

Rapid and Accurate Amperometric Determination of Acetaminophen in Pharmaceutical Preparations and Spiked Human Blood Serum Samples at Cadmium Pentacyanonitrosylferrate Modified Glassy Carbon Electrode

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The present work describes a rapid and accurate amperometric technique for the determination of acetaminophen (ACT) in pharmaceutical preparations and human blood serum, based on electrocatalytic oxidation of ACT at a glassy carbon electrode modified by cadmium pentacyanonitrosylferrate (CdPCNF) film. The electrocatalytic response of the modified GC electrode was linear over the concentration of 1.64-52.90 μM . The limit of detection was found to be 2.04 μM by amperometric technique. The method was successfully utilized for the determination of ACT in various pharmaceutical preparations and the results have been statistically compared with those obtained by the official method. The interference of some pharmaceutical and biological compounds was investigated. The results of interference study showed that the Nafion-coated CdPCNF|GC electrode can be utilized as a selective amperometric sensor for acetaminophen determination in human blood serum. The mean value of rate constant k for catalytic reaction, and the diffusion coefficient of ACT (D) in the phosphate buffer solution of pH 7.2 were found to be $4.27 \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$, and $(4.25 \pm 0.33) \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, respectively.

Keywords: Acetaminophen, Modified electrode, Amperometric determination, Cadmium pentacyanonitrosylferrate

INTRODUCTION

Paracetamol (N-acetyl-*p*-aminophenol, acetaminophen) is a long-established and one of the most extensively employed "over the counter" drugs in the world. It was first used in medicine by Von Mering in 1893. However, it was first discovered to have both analgesic and antipyretic properties in the late 19th century. It is noncarcinogenic and an effective substitute to aspirin for patients with sensitivity to aspirin [1]. Unlike aspirin, however, paracetamol's anti-inflammatory activity is considered weak and is, thus, not routinely used in inflammatory conditions such as rheumatoid arthritis.

Nevertheless, it is used to reduce fever cough and cold, and reduce mild to moderate pain, including instances of tension headache, migraine headache, muscular aches, chronic pain, neuralgia, backache, backache, joint pain, general pain and toothache [2-4]. Acetaminophen blocks pain messages to the brain by stopping a chemical called prostaglandin, which causes pain and fever.

Numerous methods have been used for the determination of paracetamol in pharmaceutical formulations and biological fluids including titrimetry [5,6], UV-Vis spectrophotometry [7-10], spectrofluorimetry [11], near infrared transmittance spectroscopy [12], electrochemical methods [13,14], and chromatography [10,15-19]. Recently, several flow injection (FI) methods for the determination of paracetamol have been

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proposed using an UV-Vis spectrophotometer [20-22], fluorimeter [23], multiptosensor [24], or Fourier transform infrared spectrophotometer [25] as a detector.

However, some of these methods are less convenient for the determination of paracetamol in pharmaceutical formulations because the methods are based on the hydrolysis of paracetamol sample to 4-aminophenol, which then produce a colored complex compound by an appropriate reaction which are time-consuming. The more rapid FI method is therefore sought. Flow-injection (FI) with chemiluminescence (CL) seems promising to serve this purpose. Chemiluminescence methods provide many advantages for pharmaceutical determinations such as high sensitivity, high selectivity, small amount of chemical consumption, cost effectiveness, simple sample preparation and instrumentation [26-28].

Three procedures have been reported in the literature for the determination of paracetamol in pharmaceutical formulations or biological fluids by using flow injection with chemiluminescence detection [29-31]. The procedures are based on the oxidation of paracetamol with cerium(IV) [29] and the inhibition of luminal- H_2O_2 - $\text{Fe}(\text{CN})_6^{3-}$ or luminal-permanganate systems [30,31].

In last few years, we published some papers indicating a potential electrocatalytic activity of transition metal hexacyanoferrate film modified electrodes [32,33]. The high conductivity, stability, ease of preparation and operation of these PB analogous encourage us to investigate their pharmaceutical and biological applications. The present paper describes a simple amperometric technique for the sensitive and selective determination of well-known analgesic drug paracetamol at CdPCNF-modified glassy carbon electrode at physiological pH of 7.2. The electrode has a catalytic function towards the oxidation of paracetamol. The electrode modified with CdPCNF film have been found to result in much better results in comparison to bare electrodes and can be have a lot of potential applications in electroanalytical studies. The proposed method does not require any sample pre-treatment and also is not sensitive to the presence of the *p*-aminophenol which is one of the paracetamol metabolites. The amperometric response of paracetamol in the presence of other interferents such as ascorbic acid, oxalic acid, citric acid, tartaric acid, glucose and urea has also been studied. Various

paracetamol containing tablets, syrups, and suppositories were also examined for their paracetamol content and experimental determinations are in good agreement with the declared content. Finally, the proposed method was successfully used for the determination of paracetamol in human serum samples.

