

Voltammetric Behavior and Amperometric Determination of Ascorbic Acid at Cadmium Pentacyanonitrosylferrate Film Modified GC Electrode

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The present work, describes electrochemical preparation of a novel cadmium pentacyanonitrosylferrate (CdPCNF) film as a Prussian Blue analogous on the glassy carbon (GC) electrode from a solution of 5 mM CdCl₂ and 5 mM Na₂[Fe(CN)₅NO]. The effect of experimental conditions such as *pH* and composition of electrodeposition solution on the peak current of the modified electrode and its stability were investigated. In the optimized conditions a controllable and repeatable value of CdPCNF film was obtained. The modified GC electrode showed a reversible cyclic voltammograms due to the redox reaction of Fe(III) / Fe(II) in the film. The CdPCNF film showed a good electrocatalytic activity toward the oxidation of ascorbic acid. The mean value of rate constant *k* and the diffusion coefficient of ascorbic acid in the solution *D*, were found to be $3.6 \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$, and $4.4 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ respectively. A calibration graph was obtained over concentration of 5 – 50 μM. The limit of detection was found to be 2.52 μM by hydrodynamic amperometry. This technique was used to the selective determination of ascorbic acid in the pharmaceutical preparations and fruit juices.

Keywords: Modified electrode; Amperometric determination, Ascorbic acid; Electrocatalytic oxidation; cadmium pentacyanonitrosylferrate

1. INTRODUCTION

During the past years inorganic film modified electrodes have received increased attention due to their potentials in technical applications. Various inorganic materials such as clays [1], zeolites [2], metal oxides [3], metal phthalocyanines [4], metal porphyrines [5], transition metals [6], polyoxometallates [7], and polynuclear transition metal cyanides [8-10], have been used to fabricate inorganic film modified electrodes. Of these, the use of polynuclear transition metal cyanides as modifier appears to be especially attractive because a large number of these compounds can be easily

prepared. In addition the well-defined zeolite-like structure makes the description and understanding of the charge-transfer process easier compared with that of organic polymer films [11].

Since the pioneering work of Neff and Itaya [8] on Prussian blue modified electrode, the preparation and electrochemical investigation of some PB analogue modified electrodes have been reported based on different transition metal hexacyanoferrate such as chromium [12], nickel [13, 14], cobalt [15], copper [16], palladium [17], molybdenum [18], vanadium [19], platinum [20], cadmium [21], and zinc [22]. Electrodes modified with Prussian blue, i.e. iron(III) hexacyanoferrate(II), showed two reversible redox centers due to low spin iron(II) and high-spin iron(III), in which both iron centers are electroactive [23]. Among the many metal hexacyanoferrates, cobalt hexacyanoferrate showed electrochemical behavior similar to PB. The other PB analogues indicated only one redox center due to hexacyanoferrate (III)/(II) system. In order to extend the electroanalytical methods based on the modified electrode, some research activities have been oriented on the electrochemical synthesis of new PB analogous, mixed transition metal hexacyanoferrates [24], and rare earth hexacyanoferrates [25]. In this regard we reported the preparation of cadmium pentacyanonitrosylferrate (CdPCNF) film modified electrode prepared by electrodeposition of metallic cadmium film followed by chemical derivatization of cadmium to CdPCNF film in solution of sodium pentacyanonitrosylferrate [26]. This method was not a suitable procedure because the electrode surface coverage could not be controlled during modification process. In this work we describe an alternative voltammetric procedure for accumulation of CdPCNF film. Using this method a controllable and repeatable amount of CdPCNF film can be produced at the electrode surface. The stability of modified electrode is also increased. The electrocatalytic oxidation of ascorbic acid and its determination in the pharmaceutical preparations and fruit juices were also investigated at the proposed electrode for first time.

2. EXPERIMENTAL PART

2.1. Reagents and apparatus

Sodium pentacyanonitrosylferrate and other chemicals were of analytical grade from Merck. Phosphate buffer solution (PBS) was made up from KH_2PO_4 and adjusted to desired *pH* by adding 1 M KOH or HNO_3 solution. 2, 6-Dichlorophenol-indophenol sodium salt hydrate was purchased from Sigma-Aldrich. Pharmaceuticals were from Hakim Pharmacy Co. and fruit samples were obtained from local sources. All solutions were prepared with twice distilled water.

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.

2.2. Fabrication of the modified electrode

The glassy carbon electrode surface is polished with 0.05 μm alumina powder on the wet polishing cloth. The polished electrode surface is rinsed with distilled water for several times. The

electrode is then immersed in a solution containing 5 mmol L⁻¹ CdCl₂, 5 mmol L⁻¹ Na₂[Fe^{III}(CN)₅NO] and 0.1 mol L⁻¹ KNO₃ as supporting electrolyte with a *pH* adjusted in the range 2 – 3 by sulfuric acid (electrode modifying solution). The modification of electrode surface was performed by 30 - 50 cycles of potential swept between -0.2 to 1.0 V 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.

