



Paracetamol Sensing using GNP-Cys-CdHCF Composite Electrode

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ABSTRACT

A surface modified CdHCF film electrode using cys-Au nanoparticles mixed graphite was prepared to resolve the paracetamol (pH 7). The voltammetric behaviour of the CdHCF customized electrode incidence in different alkali metal cations was investigated by electrochemical instrument. It's also characterized using impedance spectroscopy. Surface of the electrode was characterized by Transmission electron microscopy (TEM) it's represent that the modified surface of the CdHCF particles were discrete homogeneously on the electrode surface in the range of 10-15 nm. The modified surface is exhibiting good catalytic response on paracetamol sensor with high solidity and reproducibility. The Potential of oxidation towards paracetamol in differential pulse voltammetry was lowered 110 mV and ampere response was enhanced significantly relative to a bare electrode. 7.2×10^{-7} to 2.6×10^{-3} M range was observed in linear calibration curve for paracetamol sensor with 0.999 correlation coefficient. The exposure frontier anticipated to be 2.4×10^{-8} M. The common interferences viz., ascorbic acid, dopamine and uric acid did not affect the determination of paracetamol significantly. This method is illustrated by sensing paracetamol in pharmaceutical preparations.

Keywords: GNP; Cadmium hexacyanoferrate; Electro catalysis; Paracetamol

INTRODUCTION

Acetaminophen (Paracetamol (PA)) is used to mitigate numerous kinds of trivial aches and pains-headaches, muscle aches, backaches, toothaches, menstrual cramps, arthritis, and the aches and pains that often accompany colds [1-5]. It has no anti-inflammatory action which made it an obvious choice for home medication for more than three decades [4]. PA is hastily and utterly metabolized towards glucuronidation and sulfation to figure immobile metabolites which are eliminated in the urine at standard salutary doses [4]. However, an accretion of lethal metabolites occurs an overdose of PA, it cause sensitive hepato and nephrotoxicity [6-9]. Thus, it is essential to establish a simple inexpensive method to determine the concentration of PA with high selectivity and sensitivity. Numerous analytical techniques like titrimetry [10], spectrophotometry [11], spectrofluorometry [12], voltammetry [13], HPLC [14], TLC [15], colorimetry [16], FTIR [17], numerous methods have been projected to sense paracetamol. In view of the fact that voltammetric performances are highly discriminating, cost-effective and minimum time-consuming, in pharmaceutical preparation they are extensively worn towards paracetamol sensor. Shuyan et al. distinguished a moderately trouble-free and swift electrochemical technique by glassy carbon electrode towards oxidation of paracetamol in 1.0 M HCl [18]. Voltammetric fortitude paracetamol using chemically modified electrodes [19,20], diamond film electrode dazed on boron [21] then additional electrodes [22-25]. Due to its narrative to the properties of optical, electronic, magnetic and catalytic GNP (gold nanoparticles) is one of the most effectively deliberated and one of the most trendy resources to be massed on electrodes [26]. It has been discussed that the tiny of GNP permit the conductive materials to reach the environs of the energetic process given that bio electrocatalytic action, which can be utilized in the edifice of biosensors [27]. Also gives such valuable functions for electroanalysis [28]. GNP modified electrodes are used in various applications in electrochemical method. While

they boast the knack to augment the electrode conductivity and make easy the electron transfers, thus, civilizing the sensitivity and selectivity. Basically strange binding molecules are worn to accumulate GNP on the surface of electrode. [29,30] other than this may modify the conducting properties of the modified electrodes [31].

Here, we describe an amperometric method to sense paracetamol at a GNP composite electrode with CdHCF as electrocatalyst and the sensor produce an outstanding electrocatalytic bustle towards paracetamol sensor. This electrode has good sensitivity, rapid response time, reproducibility, stability and low detection limit and the application this electrode towards the quantification of paracetamol in commercial samples was originate to be hopeful.