EXPERIMENTAL

Reagents and Apparatus

Reagents and chemicals. Acetaminophen (paracetamol), sodium pentacyanonitrosylferrate and other chemicals were of analytical grade from Merck. Pharmaceutical preparations were obtained from a local pharmacy. Human blood serum samples were obtained from Imam Khomeini hospital, Tabriz, Iran. Phosphate buffer solutions (PBS) were made up from KH_2PO_4 and adjusted to desired pH by adding 1 M KOH or HNO_3 solution. Nafion solution (5% in alcohol) was from Aldrich and used as received. All solutions were prepared with twice distilled water.

Instrumentation. A Shimadzu UV-Vis spectrophotometer model UV-160, with a 1.0 cm optical path quartz cell was used for spectrophotometric measurements. The electrochemical experiments were carried out using an Autolab Potentiostat/Galvanostat Model 100 and 744-pH meter from Metrohm. A conventional three-electrode cell was used at room temperature. The GC electrode was used as working electrode. A silver|silver chloride electrode ($\text{Ag}|\text{AgCl}$, KCl 3 M) and a platinum wire were used as reference and auxiliary electrodes, respectively.

Sample Preparation for Electroanalysis

ACT tablets. An accurately weighed portion of finely powdered sample obtained from three tablets, equivalent to about 25 mg of ACT was dissolved in 20 ml aqueous solution of 0.1 M acetic acid and filtered. The filtrate was diluted to a final volume of 25 ml with de-ionized water. A 0.5 ml portion of the last solution (~6.6 mM) was added to an amperometric cell containing 10 ml 0.25 M PBS (pH 7.2) and 0.5 M KNO_3 ; and catalytic current was measured, while the electrode potential was kept at 0.9 V, and the solution was stirred. The amount of ACT was determined by means of the in situ standard addition method.

Suppositories for adults and pediatric suppositories. An

accurate weight of drug equivalent to about 25 mg of ACT was added to 5 ml chloroform in the separating funnel. The ACT content was extracted with two 10 ml aqueous solution of 0.1 M acetic acid. The extracts were combined in a 25 ml flask and diluted to volume and used to analyze its ACT content by amperometric method as mentioned for ACT tablets. In order to know the yield of ACT extraction to aqueous solution, we performed a recovery test as follows. A defined value of ACT (analytical grade) was added to 5 ml chloroform, and then re-extracted to 25 ml aqueous solution and analyzed by the amperometric technique as described above. The experimental result of the analysis was close to the real value. The recovery for ACT extraction was found to be % (99.4 ± 0.8) for $n = 5$.

Other ACT preparations. Other dosage forms of ACT including suspension, oral solution and pediatric oral drops were also analyzed. To do that, an accurate volume (~1 ml) of the drug equivalent to about 20-25 mg of ACT was combined in a 25 ml flask and diluted to volume with aqueous solution of 0.1 M acetic acid. The mixture was shaken and then filtered using a suitable filter paper. The clear solution obtained was used for the determination of ACT by the amperometric method as mentioned for ACT tablets.

Standard solution. Pure paracetamol (75.6 mg) (Merk, Germany) was dissolved in 50 ml acetate buffer solution (0.1 M) prepared with de-ionized water which results a standard ACT solution of 0.01 M.

Electrode Preparation and Procedure

The glassy carbon electrode surface was polished with 0.05 µm alumina powder on the wet polishing cloth. The polished electrode was rinsed with distilled water for several times. The electrode was then immersed in a solution containing 5 mM CdCl₂, 5 mM Na₂[Fe^{II}(CN)₅NO] and 0.5 M KNO₃ as supporting electrolyte with a pH adjusted in the range 2-3 by sulfuric acid (electrode surface modifying solution). The modification of electrode surface was performed by cycling electrode potential between -0.2 to 1.0 V for 30-50 times with a scan rate of 100 mV s⁻¹. The solid film of cadmium pentacyanonitrosylferrate (CdPCNF) was deposited by the potential cycling and its thickness increased by each cycle. Finally, the electrode was removed from the electrode surface modifying solution and washed with

distilled water before use.

The GC modified electrode was stored in air for drying and then used as working electrode. The CdPCNF|GC electrode showed a reversible redox peaks (Fig. 1, curve a) related to the following reaction:



The surface concentration of the mediator in the film per unit surface area of the electrode (Γ) was determined from the area under the anodic part of the cyclic voltammogram peak of the modified electrode recorded at a low scan rate (20 mV s⁻¹). A Nafion-coated CdPCNF|GC electrode was prepared by casting a desired amount of Nafion dissolved in the ethanol on the surface of modified electrode.