The mechanism of film formation may be described as follows: The Cd²⁺ cations do not undergo to a redox reaction at potential range -0.2 - 1.0 V [26], while the [Fe^{III}(CN)₅NO]²⁻ is reduced to [Fe^{II}(CN)₅NO]³⁻ during cathodic scans. It seems that the [Fe^{II}(CN)₅NO]³⁻ species have further affinity to reaction with Cd²⁺ cations to form solid CdPCNF film. This supposition was examined by a simple test; the cleared solution of [Fe^{III}(CN)₅NO]²⁻ (2 mM) and Cd²⁺ (2 mM) is stable at least 24 h without formation of any participate, but it is quickly changed to a cloudy solution and formed light-brown participate by addition of a reducing reagent as ascorbic acid. A reducing compound could produced [Fe^{II}(CN)₅NO]³⁻, that can be participate by Cd²⁺ cations. However, we understood that the growth of film is proceeded via electrochemical production of [Fe^{II}(CN)₅NO]³⁻ followed by chemical reaction with Cd²⁺. The solid film is doped by alkali-metal cations as K⁺ present in the solution. The value of mediator film can be satisfactory controlled by the number of potential cycles. The total surface coverage of the electrode, *Γ* was determined from the area under anodic peak of cyclic voltammogram recorded for CdPCNF/IGC electrode.

2.3. Analysis of real samples

Vitamin C tablets: An accurately weighed portion of finely powdered sample obtained from three tablets, equivalent to about 25 mg of ascorbic acid was transferred to a 20 mL assay tube and ascorbic acid was extracted with two 10 mL portions of 0.5% citric acid in doubly distilled water. The extract were combined in a 50 mL flask and diluted to volume. Finally, 1 mL of the extract (~ 2.8 mM) was transferred to 25 mL flask and diluted to the volume. A 0.5 mL portion of diluted extract (~ 0.11 mM) was added to amperometric cell containing 12 mL PBS (*pH* 7.0) and 0.1 M KNO₃; and catalytic current of the modified electrode was measured at an optimized potential (0.9 V) while the electrode was rotated at 1000 rpm. The amount of ascorbic acid was determined by means of the standard addition method.

Vitamin C ampoules: An accurate ampoule volume equivalent to about 25 mg of vitamin C was transferred to a 50 mL flask and diluted to volume with 0.5% citric acid solution. Then, 1 mL of the former solution was transferred to 25 mL flask and diluted to the volume. A 0.5 mL portion of the diluted extract was subjected for amperometric measurement as described for tablets.

Fruit juices: Fresh juice of orange, apple, kiwi, and tomato was obtained using a hand squeezer (polymer material), whereas the juice-orange, apple, Kiwi and tomato were obtained using a mechanical squeezer. The juices obtained were filtered into a beaker and acidified slightly (*pH* 2) with sulfuric acid and diluted with distilled water to the suitable concentration level. A 0.5 mL portion of the sample was added to the supporting electrolyte solution in amperometric cell. In all cases the amounts of vitamin C in the samples were determined by the standard addition method.

3. RESULTS AND DISCUSSION

3.1. Optimization of electrode modification conditions

Fig. 1A shows the repetitive cyclic voltammograms recorded at the GC electrode in the solution of 10 mM $\text{Na}_2[\text{Fe}^{\text{III}}(\text{CN})_5\text{NO}]$ + 1 mM CdCl_2 + 0.5 M KNO_3 during electrode modification process. There are two pairs of redox peaks in each cyclic voltammograms. The corresponding formal potentials, $E^{0'}$ are 480 mV (average of a_{I} , c_{I} redox peaks) and 660 mV (average of a_{II} , c_{II} redox peaks) at scan rate of 100 mV s^{-1} . The electrochemical processes of redox couple (I) and (II) can be attributed to the reduction / oxidation of $[\text{Fe}^{\text{III}}(\text{CN})_5\text{NO}]^{2-}$ species in CdPCNF solid film and its solution species in the reaction layer (electrode | solution interface), respectively. Because, the bare GC electrode did not show redox couple (I) in supporting electrolyte containing only $[\text{Fe}^{\text{III}}(\text{CN})_5\text{NO}]^{2-}$ species, and the redox couple (II) was disappeared when the GC modified electrode was immersed in the supporting electrolyte (0.5 M KNO_3) and scanned at the same potential range as shown in Fig. 3A curve c.