EXPERIMENTAL SECTION

Reagents

Whole the chemicals are analytical grade; Graphite powder at Aldrich (1-2 μm), (Aldrich, Steinheim, Germany). Paracetamol purchased in Himedi (P) Ltd, India, CdCl_2 , $\text{K}_4[\text{Fe}(\text{CN})_6]$ were purchased from Merck (Mumbai, India). For electrochemical technique DD (Double distilled) water was used. 0.1 M HCl and 0.1 M NaOH are used to carry out the effect of pH. 20 mM of L-cysteine was equipped by DD water and 0.01 M of cadmium solution equipped in ethanol. Potassium ferrocyanide (0.02 M) with KNO_3 (0.1 M) was used as a electrolyte to coordinated cadmium ion as hexacyanoferrate by derivatization.

Apparatus and Material

Using Electrochemical workstation (CH Instruments (660B)), all the Electrochemical measurements were done with IBM computer with typical three-electrode configuration., saturated calomel is working as reference by the way platinum wire as the counter and the surface modified CdHCF composite electrode was used as the working electrode. This electrode is characterized by Fourier transform infrared (FTIR) spectra using Bruker tensor 27.

Michlins peristaltic pump PP10 (India) used in FIA and a model of Rheodyne (Cotati, CA) 7725 injection valve prepared with a 20 μL loop, attached to a routine built radial flow cell with a reference of Ag/AgCl and counter of platinum wire electrode connected to CH Instruments (660B), connected with IBM personal system for data acquirement. Working electrode was a 3 mm dia CdHCF composite electrode.

Fabrication of GNP-CdHCF Electrode

The citrate trumped GNP was equipped by the reported methods [32]. 530 nm of absorption is noted for GNP in UV. 125 ml of 0.6 mM of GNP solution is mixed with 1 g of graphite powder and this enthused for 120 mins at 300 rpm at normal temperature. It's centrifuged at 1450 rpm for half an hour and the filtrate was kept during the night for drying. 900mg of GNP adsorbed graphite powder mixed with paraffin wax in the ratio of 4:1 finally this was make it as a electrode by a small glass tube of 3 mm diameter. Electrode was detached gently from the tube following it turns hard and it shows an excellent conductivity and hardness. This electrode surface was first dipped in 20 mM of L- cysteine solution for 120 minutes. Then, this surface modified L-cysteine - GNP electrode was curved in ethanolic solution of 0.01 M CdCl_2 for 15 minutes. This composite electrode has a superior affinity towards transition metal ion like Cd^{2+} . By derivatize the Cd^{2+} ions using 0.02 M potassium ferrocyanide solution dissolved in 0.1 M KNO_3 in the potential range of -0.2 V to 1.0 V at the scan rate of 50 mV s^{-1} [33] to coordinates amine group.

Commercially Sample Analysis

A stock was primed by dissolving 350 mg of the solid product in 500 ml distilled water using an ultrasonic water bath to ensure a complete dissolution. This solution was kept 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.

RESULTS AND DISCUSSION

Microscopic Characterization

TEM characterization of the surface:

TEM descriptions of the electrode surfaces are shown in Figure 1. Figure 1a show the image of the bare electrode surface while, Figure 1b shows the GNP incorporated graphite surface. The presence of 25-40nm size GNPs are seen, which confirm the presence of GNP on the surface.

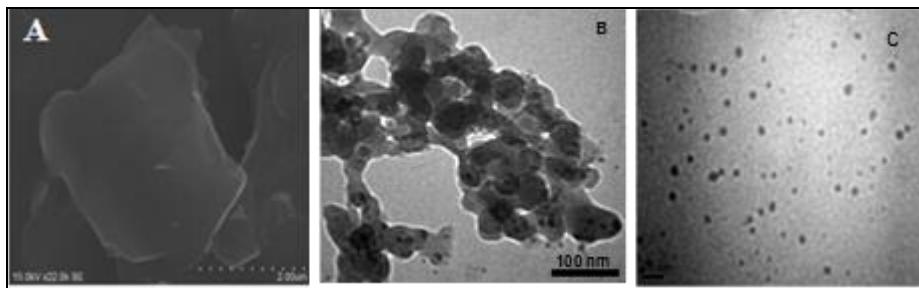


Figure 1: Shows the CdHCF nanoparticle on the surface which is about 10-15 nm in size. The TEM images (A) unmodified, (B) presence of GNP (C) CdHCF composite electrode