RESULTS AND DISCUSSION

Electrocatalytic Oxidation of Acetaminophen on GC Modified Electrode

The cyclic voltammograms (CVs) recorded at the bare GC and CdPCNF|GC electrodes in the absence and presence of acetaminophen are shown Fig. 1. A phosphate buffer solution (PBS) of pH 7.2 containing 0.5 M KNO₃ was used as supporting electrolyte. As can be seen from curve a, a pair of symmetrical redox peaks appeared at 0.553 and 0.463 V. The mean peak potential $E^{\prime} = (E_{pa} + E_{pc})/2$ was calculated as 0.508 V (vs. Ag|AgCl). The peak potential separation was 0.09 V at a scan rate of 100 mV s⁻¹. The redox peak corresponded to a one-electron reduction and oxidation process. However, it can be seen that in the potential range from 0 to 1 V, no redox peaks were obtained at the bare GC electrode (curve b). Upon addition of 10 mM acetaminophen, a small anodic peak is observed at 0.465 and a flat current at more positive potentials (curve c) due to very slow oxidation reaction of acetaminophen. In contrast, at the modified electrode, a well-defined and sharp catalytic current is observed in anodic scan and corresponding cathodic current is depressed (curve d). The enhancement in the anodic peak current is attributed to the regeneration and re-oxidation of Fe(II)-redox sites produced by chemical reaction between acetaminophen and Fe(III)-redox sites in the film. The oxidation current of acetaminophen at the modified electrode was more 50 times than that observed at the bare GC electrode. These results indicated that CdPCNF|GC electrode could accelerate the rate

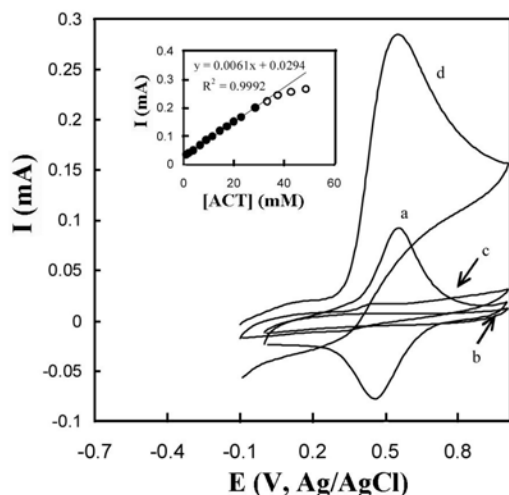
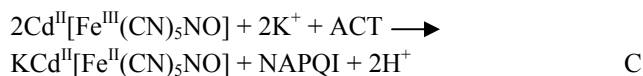
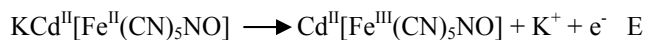


Fig. 1. Cyclic voltammograms recorded at the CdPCNF modified GC electrode (a, d) and bare GC electrode, (b, c) in the absence (a, b) and presence (c, d) of 10 mM acetaminophen at a scan rate of 100 mV s^{-1} . The supporting electrolyte was 0.25 M PBS (pH 7.2) containing 0.5 M KNO_3 . Inset shows the plot of anodic peak current vs. ACT concentration, obtained at the CdPCNF|GC electrode at a potential sweep rate of 25 mV s^{-1} .

of electron transfer of acetaminophen in pH 7.2 (curve d). The chemical reaction of acetaminophen species with CdPCNF on the electrode surface can be shown as follows:



The symbols E and C imply the electrochemical and chemical reactions. The terms ACT and NAPQI are the abbreviated form of acetaminophen and its oxidation form, respectively. ACT is electrochemically oxidized in a pH-dependent, 2-electron, 2-proton process to N-acetyl-*p*-quinone-imine (NAPQI).

The anodic peak current dependence to ACT concentration was investigated in the range 1-60 mM. The inset of Fig. 1 clearly shows that the plot of peak current is linearly changed with ACT concentration in the range 1-33 mM. For ACT

concentration larger than 35 mM, the variation of peak current deviated from the linearity. The regression equation for the linear part of the calibration plot was obtained as: $I (\mu\text{A}) = 0.0061C + 0.0294$, with $r^2 = 0.9992$. The modified electrode showed a poor response for concentrations smaller than 1 mM, for which the catalytic current can not be distinguished from the base line. The limit of detection was found to be 1.1 mM, which is too large for some analytical purposes. In order to improve the limit of detection, we will investigate the catalytic activity of the modified electrode by constant potential amperometric technique in the next sections of this paper.