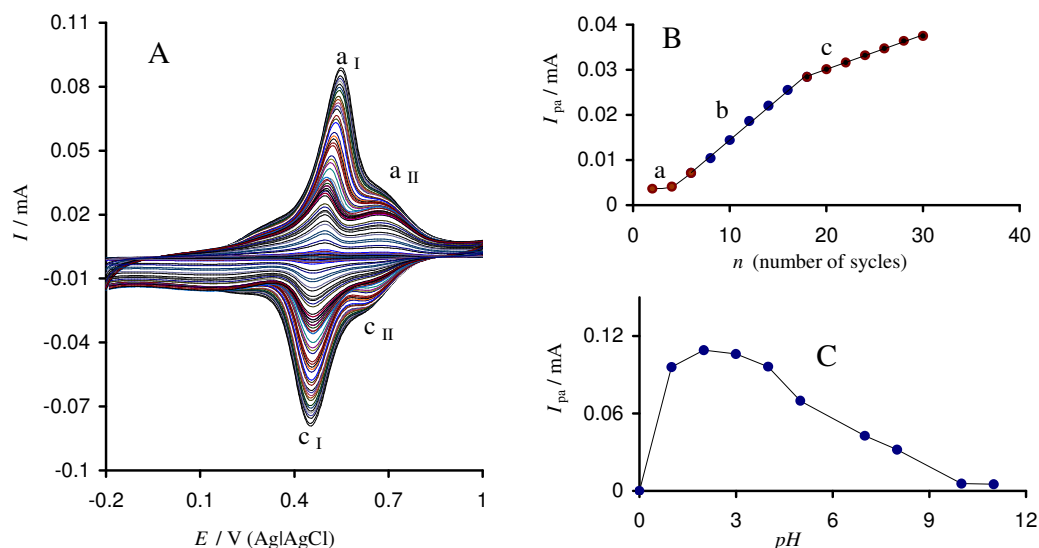


Figure 1. (A) The repetitive CVs of the GC electrode recorded in a solution of 1 mM CdCl_2 , 10 mM $\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]$, and 0.5 M KNO_3 during electrodeposition of CdPCNF film, at scan rate of 100 mV s^{-1} . (B) The variation of anodic peak current for CdPCNF deposited vs. the number of voltammetric cycles. (C) The plot of anodic peak current for CdPCNF film prepared at different pH values.

The effect of both CdCl_2 and $\text{Na}_2[\text{Fe}^{\text{III}}(\text{CN})_5\text{NO}]$ concentrations on the rate of immobilization of CdPCNF film and its stability were examined. In some experiments the concentration of CdCl_2 was changed from 1 to 10 mM, while the concentration of $\text{Na}_2[\text{Fe}^{\text{III}}(\text{CN})_5\text{NO}]$ was kept at 10 mM. The other experiments was performed using a constant concentration of 10 mM CdCl_2 , and increasing concentration of $\text{Na}_2[\text{Fe}^{\text{III}}(\text{CN})_5\text{NO}]$ in the range 1 – 10 mM. In all cases we obtained surface attached CdPCNF film. However, from view point of the reversibility of electrode reaction and its stability, the best response of modified electrode was belonged to the case that the concentrations of both CdCl_2 and $\text{Na}_2[\text{Fe}^{\text{III}}(\text{CN})_5\text{NO}]$ were equal to 5 mM.

Fig. 1B shows the variation of anodic peak current related to CdPCNF film during its electrodeposition on the electrode surface. As can be seen the anodic peak current thus the amount of surface coverage increases with the numbers of voltammetric scans (n). The plot of peak current vs. n consists of three segments with different slopes. For the first five cycles the value of CdPCNF electrodeposited is negligible (region a), this may be attributed to the slow nucleation process. When the primary nuclei were formed on the electrode surface, the rate of film formation is increased (region b) until the number of scans reaches to ~ 20 . For scan numbers greater than 20 cycles, the slope is decreased (region c). It seems that the electrode surface is completely covered with CdPCNF film during first 20 cycles. Further increasing in scan number makes film formation on CdPCNF film increasing its thicknesses. Comparing the slopes of regions b and c shows that the rate of film formation at GC electrode is greater than that of CdPCNF film. This may be attributed to the electrical resistance of CdPCNF film.

The solution pH affects markedly the formation of CdPCNF film. Fig. 1C shows the anodic peak current of the modified electrode prepared in solution of $CdCl_2$ (5 mM) and $Na_2[Fe^{II}(CN)_5NO]$ (5 mM) with different pH s ranging 0 – 11 adjusted by H_2SO_4 and $NaOH$. As can be seen we could not immobilize CdPCNF to the electrode surface at zero pH value. It seems that in the highly acidic solution, the dissolution of the film over come to the film formation process. The pH range 2 - 3 is very suitable for immobilization of CdPCNF film. Further increasing in pH made an inhibiting effect on film formation due to partial precipitation of Cd^{2+} by hydroxide ions. In hardly alkaline solutions we could not immobilize CdPCNF film because of the degradation effect of these media on the film and precipitation of Cd^{2+} cations.

The stability of modified electrode under voltammetric experiments was studied. It was found that the storing of electrode in air enhanced markedly its stability. Fig. 2A shows the variation of (I_n / I_1) vs. n for a freshly prepared electrode (curve a) and for a similar electrode dried in air for 24 h period of time (curve b). Where, I_1 and I_n are the anodic peak currents observed for the first and n^{th} cyclic voltammograms, respectively. The stability of (I_n / I_1) with the number of CV scans, n is an indicative for strong attachment of the CdPCNF film with the electrode surface. For the both modified electrodes, decreasing in the term (I_n / I_1) is similar for the first 30 cycles. Continuing voltammetric scans makes further decrease in the term (I_n / I_1) for freshly prepared electrode, as it reaches to zero after 170 cycles. In contrast, the electrode dried in air for 24 h (curve b) shows a considerable stability in the term (I_n / I_1) against voltammetric scans.

In order to investigate the rate of film degradation and its mechanisms, the logarithm of peak current was drawn against voltammetric scan number n (Fig. 2B). The plot obtained shows two different segments with different slopes (Fig. 2B, regions a and b). This may be attributed to the presence of CdPCNF in two different structures with different stability. The linearity of this plot shows that the electro-degradation reaction obeys the first order kinetics.