FTIR studies:

FTIR spectra of the composite electrode shows in Figure 2a uncontaminated cysteine, (b) cysteine adsorbed GNP electrode and (c) Final electrode. The peaks denoted at 1126 cm^{-1} , 1535 cm^{-1} and 2528 cm^{-1} , in Figure 2a which can in the same way be endorsed to NH_3^+ rocking, $-\text{COO}^-$ asymmetric stretching, and SH stretching vibrations [34].

The $-\text{SH}$ peak was tainted among the cysteine and GNP, instead of that the $-\text{SH}$ group on cysteine is occupied with binding to GNP (Figure 2b). The $-\text{COO}^-$ peak on cysteine did not modify hugely compared with Au-cysteine, indicative of that the $-\text{COO}^-$ was not efficiently implicated in Cd binding. Figure 2c shows peak of NH_3^+ shifted from 1126 cm^{-1} of the unique cysteine range to 1231 cm^{-1} to bounded cysteine- GNP-Cd complex. The nonappearance peak of the S-H group around 2530 cm^{-1} demonstrates the configuration of GNP-cysteine-Cd composite by the chemical reaction among cysteine adsorbed GNP and Cd.

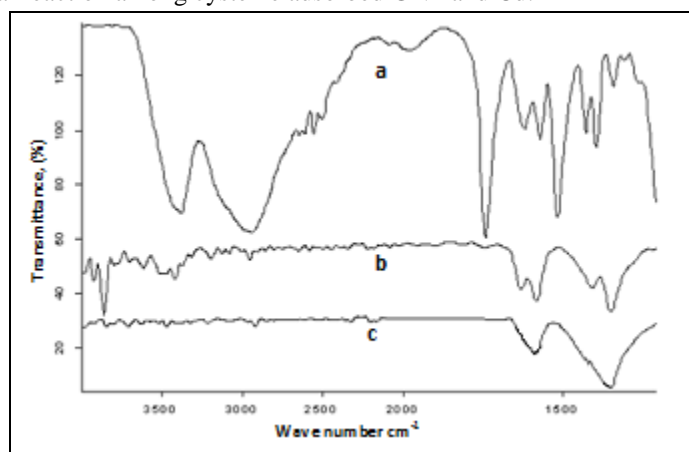


Figure 2: FTIR spectra of (a) uncontaminated cysteine (b) cysteine -GNP and(c) GNP-Cys- CdHCF composite electrode

Electrochemical characterization:

Effect of supporting electrolyte: CdHCF customized electrode was systematically deliberated by cyclic voltammetry (CV). The effect of electrolyte was studied recording the CVs of the CdHCF modified electrode in different background electrolytes of the same concentration. The effect of various cations such as K^+ , Na^+ , NH_4^+ , Li^+ , Ba^{2+} , Ca^{2+} and Cs^+ on the response of the composite electrode was studied electrochemically. The modified electrode presented a better voltammogram when K^+ was worked as cation of the background electrolyte. The mediator film differs with the nature or size of the alkali metal cations in the solution. The CV studies of the modified surface were considerably exaggerated by the anion present. The retort of the electrode was good when the anion was NO_3^- . In presence of other anions the response was found to decrease in the order of $\text{Cl}^- < \text{HCO}_3^- < \text{SO}_4^{2-}$. Hence 0.1 M KNO_3 was chosen as the background electrolyte for the subsequent studies. The peak current and peak potential values of the electrode in different electrolytes at 20 mVs^{-1} .