The effect of potential scan rate on the anodic peak current of acetaminophen was investigated. The cyclic voltammograms of CdPCNF|GC electrode, recorded at various scan rates in PBS (pH 7.2) containing 10 mM ACT, are shown in Fig. 2A. As can be seen, the anodic peak current, I_{pa} changes proportional to the square root of potential scan rate (Fig. 2B, region a) with a slope of $0.0134 \text{ mA (mV s}^{-1}\text{)}^{-1/2}$, while the oxidation peak potential, E_{pa} remains constant for scan rates ranging 10-200 mV s^{-1} (Fig. 2C, region a), indicating the reversibility of the diffusion controlled electrochemical reaction which can be expressed by Randles-Sevcik equation [34]:

$$I_{\text{pa}} = 2.69 \times 10^5 n^{3/2} AC^* D^{1/2} \nu^{1/2} \quad (1)$$

Increasing potential scan rate to upper values made a considerable decrease in the time scale of the voltammetric method, at which, the electrode process may be dominated by kinetic parameters of the electrochemical reaction. This conditions was appeared for scan rates between 300 to 700 mV s^{-1} , where, I_{pa} vs. $\nu^{1/2}$ plot was deviated from the straight line (Fig. 2B, region b), and E_{pa} shifts with increasing scan rates towards a more positive potential (Fig. 2C, region b), confirming the irreversibility of the electrochemical reaction. For an irreversible process, the anodic peak current, I_{pa} and peak potential (E_{pa}) could be given by the following equations [35]:

$$I_{\text{pa}} = 3.01 \times 10^5 n [(1-\alpha)n_{\alpha}]^{1/2} AC^* D^{1/2} \nu^{1/2} \quad (2)$$

$$E_{\text{pa}} = K + \frac{2.3RT}{2(1-\alpha)n_{\alpha}F} \log \nu \quad (3)$$

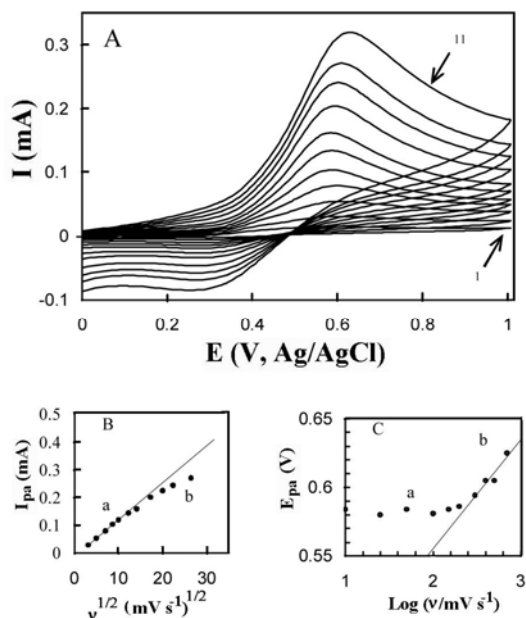


Fig. 2. (A) Cyclic voltammograms of 10 mM ACT at the CdPCNF|GC electrode in 0.25 M PBS (pH 7.2) at different scan rates of 10, 25, 50, 75, 100, 150, 200, 300, 400, 500, and 700 mV s^{-1} (from 1 to 11). (B) Dependence of the anodic peak current on the square root of the scan rate. (C) Dependence of the anodic peak potential on the logarithm of the scan rate.

$$K = E^{\circ'} + \frac{RT}{(1-\alpha)n_a F} \times \left(0.78 + \frac{2.3}{2} \log \left(\frac{(1-\alpha)n_a F D}{k^2 RT} \right) \right) \quad (4)$$

where α is transfer coefficient, n_a is the number of electrons involved in the rate-determining step, ν is scan rate, $E^{\circ'}$ is formal electrode potential, k is heterogeneous electron transfer rate constant, D is diffusion coefficient for ACT. Based on Fig. 3C and Eq. (3), the value of $(1-\alpha)n_a$ was calculated as 0.3721; and considering $D = 4.2 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, $A = 0.034 \text{ cm}^2$, and Eq. (2) it is estimated that the total number of electrons involved in the anodic oxidation of ACT is $n = 1.899 \approx 2$. The experimental intercept of Eq. (3), K was obtained as 0.3963, applying this in Eq. (4), we found that the rate constant for catalytic reaction is $k \approx 2.56 \times 10^{-2} \text{ cm s}^{-1}$ or $4.27 \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$ assuming a surface coverage of $6 \times 10^{-8} \text{ mol cm}^{-2}$ obtained from voltammetric data.