The modified electrode prepared at optimum conditions exhibited symmetrical cyclic voltammograms as expected for a highly reversible surface immobilized redox couple (Fig. 3A curve c). The anodic and cathodic peak currents were found to be linear with the potential scan rate up to 250 mV s^{-1} , and the ratio of the anodic to the cathodic charge obtained by integrating the I-E curve at a low potential scan rate was close to unity. The variations of peak separation ΔE_p , are relatively small

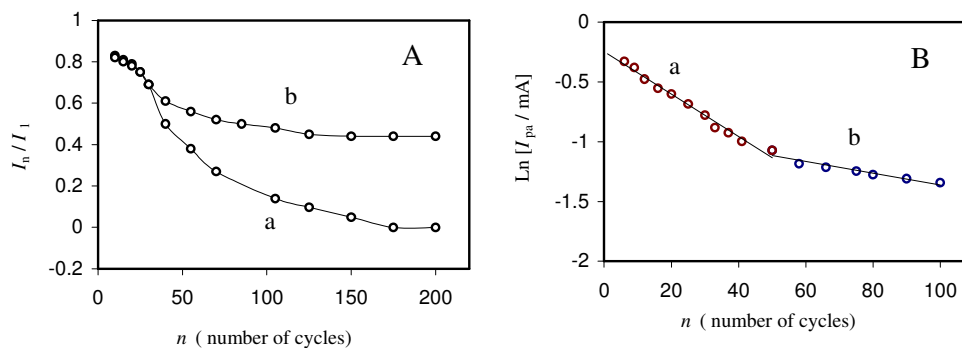
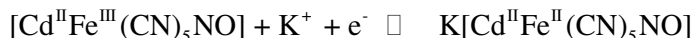


Figure 2. (A) The variation of I_n / I_1 vs. n for a freshly prepared electrode (curve a) and a similar electrode dried in air for 24 h (curve b). (B) The logarithm of peak current against voltammetric scan number, n , obtained from cyclic voltammograms of CdPCNF/GC in 0.5 M KNO_3 at scan rate of 100 mV s^{-1} .

for scan rates ranging 25 to 100 mV s^{-1} . These show that the redox reaction is reversible and involves the redox reaction of Fe(II)/Fe(III) in CdPCNF film as follow:



Ideal surface-confined, diffusionless species are characterized by zero ΔE_p values. The smallest ΔE_p was found equal to 30 mV at scan rate of 10 mV s^{-1} . This deviation might suggest kinetic limitations or electrostatic interactions of the redox sites in the film. The surface coverage (Γ) was estimated from the usual equation:

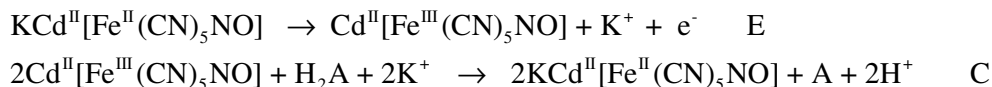
$$\Gamma = Q/nFA \quad (1)$$

Where Q is the electric charge obtained by integrating anodic peak obtained at 20 mV s^{-1} , with the background correction; n , F and A present the number of electrons transferred in redox reaction, faraday constant and geometric electrode area, respectively.

3.2. Electrocatalytic oxidation of ascorbic acid (AA) at the GC modified electrode

The electro-oxidation of AA at the bare GC electrode requires a large overpotential, and a poor anodic peak was observed at 0.85 V due to the oxidation of AA, but no cathodic current peak was found, indicating an irreversible heterogeneous charge transfer in this system, as shown in Fig. 3A curve b. Further, the repetitive scans in the presence of AA made a decrease in current due to the adsorption of reaction products. The catalytic oxidation of AA at the GC electrode modified with CdPCNF film can be clearly seen in Fig. 3A curve d; where the peak current was markedly enhanced and the peak potential was shifted negatively about 340 mV. Fig. 3A curve c shows the cyclic voltammogram of the GC modified electrode in PBS ($\text{pH } 7.0$). Adding AA to the cell produced a

dramatic change in the cyclic voltammogram with an obvious increase in anodic current and a concomitant decrease in cathodic current. This behavior is a typical of that expected for mediated oxidation of an irreversible process. The chemical reaction of AA 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 H₂A and A is the abbreviated form of ascorbic acid and its oxidation form.

A calibration plot was provided using the linear sweep voltammograms recorded for a series of AA solutions with various concentrations and it was shown in Fig. 3B. The linear part of this curve (1 - 55 mM) shows a sensitivity of (6.3 ± 0.22) $\mu\text{A}/\text{mM}$ with a correlation coefficient $r = 0.9982$ ($n = 16$). The limit of detection was found to be 1.3 mM. At high concentrations of AA a negative deviation from the straight line was observed. This deviation may be attributed to the saturation of the electroactive sites, i.e. all AA species can not be oxidized via electrocatalytic process at high concentrations levels.

Fig. 4A shows the linear sweep voltammograms for the anodic oxidation of AA (10 mM) on the CdPCNF/IGC electrode at various scan rates (10 - 600 mV s^{-1}). It is clear that the peak current is increased with the rising of scan rate, while the peak potential is shifted positively. I_{pa} for oxidation of AA increases linearly with $v^{1/2}$, as expected for a diffusion-controlled reaction (Fig. 4B). The slope of the straight line ($r = 0.9993$) of I_{pa} versus $v^{1/2}$ is $0.0084 \text{ mA (mV)}^{-1/2} \text{ s}^{1/2}$. On the other hand, the peak potential (E_{pa}) was positively shifted at high potential scan rates due to the depressing the time scale of the method. This shows that the catalytic current can be controlled by the kinetics of catalytic reaction. In this case the plot of E_{pa} versus the logarithm of the scan rate, a nice straight line ($r = 0.9988$) is obtained (Fig. 4C), from the straight line, the slope and the intercept can be given as 0.0673 and 0.3474, respectively. For an irreversible process, the peak potential (E_{pa}) could be present by the following equation [27].