Effect of scan rate:

The consequence of scan rate (ν) on the surface of the composite electrode was premeditated in the range of 10-700 mVs^{-1} . The anodic and the cathodic currents increase on increasing scan rate. A beeline relation was observed between the current and scan rate's square root in the range of 10-100 mVs^{-1} (Figure 3a) signifying diffusion controlled reaction at the electrode surface. The ΔE_p values at higher scan rates show an increase, indicating the

limitation in charge transfer kinetics. The peak current of cathodic and anodic are linearly reliant on the logarithm of ν (Figure 3b). It is predictable for diffusion-controlled process. The electrochemical transfer coefficient was calculated from the plot of current peak against \log of ν , the transfer coefficient and rate constant of heterogeneous electron transfer were intended to exist 0.63 and 0.964 s^{-1} respectively [35].

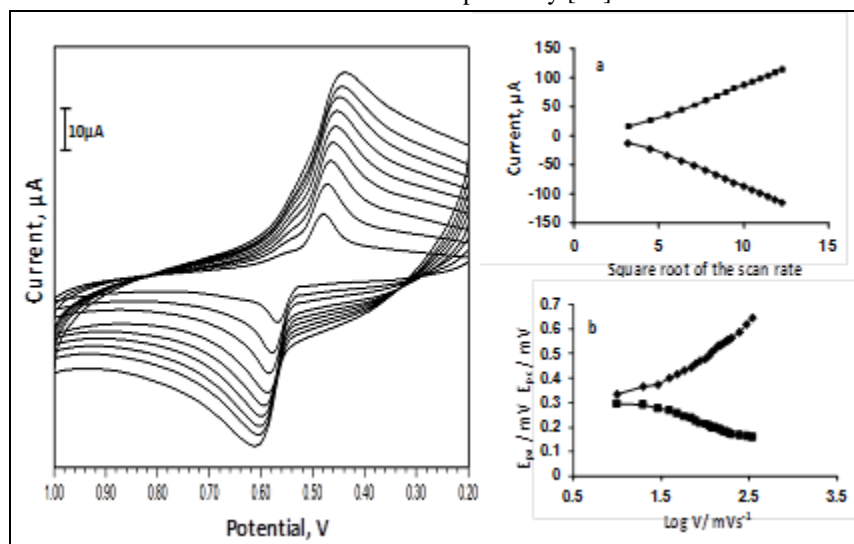


Figure 3: Scan rate variation of the modified electrode (10-70 mV). Inset figure a dependence of peak current I_{pa} and I_{pc} on scan rate's square root (ν), b. peak potential variation vs. ($\log \nu$)

Effect of pH:

Neutral and weakly acidic electrolytes are most commonly used to maintain the stable electrochemical response in metal hexacyanoferrates (MHCs) [36], in alkaline media cations will hydrolyzed, so the film can cause worsening of the scaffold of MHCs [37]. To investigate the stability of CdHCF under different pHs, we compared the electrochemical response of CdHCF electrode in solutions at different pHs ranging from 1–10. Figure 4 exhibits the CV of the composite electrode recorded in 0.1 M KNO_3 of pH 1–10 (adjusted by 1 M HNO_3 or KOH solutions). Based on the experimental results, the CdHCF film modified electrode showed a good response in neutral solutions (pH 7). However, a strong alkaline solution reduces the response of modified electrode. Therefore, a pH of 7 can be used for the electrochemical experiments.

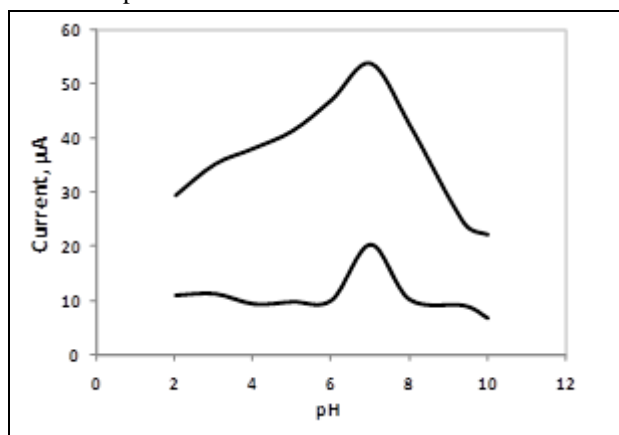


Figure 4: Effect of pH and catalytic oxidation of paracetamol pH

Studies on EIS:

Figure 5 explain the results obtained from EIS studies. It presents the complex plane impedance plot for the bare composite (\bullet) and the composite electrode (\square). Charge transfer process occurs at the electrode surface was concluded. For the modified electrode, only the dissemination and charge transfer processes are observed; however, for bare electrode a supplementary capacitive method at liaison frequencies were observed. The electron transfer resistance was determined from the intersection of the semicircle, which can be related to iron oxidation and

reduction. For the bare electrode, the R_s , R_{ct} , CPE and Z_w values were estimated as $99.2 \text{ k}\Omega\text{cm}^{-2}$, $27.3 \text{ k}\Omega\text{cm}^{-2}$, 3.6 nFcm^{-2} and $1.2 \times 10^{-4} \Omega\text{cm}^{-2}\text{s}^{1/2}$, respectively. For CdHCF modified electrode, values of $91.3 \text{ k}\Omega\text{cm}^{-2}$, $18.54 \text{ k}\Omega\text{cm}^{-2}$, and 9.2 nFcm^{-2} and $5.96 \times 10^{-4} \Omega\text{cm}^{-2}\text{s}^{1/2}$ were obtained for the R_s , R_{ct} , CPE and Z_w , respectively. From these results, From the R_{ct} value we can concluded that the composite electrode exhibits the best electrocatalytic activity compare with unmodified electrode, hexacyanoferrate films have the faster diffusion of counter-ions.

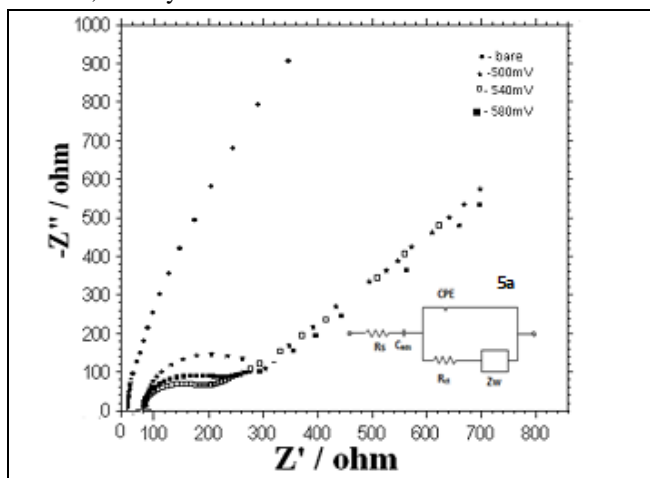


Figure 5: EIS plots of CdHCF electrode in 0.1 M KNO_3 , three different potential were made in Nyquist plot (\blacktriangle -500 mV), (\square -540 mV), (\blacksquare -580 mV) and bare wax composite electrode (bare- \bullet); 5a circuit diagram of Randels equivalent for modified electrode

Electrochemical oxidation of paracetamol at CdHCF-GNP modified electrode:

The electrocatalytic activity of the CdHCF modified electrode for paracetamol oxidation was examined by cyclic voltammetry (Figure 6a). The CdHCF modified electrode exhibits significant oxidative activity in the existence of paracetamol. Figure 6a shows the CV of the CdHCF electrode with rising analyte concentrations, Figure 6b shows the catalytic current plot response to the rising attentiveness of paracetamol. Anodic peak and catalytic current are directly proportional to the concentration of paracetamol. The range of the catalytic current observed in the presence of paracetamol is 7.2×10^{-7} to 2.6×10^{-3} and the detection limit found to be $2.4 \times 10^{-8} \text{ M}$.