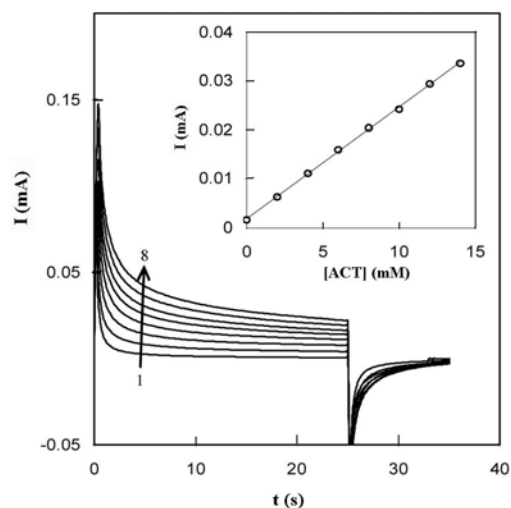


Fig. 3. Chronoamperograms obtained at the CdPCNF|GC electrode in the absence (1) and presence of 2, 4, 6, 8, 10, 12, and 14 mM ACT (from 2 to 8); first and second potential steps were 0.9 and 0.0 V, respectively. Inset shows the plot of fixed-time currents vs. ACT concentrations for elapsed time of 4 s, derived from data of the main panel. Supporting electrolyte was as in Fig. 1.

Chronoamperometric Experiments

The electrocatalytic oxidation of ACT at CdPCNF|GC electrode was investigated by double-step chronoamperometric technique in order to evaluate the diffusion coefficient and rate constant for the catalytic reaction of ACT in PBS (pH 7.2). The oxidative and reductive chronoamperograms were drawn by setting the electrode potentials at 0.9 and 0.0 V, respectively, for a certain period of time. Figure 3 shows the chronoamperometric measurements of ACT (2–14 mM) at the CdPCNF film modified GC electrode. The forward and backward chronoamperograms obtained at the modified electrode in 0.25 M PBS appeared very symmetrical, with an equal charge consumed for the oxidation and reduction of surface confined CdPCNF redox sites (Fig. 3, curve 1). However, in the presence of ACT, the charge value associated with the forward chronoamperometry, Q_f , (potential step 1:0.9 V) is significantly greater than that observed for the backward chronoamperometry, Q_b , (potential step 2:0.0 V). Inset of Fig. 3 shows the plot of currents

sampled at an elapsed time of 4 s after application of the potential step 1, as a function of ACT concentration, added to the electrochemical cell. The linear dependence of current to the substrate concentrations allows us to suggest that the mass transfer by diffusion has fulfilled for all chronoamperometric experiments illustrated in Fig. 3. For an electroactive substrate with diffusion coefficient D , the current corresponding to the electrochemical reaction (under diffusion control) is described by Cottrell's law [36]:

$$I = nFA(D/\pi t)^{1/2} C^* \quad (5)$$

where n is the number of electrons transferred in the electrocatalytic reaction, A is the geometric surface of electrode (cm^2), D is the diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$) and C^* is the bulk concentration of substrate (mol cm^{-3}). Other terms have their conventional meanings. On the basis of Eq. (5), the plots of I vs. $t^{1/2}$ are the straight lines (Fig. 4) for the time range of 4-25 s (or $t^{1/2}$: 0.55-0.2), with the slopes of $nFAD^{1/2} C^*/\pi^{1/2}$. From the slope of these plots the mean value of D was found to be $(4.21 \pm 0.18) \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$.

Chronoamperometry can be used for the evaluation of the kinetics of an electrocatalytic reaction. For an electroactive material with diffusion coefficient D , the current corresponding to the electrochemical reaction (under diffusion control) is described by Cottrell's law. But for the short times after step potential duration ($t \approx 0.4$ -1.5 s, or $t^{1/2}$: 0.6-1.2, in the present work), where a high concentration of substrate is still present at the electrode|solution interface, the catalytic current (I_{cat}) is dominated by the rate of the electron cross-exchange between CdPCNF redox sites and ACT. In this case, the rate constant can be determined according to the method described in the literature [37], using the following equation:

$$I_{\text{cat}}/I_L = \gamma^{1/2} [\pi^{1/2} \text{erf}(\gamma^{1/2}) + \exp(-\gamma)/\gamma^{1/2}] \quad (6)$$

where I_{cat} and I_L are the currents recorded at the CdPCNF|GC electrode in the presence and absence of ACT respectively, and $\gamma = kC^*t$, (C^* is the bulk concentration of ACT) and $\text{erf}(\gamma^{1/2})$ is the argument of the error function. In the cases where γ exceeds 2, the error function is almost equal to 1 and the above equation can be reduced to:

$$I_{\text{cat}}/I_L = \gamma^{1/2} \pi^{1/2} = \pi^{1/2} (k C^* t)^{1/2} \quad (7)$$