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

$$K = E^{\circ} + \frac{RT}{(1-\alpha)n_{\alpha}F} \times \left(0.78 + \frac{2.3}{2} \log \left(\frac{(1-\alpha)n_{\alpha}FD}{k^2RT} \right) \right) \quad (3)$$

Where α is transfer coefficient, n_{α} is the number of electrons involved in the rate-determining step, v is scan rate, E° is formal electrode potential, k is heterogeneous electron transfer rate constant, D is diffusion coefficient, for AA, it is $3.4 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ [28]. So, $(1-\alpha)n_{\alpha}$ can be calculated as 0.4393, k is $5.6 \times 10^{-3} \text{ cm s}^{-1}$ or $3.5 \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$ assuming a surface coverage of $1.6 \times 10^{-8} \text{ mol cm}^{-2}$.

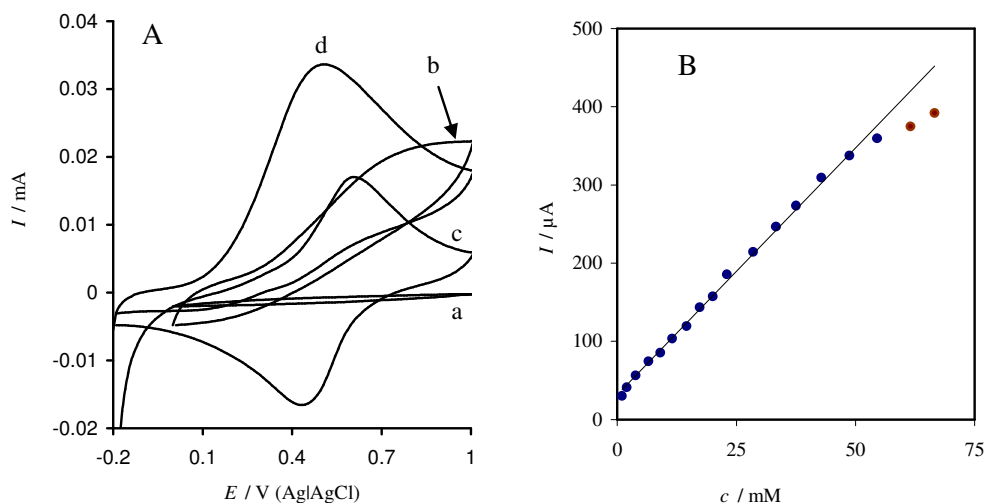


Figure 3. (A) Cyclic voltammograms recorded at the bare GC electrode (a, b) and CdPCNFIGC electrode (c, d) in the absence (a, c) and presence (b, d) of 5 mM AA at scan rate of 100 mV s^{-1} . Supporting electrolyte was 0.25 M PBS ($\text{pH } 7.0$). (B) The plot of anodic peak current versus concentration of AA, obtained from linear sweep voltammograms recorded at the CdPCNFIGC electrode; Potential scan rate: 100 mV s^{-1} .

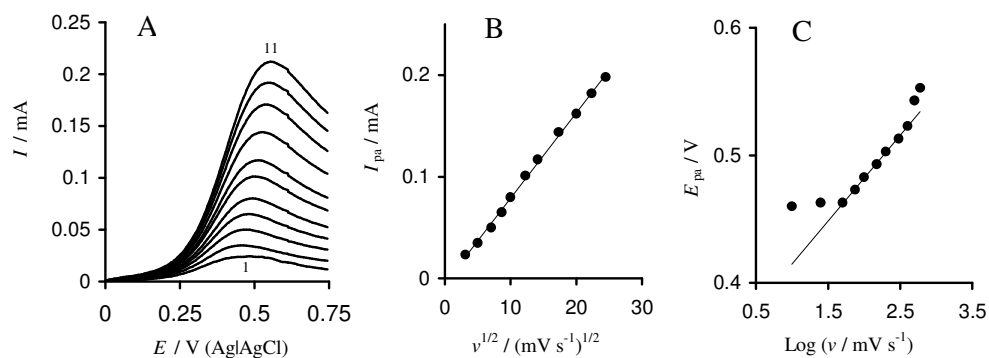


Figure 4. (A) Cyclic voltammograms of the CdPCNFIGC electrode in the presence of 10 mM AA at various potential scan rates: 1 - 11) 10, 25, 50, 75, 100, 150, 200, 300, 400, 500, and 600 mV s^{-1} , respectively. Supporting electrolyte was 0.25 M PBS ($\text{pH } 7.0$). (B) The variation of anodic peak current, I_{pa} vs. square root of potential scan rate, $v^{1/2}$. (C) The dependence of the anodic peak potential E_{pa} versus $\text{Log}(v)$ derived from the voltammetric data.