Table 1 is explained the previously reported the electrochemical behavior of CdHCF-GNP electrode. From this table 1, CdHCF showed a low oxidation potential, broad linearity range, and a low detection limit. A significant catalytic activity of the CdHCF electrode is proved by extensive shift to the negative potential of analyte oxidation (over potential reduced by 110 mV) as well as a drastic rising in anodic current. It's also exhibiting good antifouling properties for paracetamol sensor and its oxidation byproducts. Moreover 5-6 weeks leaving the electrode in an airtight container, the peak potential does not change for paracetamol oxidation and 2% decrease of current signals only showed. 2-4% of relative standard deviation (R.S.D) was observed in current peak. Exactly 6 electrodes were made to study the reproducibility of its preparation, and their CV responses were recorded with $2.65 \times 10^{-5} \text{ M}$ of analyte. RSD is found to be only 2% at 0.54 V.

To determine the paracetamol Differential Pulse Voltammetry (DPV) was also used. It's a more sensitive instrument than CV, which causes superior severance among the redox peaks. So, it uses to reduce the errors in determination of various species. In this work the same potential is observed while the oxidation of paracetamol in DPV and CV. (Figure 6c).

Modified electrode was worked with dynamic condition was evaluated by hydrodynamic studies (HDV). CdHCF electrode was measured in the range of 0–1.0 V at a stirring rate of 300 rpm in the presence of the analyte. In Figure 7, curve 'a' depicts the hydrodynamic study for the oxidation of $2.196 \times 10^{-5} \text{ M}$ of paracetamol at the surface of the electrode while curve 'b' is the bare one, it exhibit a very tiny current response towards the same amount of analyte sensor. Oxidation potential is started from 0.2 V and a maximum was observed at 0.54 (Figure 7) and finally reaches a plateau. Only at 0.42 V oxidation starts at unmodified one, and reaches a maximum at around 0.54 V and cascade back to a steady value. While compared with bare modified electrode, the greatest response is establish to be at an inferior potential for the modified electrode and the response of the bare one is insignificant. Hence, an applied potential of 0.55 V was experimentally determined as optimum for amperometric experiments. As expected the activity of the CdHCF electrode permits the expedient exposure of paracetamol at highly sensitive with lower potential.

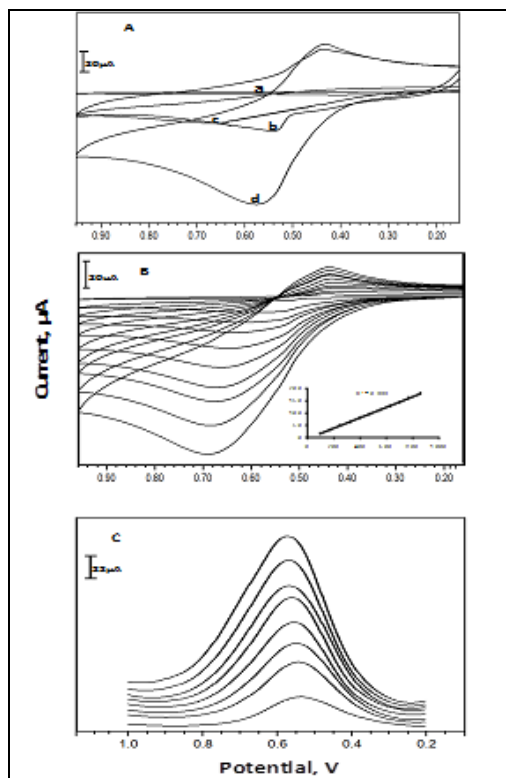


Figure 6: CV in 0.1 M KNO_3 (pH 7.0) at 20 mVs^{-1} (a) bear without paracetamol (b) modified electrode without paracetamol (c) bare in presence of $2.65 \times 10^{-5} \text{ M}$ paracetamol (c) CdHCF electrode in presence of $2.65 \times 10^{-5} \text{ M}$ paracetamol

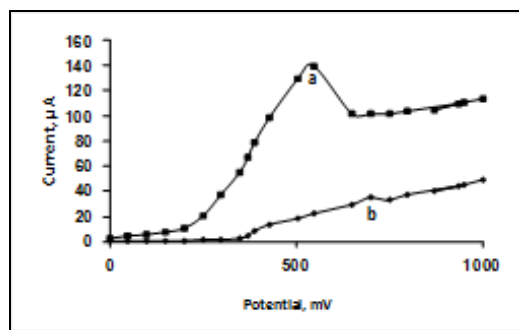


Figure 7: HDV of (a) CdHCF electrode with the concentration of $2.196 \times 10^{-5} \text{ M}$ paracetamol. (b) Bear with the concentration of $2.196 \times 10^{-5} \text{ M}$ paracetamol