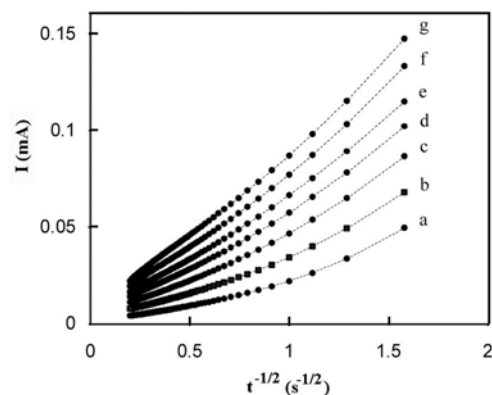


Fig. 4. The experimental plots of I vs. $t^{1/2}$ derived from chronoamperograms illustrated in Fig. 3, for different ACT concentrations of 2, 4, 6, 8, 10, 12, and 14 mM, (from a to g).

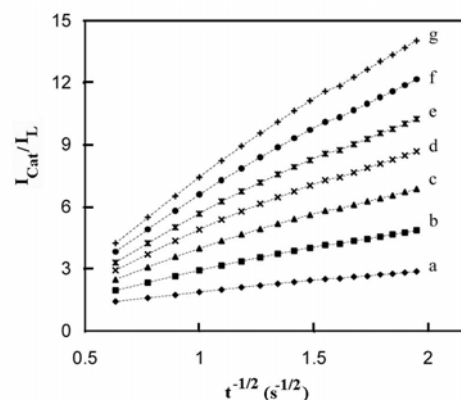


Fig. 5. The experimental plots of I_{cat}/I_L vs. $t^{1/2}$ derived from chronoamperograms illustrated in Fig. 3; I_L and I_{cat} are the currents recorded in the absence and presence of various ACT concentrations of 2, 4, 6, 8, 10, 12, and 14 mM (from a to g).

where k , C^* , and t are the catalytic rate constant ($\text{M}^{-1} \text{s}^{-1}$), the bulk concentration of ACT (M) and time elapsed (s), respectively. From the slope of the I_{cat}/I_L vs. $t^{1/2}$ plots, we can calculate the value of k for a given concentration of ACT. Figure 5 illustrates some I_{cat}/I_L vs. $t^{1/2}$ plots constructed from the data presented in Fig. 3. The mean value for k was found to be $4.1 \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$, which is in good agreement with those obtained from voltammetric technique.

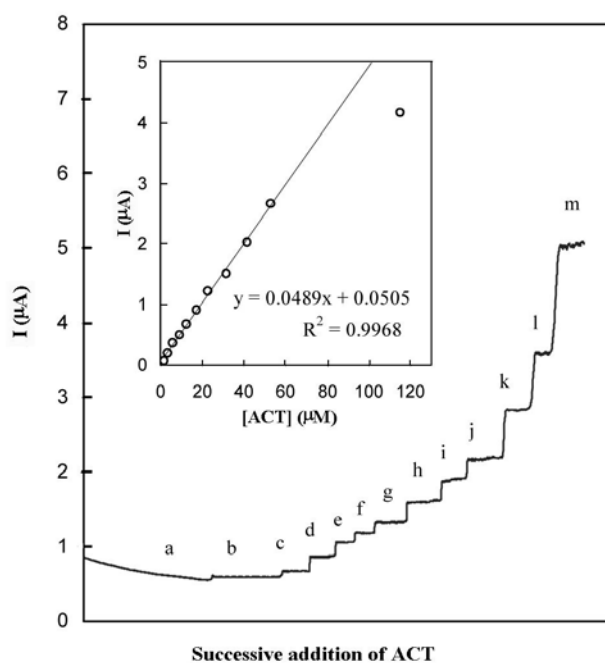


Fig. 6. The current-time recordings obtained at the CdPCNF|GC electrode with operating potential kept at 0.9 V. The step-current responses were appeared for successive addition of 0.1 mM standard solution of ACT to 10 ml supporting electrolyte (as in Fig. 1), at different concentrations of 0.0, 1.64, 3.28, 5.7, 9.1, 12.4, 17.2, 22.5, 31.4, 41.46, and 52.9 μM (from a to k). Inset shows a typical calibration curve obtained from the amperometric data with base-line correction. Supporting electrolyte was as in Fig. 1.