3.3. Rotating disk electrode (RDE) voltammetry

In order to investigate the electrocatalytic oxidation of AA and estimate heterogeneous electron transfer rate constant, k the electrochemical measurements were also carried out using RDE voltammetry (low potential scan rate technique). In this technique, the mass transfer limited current in A is expressed by the Levich equation [29]:

$$I_{Lev} = 0.62nFAD^{2/3}\nu^{-1/6}\omega^{1/2}c_0 \quad (4)$$

where n is the number of electrons, D the diffusion coefficient of the substrate (AA), F the faradaic constant, c_0 the bulk concentration of the substrate (AA), A is the electrode area, ν the kinematic viscosity of the solution and ω the rotation rate. For all calculations, the angular velocity is taken as $\omega = (2\pi/60)f$, where f is the frequency of electrode rotation in rpm (round per minute).

In the case that the mass-transfer process in the solution controls solely the oxidation of AA at the surface of modified electrode, the relationship between the limiting current (I_{lim}) and rotation speed should obey the Levich equation. The linearity of I_{lim} versus $\omega^{1/2}$ (Levich plot), implies that the electrocatalytic reaction is faster than the rate of substrate delivery to the electrode surface, so that the current is determined only by how fast the substrate is transported to the surface of the modified electrode. Fig. 5A shows the typical RDE voltammograms of 10 mM AA at the CdPCNFIGC electrode recorded by sweeping the potential of the modified electrode with a surface coverage of 5×10^{-6} mol cm^{-2} , at 10 mV s^{-1} and a rotation rate of 200 – 3000 rpm in PBS of $pH = 7.2$ as supporting electrolyte. By means of the slope of Levich plots shown in Fig. 5B, the diffusion coefficient of the AA was determined as $4.4 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$. The electrocatalysis however cannot keep up with the increased flux of AA at high rotation rates, so the currents level off. The lack of linearity at high rotational speed suggests that the reaction is limited by the kinetics and not by mass-transport. As the rotation speed increased, the limiting electrocatalytic current approached value independent of the rotation speed.

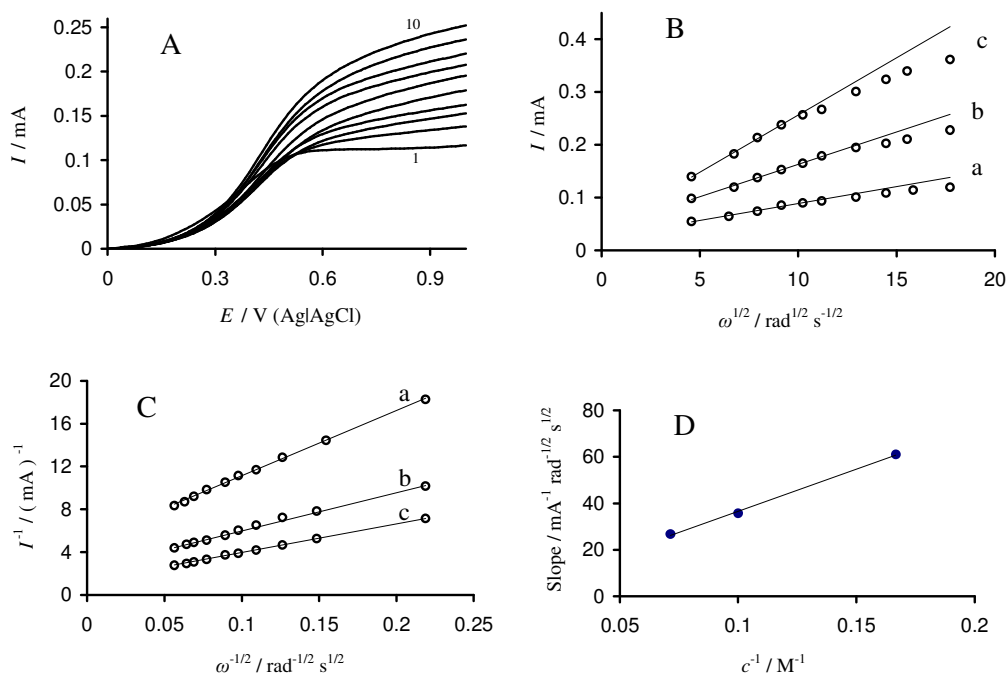


Figure 5. (A) Rotating disk electrode voltammograms recorded at the CdPCNFIGC electrode for 10 mM AA. The rotation rates are in rpm: 1 - 10) 400, 600, 800, 1000, 1200, 1400, 1800, 2000, 2400, and 3000, respectively. Potential sweep rate: 10 mV s^{-1} , supporting electrolyte was 0.25 M PBS ($pH 7.0$), $\Gamma = 4.8 \times 10^{-8} \text{ mol cm}^{-2}$. (B) and (C) represent the Levich and Koutecky-Levich plots (respectively) for various concentrations of AA: (a) 6, (b) 10 and (c) 14 mM. (D) The variation of slope of Koutecky-Levich plots versus the reverse of the AA concentration.

