fact that this method of determination may be suitable for analysis of paracetamol and the added features like stability, excellent rugosity, reproducibility and low cost of fabrication confirms the above fact and makes this sensor a definite candidate for efficient determination of paracetamol.

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