Amperometric Determination of Acetaminophen

Before the use of CdPCNF|GC electrode to the amperometric determination of ACT content in some pharmaceutical preparations, its efficiency was demonstrated by evaluation of the sensitivity, limit of detection, and dynamic range of the amperometric method. Figure 6 shows an amperogram recorded at a stirred solution under conditions where the potential of working electrode was kept at 0.9 V in PBS (pH 7.2), during addition of ACT by various increments. As seen, a well-defined response was observed. Unlike voltammetric method, linear response range of amperometric procedure was appeared at lower concentrations of ACT. The

calibration graph is linear up to about 50 μM and is described by the equation $I = 0.0489C + 0.0505$, $r^2 = 0.9968$, ($n = 10$), where C is the ACT concentration (μM), r is the correlation coefficient and n represents the number of determinations. The limit of detection (LOD) was found to be 2.04 μM , using the equation $\text{LOD} = 3\sigma_B/m$, where σ_B is the standard deviation (SD) of the blank (or SD for intercept of regression line) and m is the slope of the calibration graph.

Interference Study

The electroanalytical determination of ACT is often hampered by other electroactive species. For example, the interferences of citric acid, oxalic acid, and tartaric acid, benzoic acid, salicylic acid, adipic acid, maleic acid, manitol, glucose, and saccharose were investigated by cyclic voltammetry and constant potential hydrodynamic amperometry. No interference effects on amperometric response of ACT (5 mM) were observed due to the presence of these compounds even up to 100-fold excess.

ACT determination generally suffers from the interference of *p*-aminophenol as well as ascorbic acid (AA), glucose (G), sucrose (SC) and uric acid (UC) which coexist in biological systems. Hence, a systematic study was carried out for the evaluation of interference of these compounds. Specificity of the CdPCNF|GC electrode to 5 mM of ACT in the presence of these compounds was checked by recording amperogram for oxidation of ACT (5 mM) after addition of varying concentration of each interfering compound. No interfering effect due to the presence of glucose, sucrose, *p*-aminophenol even up to 50-fold-excess was observed. In contrast, the presence ascorbic acid and uric acid changes markedly the analytical signal related to ACT, limiting the application of proposed method in biological systems. In order to solve this problem, we used a cation exchange polymer (Nafion) film on the surface of CdPCNF|GC electrode. Nafion is a perfluorinated cation exchange polymer with a hydrophobic perfluoro-backbone and pendant sulfonic acid groups. The Nafion film repelled ascorbic acid ($pK_{a1} = 4.17$, $pK_{a2} = 11.57$) and uric acid ($pK_{a1} = 5.7$, $pK_{a2} = 9.8$) which exist as anionic species at physiological pH of 7.2, while ACT can freely penetrate into the Nafion film due to the cationic form (protonated form, $pK_a = 12$) at working pH. Thus, we used a negatively-charged Nafion-coated modified electrode for the

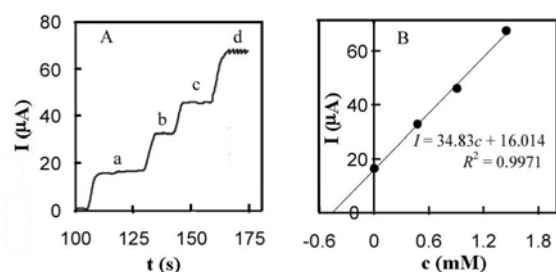


Fig. 7. (A) Amperometric response of the CdPCNF|GC electrode to a 0.5 ml ACT tablet solution added to 10 ml supporting electrolyte as in Fig. 1 (step current a), followed by successive addition of standard solution of ACT (10 mM) with various volumes of 0.5, 0.5, and 0.7 ml (from b to d). (B) The plot of catalytic current vs. standard ACT concentration.

determination of ACT spiked in human blood serum, containing uric acid.

Analytical Applications

The CdPCNF modified electrode was used as a sensor for the determination of acetaminophen in some pharmaceutical samples, such as acetaminophen tablets, suppositories for adults, pediatric suppositories, suspension, oral solution, and pediatric oral drops. The determination of ACT in pharmaceutical samples was carried out by the standard addition method in order to prevent of any matrix effect.