A detailed description of the kinetic process at the modified electrodes by RDE voltammetry has been treated comprehensively by Andrieux et al [30]. If the electron exchanges process at the electrode | mediator film interface and electron diffusion process within the film is assumed to be fast, for a reaction mediated by a surface-bound modifier, the kinetically controlled current (I_{kin}) is given by:

$$I_k = nFAc_0k\Gamma \quad (5)$$

where Γ is the surface coverage of the electrode modifier and k is the rate constant for the electron cross-exchange reaction between the reactants in the solution and the redox centers in the modifier film. So, combining Equations (4) and (5) leads to the Koutecky–Levich expression [29], which describes the overall RDE plateau current for the electrocatalytic reaction:

$$\frac{1}{I_{lim}} = \frac{1}{I_{Lev}} + \frac{1}{I_{Kin}} \quad (6)$$

According to this expression, the limiting current (I_{lim}) corresponding to the mediated reaction is a function of the Levich current (I_{Lev}) representing the mass transfer of AA in the solution and the kinetic current (I_{kin}) corresponding to the electron cross exchange between AA and the CdPCNF redox site. Based on the Koutecky–Levich equation, a plot of $(I_{lim})^{-1}$ versus $\omega^{1/2}$ should yield a straight line whose intercept gives the value of $K' = k\Gamma$. The Koutecky – Levich plots, obtained from the data of Fig. 5A, are shown in Fig. 5C. From the intercepts of these plots, corresponding to the infinite rotation speed, the rate constant for the catalyzed oxidation of AA was evaluated. The average value of k was found to be $3.7 \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$ for AA concentration in the range 6 – 14 mM, using $\Gamma = 5 \times 10^{-8} \text{ mol cm}^{-2}$. The slope of the Koutecky–Levich plot is dependent on the reactant concentration c_0 . As shown in Fig. 5D, the linear relationship between the slope of Koutecky– Levich plots and inverse AA concentration confirms that the catalytic reaction shows a first order dependence on the concentrations of AA.

3.4. Analytical applications

The efficiency of CdPCNFIGC electrode was demonstrated by applying it to determine the vitamin C content in some fruit juices and pharmaceutical preparations. These samples were diluted and then added to the electrochemical cell directly, without any pretreatment. The hydrodynamic amperometric measurement at an optimized operation potential of 0.9 V was utilized as analytical method. An advantage of this method is an easy base-line correction of catalytic current. Fig. 6A shows a typical hydrodynamic amperogram obtained by successively addition of 10^{-4} M standard AA solution by different increments to 12 mL phosphate buffer solution (pH 7). According to the Fig. 6B, the catalytic current is linearly increased after addition of different increments of standard AA solution. The regression equation is: $I_p (\mu\text{A}) = (0.2253 \pm 0.0159) c + (0.3641 \pm 0.4858)$ with $r = 0.9977$ ($n = 7$), where c is AA concentration in μM . The best performance of the modified electrode as an amperometric sensor was achieved at pH 7, and limit of detection (3σ) was found to be 2.52 μM .

In order to demonstrate the analytical applications of CdPCNF|GC electrode, we examined the oxidation of AA in the real samples including pharmaceutical preparations and fresh fruit juices purchased from local sources. The determination of AA (vitamin C) in these samples was carried out by the standard addition method in order to prevent of any matrix effect.

Fig. 7A shows typical chronoamperogram recorded at the modified electrode in PBS (region a) and after adding a certain volume from solution of vitamin C tablet prepared in twice distilled water (step b) and standard concentration solution of AA (steps c, d, e). Fig. 7B shows a typical linear plot of I (current step on chronoamperogram) versus the concentration of AA added to the electrochemical cell. The amount of AA in the unknown solution of vitamin C tablet was calculated with the aid of this plot.

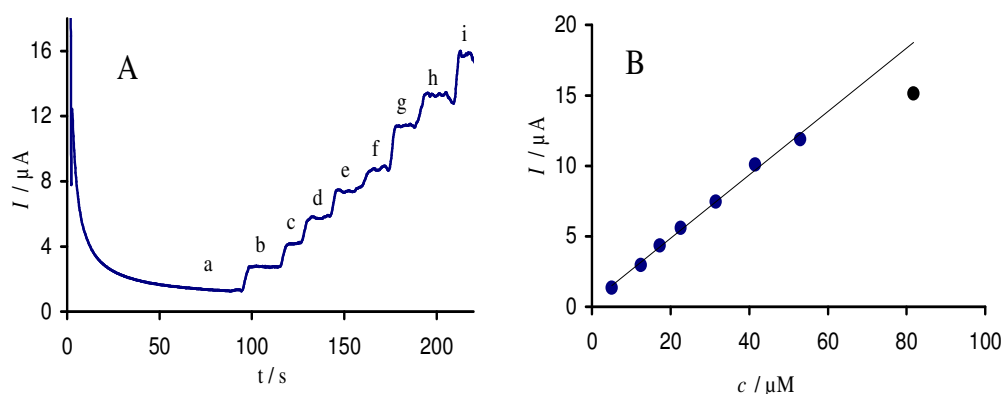


Figure 6. (A) A hydrodynamic amperogram showing the current response of the modified electrode for different concentration of AA added from 0.1 mM standard solution to 12 mL PBS (pH 7): a) 0.0, b) 5, c) 12.4, d) 17.2, e) 22.5, f) 31.43, g) 41.46, h) 52.9, and i) 81.7 μM . The rotation speed of the modified electrode was 1000 rpm. (B) A typical calibration curve obtained from the amperometric data with base-line correction.