Flow injection analysis:

The flow injection response obtained for paracetamol sensor on CdHCF composite electrode at different concentration ranges at 0.55 V is shown in Figure 8. Peak current is directly proportional with the concentration of the analyte. The sensitivity of CdHCF modified electrode was $1.9532 \mu\text{A}/\mu\text{M}$. A stable and fast response was obtained upon repeated injection of paracetamol. The relative standard deviation of 8 successive determination of $2.196 \times 10^{-5} \text{ M}$ paracetamol was 1.8% indicating that the CdHCF modified electrode exhibited good reproducibility.

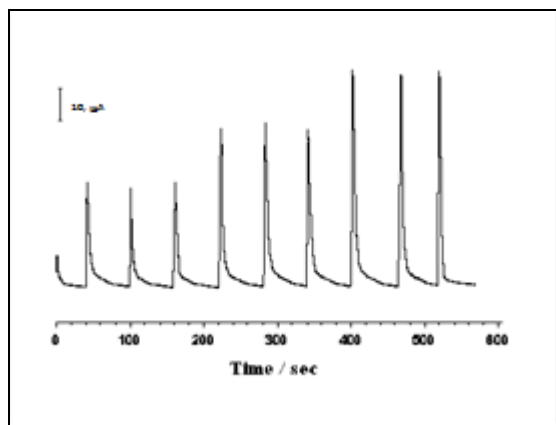


Figure 8: Flow injection analysis of the CdHCF modified electrode for three replicate additions of 9.2 μM , 17.6 μM and 21.2 μM of paracetamol; a extracted from commercially available tablets and syrup in 0.1 M KNO_3 , operational voltage 0.55 V. Flow rate 0.3 ml min^{-1}

Pharmaceutical applications:

Numerous profitable paracetamol drugs were beached to powder and a little of this was biased and dissolved in water. After filtration a little filtrate were diluted with water again, in order that the concentration of paracetamol was in the working range. Following the procedure detailed in Section 2, this method was applied to the pharmaceutical preparations from determination of paracetamol concentration. The contents of paracetamol in four kinds of pharmaceutical preparations were determined (Table 1).

Table 1: Paracetamol in four kinds of pharmaceutical preparations

Samples	Concentration of paracetamol (μM)		Recovery (%)
	Added	Found ^a	
Sample I	30	29.80 \pm 0.70	99.3
	40	38.99 \pm 0.48	97.5
Sample II	30	28.99 \pm 0.50	96.6
	40	39.92 \pm 0.48	99.8
Sample III	30	29.76 \pm 0.48	99.2
	40	39.76 \pm 0.47	99.4
Sample IV	30	28.96 \pm 0.49	96.5
	40	39.89 \pm 0.45	97.7

^aAverage of four replicate experiments \pm R.S.D

Effect of interferon's:

The specificity of GNP-CdHCF modified electrode to paracetamol determination with some probable intrusive substances like dopamine, ascorbic acid and uric acid was determined for its authority on the voltammetric retort of paracetamol. DPV was carried out for 0.10 mM paracetamol with 0.05–1.00 mM of other interfering analytes. There was no considerable change in current retort for 0.10 mM paracetamol with less than fifty fold excess of interferents.

CONCLUSION

In this work, A GNP-L-Cysteine-CdHCF film modified electrode was fabricated for the electrochemical investigation of paracetamol by cyclic voltammetry, difference pulse voltammetry and flow injection analysis. The peak current of paracetamol has improved significantly and the oxidation peak shifted towards positive potential with GNP-L-cysteine-CdHCF composite film. The film was successful one for the determination of paracetamol. Its advantages, such as simple, sensitive, rapid and accurate, were demonstrated by paracetamol determination in the pharmaceutical samples with good result.

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