Figure 7A shows the results an amperometric experiment

Table 1. Determination of Acetaminophen in Tablets (Theoretical Value for $t = 2.78$ and $p = 0.05$)

No.	Amount of acetaminophen (mg/tablet)		
	Claimed	Proposed method	Official method ^a
1	325	323.00	324.10
2	325	323.75	323.80
3	325	324.18	325.80
4	325	324.50	324.24
5	325	326.20	323.60
Average		324.33 ± 1.48	324.31 ± 1.08

^aUV-Spectrophotometric method.

to determine ACT in tablet. The first current step (a) was appeared by adding a 0.5 ml solution of ACT tablet. The next current steps (b, c, and d) were obtained after successive addition of 0.5, 0.5, and 0.7 ml standard ACT solution of 0.01 M, respectively. Figure 7B shows a typical linear plot of I_{pa} vs. the ACT concentration constructed from amperometric data. The evaluation of the ACT concentration was found to be more suitable with the aid of these plots without interference from recipients. By considering the dilution factor, it was found that ACT concentration determined using this method is in good agreement with the declared value. The ACT tablet was also analyzed using standard method [38] and the results are shown in Table 1, which are comparable with those obtained by the proposed method, confirming the efficiency of

Table 2. Determination of Acetaminophen in Pharmaceutical Samples and Results of Recovery Tests

Sample	Amount of acetaminophen (mM)			
	Claimed	Added	Found	Recovery (%)
Suppositories for adults (mg/Suppository)	325	-	326.0	103
		20	346.6	
Pediatric suppositories (mg/Suppository)	125	-	122.6	99.8
		20	142.55	
Suspension (mg ml ⁻¹)	24	-	23.4	98
		5	28.3	
Oral solution (mg ml ⁻¹)	24	-	23	96
		7	29.72	
Pediatric oral drops (mg ml ⁻¹)	100	0	98.15	99.8
		72	170	

Rapid and Accurate Amperometric Determination of Acetaminophen

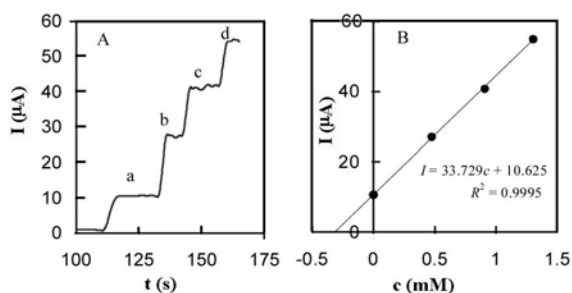


Fig. 8. (A) Amperometric response of the CdPCNF|GC electrode to a 0.5 ml human blood serum containing 10 mM ACT added to 10 ml supporting electrolyte as in Fig. 1 (step current a), followed by three successive addition of standard solution of ACT. (B) The plot of catalytic current vs. standard ACT concentration.

Table 3. Determination of Acetaminophen in Human Blood Serum Samples (Theoretical Value for $t = 2.78$ and $p = 0.05$)

No.	Amount of acetaminophen (mM)		
	Added	Found	Recovery (%)
1	10	10.3	103
2	10	9.9	99
3	10	9.83	98.3
4	10	9.86	98.6
5	10	9.84	98.4
Average		9.95 ± 0.25	9.95 ± 2.5

amperometric method for analysis of ACT tablets. Determinations of ACT in the other pharmaceutical preparations are summarized in Table 2. As can be seen, the results are in good agreement with those of labeled in the formulation, and satisfactory recoveries were obtained.

Figure 8A shows the results of an amperometric experiment recorded at the Nafion-coated CdPCNF|GC electrode to determine ACT in human serum sample. The first current step (a) was appeared by adding a 0.5 ml portion of serum containing 10 mM ACT. The next current steps (b, c, and d) were obtained after successive addition of 0.5 ml

standard ACT solution of 0.01 M. As can be seen, the response time of Nafion coated modified electrode is as good as that of the CdPCNF|GC electrode. Figure 8B shows a typical linear plot of I_{pa} vs. the ACT concentration constructed from amperometric data. The results of statistical calculations are summarized in Table 3.

The interference of AA and UC on the determination ACT (10 mM) in the serum sample was further investigated at Nafion-coated modified electrode by amperometric technique. Both AA and UC could not cause a relative error larger than %0.4 even up to 50-fold excess.

CONCLUSIONS

The glassy carbon electrode modified with CdPCNF showed good electrocatalytic response to the oxidation of ACT and a linear calibration graph was obtained over the concentration range 1.64–52.9 μM by the amperometric technique. The modified electrode was satisfactorily employed to amperometric determination of ACT in the commercial drugs and the results obtained are in good agreement with the declared values in the formulation. The results of inference study suggest that a Nafion-coated CdPCNF|GC electrode can be utilized as an amperometric sensor for acetaminophen determination in clinical analyses. The proposed method was satisfactory applied to the determination of acetaminophen spiked in human blood serum. The stability, reproducibility, and repetitive usability exhibited by the proposed modified electrode are enough to construct a flow injection analysis system with amperometric detection of ACT.

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