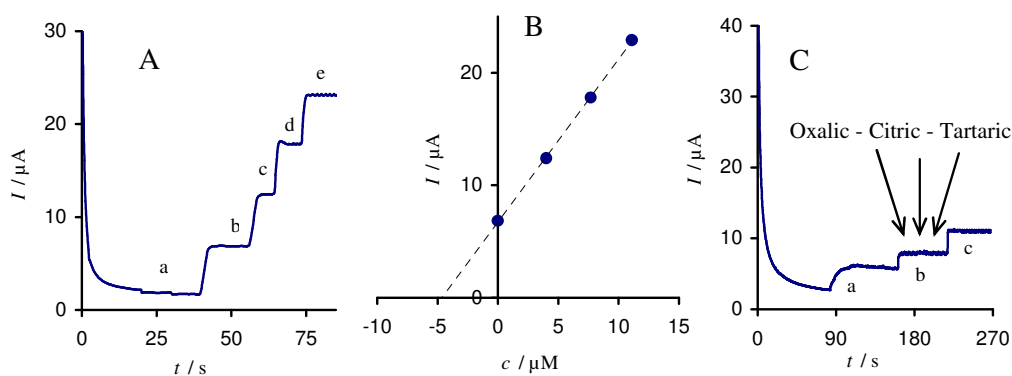


Figure 7. (A) Amperometric response of the modified electrode recorded in 12 mL PBS of $pH = 7$ in the absence of AA (region a) which was followed by addition of 0.5 mL unknown solution of vitamin C tablet (step b) and standard solution of AA added to the electrochemical cell with different concentrations: b) 0.0, c) 4, d) 7.69, e) 11.1 μM . The rotation speed of the modified electrode was 1000 rpm. (B) A typical calibration curve obtained from the amperometric data with base-line correction. (C) Amperometric response of the CdPCNF|GC electrode to AA at different concentrations: a) 0.91, b) 1.6, c) 2.0 mM, and some interfering species. Each catalytic current step started by addition of AA, followed by successive addition of oxalic acid, citric acid, and tartaric acid, respectively, with various concentrations: a) 11.5, b) 56.5, c) 116.8 mM. The potential of modified electrode was kept at 0.9 V.

In Table 1 some results for analysis of pharmaceutical preparations with the amperometric method compared favorably with those obtained by the USP standard method [31] which is iodine titration are summarized. Table 2 shows the results for analysis of some fruit juices obtained by using the proposed amperometric method, which are comparable with those obtained with standard 2, 6-Dichlorophenol-indophenol method [32].

Table 1. Determination of AA in the pharmaceutical samples (theoretical value for $t = 2.78$ and $p = 0.05$). Results based on three replicate determinations per sample.

Sample	Amount of ascorbic acid				
	Proposed method	% RSD	Official method*	% RSD	t
Effervescent tablet (mg / tablet)	1498±9.28	0.14	1492±23.35	0.364	1.78
Chewable tablet (mg / tablet)	240.66±7.31	0.71	243.4±9.16	1.61	1.11
Ampoule (mg / 5 mL)	498.00±4.94	0.23	498.33±10.72	0.5	0.21

* Iodine titration method.

Table 2. Determination of AA in the fruit juices (theoretical value for $t = 2.78$ and $p = 0.05$). Results based on three replicate determinations per sample.

Sample	Amount of ascorbic acid				
	Proposed method	% RSD	Official method*	% RSD	t
Orange (mg / mL)	49.24±2.20	1.04	50.10±2.30	1.07	2.01
Tomato (mg / mL)	28.80±1.27	1.03	28.90±1.37	1.10	0.4
Apple (mg / mL)	8.80±0.59	1.58	8.50±0.77	2.10	2.39
Kiwi (mg / mL)	40.50±2.73	1.57	41.20±3.53	2.01	1.17

* 2,6-Dichlorophenol-indophenol method.

3.5. Interference study

The electroanalytical determination of AA is often hampered by other electroactive species. For example, citric acid, oxalic acid, and tartaric acid may be existed in fruit juices. The interference of citric acid, oxalic acid, and tartaric acid, benzoic acid, salicylic acid, adipic acid, maleic acid, manitol, glucose, saccharose and formaldehyde were investigated by cyclic voltammetry and constant potential hydrodynamic amperometry. All compounds mentioned above, except formaldehyde, could not make an error larger than % 1-2, even for a concentration level bigger 100 time of AA. Based on the experimental results, formaldehyde can be persistent interfering specie in the determination of AA. Fig. 7C shows a typical comparative response of the modified electrode to the AA, oxalic acid, citric acid, and tartaric acid. As can be seen, these compounds did not make an increase in catalytic current;

in contrast it was increased markedly by addition of AA in each time, indicating the selectivity of electrode response to AA.

4. CONCLUSIONS

The CdPCNFIGC modified electrode can catalyze the oxidation of AA via surface-layer mediated charge transfer. The rate constants for the electrocatalytic reaction obtained by different approaches are in good agreement. A mean value for the rate constant k was found to be $3.6 \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$, which is in good agreement with that obtained at cobalt pentacyanonitrosylferrate (CoPCNF) film [33]. We proposed an amperometric method to show the capability of modified electrode to determination of AA in the pharmaceutical preparations and fruit juices. The limit of detection ($2.52 \mu\text{M}$) found by the proposed method was very improved respect to that reported at GC electrode modified with nickel(II) macrocycle containing dianionic tetraazaannulene ligand [34], and is larger than those obtained at poly(toluidine blue) [35], calixarene [36] modified electrodes and indirect atomic absorption spectrometry [37]. However, the efficiency of proposed method was enough for analysis of pharmaceutical preparations and some fruit juices. The results obtained by applying the proposed method were compared with those obtained by official methods. Based on the experimental results, there are no significant error between the proposed and official methods from view point of the accuracy and repeatability.

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