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Electrocatalytic Determination of Ascorbic Acid at Chemically Modified Carbon Paste Electrode with 2, 7-bis (Ferrocenyl ethynyl) Fluoren-9-one

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Cyclic voltammetry, differential pulse voltammetry and double potential step chronoamperometry were used to investigate the electrochemical behavior of ascorbic acid (AA) at a chemically modified electrode prepared by incorporating 2, 7-bis (ferrocenyl ethynyl) fluoren-9-one (2, 7-BFEFO) into carbon paste matrix. Under the optimized conditions (pH 7.00), the modified electrode showed high electrocatalytic activity toward AA oxidation; the overpotential for the oxidation of AA was decreased by more than 200 mV and the corresponding peak current increased significantly. In fact, the Fc/ Fc^+ redox couple acts as a suitable mediator for indirect oxidation of AA. The diffusion coefficient (D = 8.65×10^{-6} cm^2 s^{-1}), and the kinetic parameters such as electron transfer coefficient, (α = 0.47) and the catalytic reaction rate constant, ($k=8.55\times10^3$ M^{-1} s^{-1}) were also determined using electrochemical approaches. The voltammetric response of the modified electrode was linear against the concentration of AA in the ranges of 5×10^{-5} M-2.65×10^{-3} M and 9×10^{-6} M-3.5×10^{-3} M with cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods, respectively. The detection limits (2δ) were determined as 1.8×10^{-5} M and 4.2×10^{-6} M by CV and DPV methods, respectively. This method was also used for determination of AA in some pharmaceutical preparations.

Keywords: 2, 7-bis (ferrocenyl ethynyl) fluoren-9-one, ascorbic acid, electrocatalysis, cyclic voltammetry, differential pulse voltammetry, chronoamperometry, Modified carbon paste electrode

1. INTRODUCTION

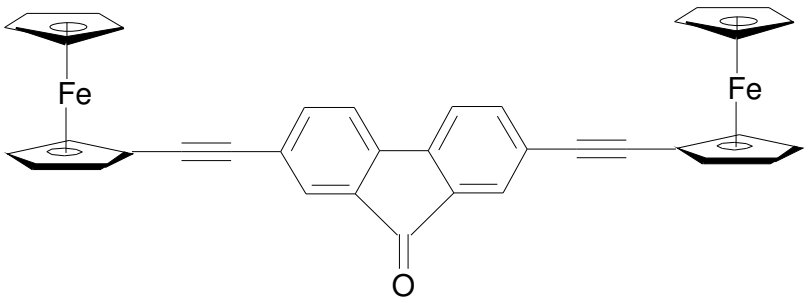
Electrochemical methods traditionally have found important applications in sample analysis and in organic and inorganic synthesis. The electrode surface itself can be a powerful tool in such applications [1, 2]. Apart from the conventional electrodes such as Au, Hg, and Pt, carbon is a preferred substrate in electroanalytical studies [3, 4]. Biological fluids are best determined at modified

carbon electrodes [5], and also at modified carbon paste electrodes [6]. The overpotential phenomena make most of the clinically important compounds difficult to analyze at conventional electrodes, which is promoted by incorporating redox mediators by various methods [7]. There are four principle enhancement techniques for voltammetric and amprometric electrodes, namely selective preconcentration, permselectivity, selective recognition and electrocatalysis [8, 9].

Redox mediators are small electroactive compounds that effectively shuttle electrons between the analyte and electrode. Several types of chemically modified electrodes (CMEs) have been designed and characterized for electrocatalysis of biological compounds particularly for vitamins such as ascorbic acid.

Ascorbic acid (AA) is known for its reductive properties and for its use on a wide scale as an antioxidant agent in foods and drinks; it is also important for therapeutic purposes and biological metabolism. Therefore, recent advances in the food and pharmaceutical industries and a need for nutritional assessment have necessitated the development of a selective, simple and accurate method to determine of AA [10-16]. Due to its selectivity and sensitivity, an electrochemical method to determine of AA has been a subject of considerable interest. A variety of examples of the electrochemical determination of AA have been proposed. These include a glassy carbon electrode (GCE) and a carbon paste electrode with complexes and organic compounds, such as, 1,5,8,12,tetraaza-2,4,9,11-tetramethyl-cyclotetradecinatonickel(II) [17, 18], cobalt hexacyano-ferrate [19], *meso*-tetrakis(*o*-nitrophenyl)tetra-benzoporphyrin with Fe(III), Ni(II), Mn(III) and Co(II) [20], ferricyanide-doped Tosflex [21], ruthenium(III) diphenyldithiocarbamate [22], ferrocene [23], ferrocene with β-cyclodextrin [24], ferrocenecarboxylic acid, ferroceneacetic acid, ferrocenemetanol [25, 26], ferrocene in lipid film [27], pentachloroiridite [13], benzoquinone [28], norepinephrine [29], pyrocatechol sulfonephthalein [30], poly(glutamic acid) [31], cellulose acetate film bearing 2,6-dichlorophenolindophenol [32] and aniline [33]. In addition, some chemically modified electrodes with various active mediators immobilized at the metal electrode surface, such as, an aluminum electrode with pentacyanonitrosylferrate films [34], a gold electrode with the electrodeposition of platinum [35], 3,4-dihydroxybenzoic acid and aniline [14] for the mediated oxidation of AA, have been used. Despite so much modified electrodes, a literature survey confirms that effort about the preparation of new patterns for electrocatalytic oxidation of ascorbic acid is still in progress.

On the other hand, the importance of ferrocene and its derivatives as a mediator for electro-oxidation processes has been known for several years. Also, according to our knowledge, there is no report on the electrochemical properties and especially the electrocatalytic activity of 2, 7-bis-(ferrocenyl ethynyl)-fluorene-9-one (2, 7-BFEFO) in aqueous media. Therefore, in continuation of our studies concerning the preparation of carbon paste modified electrodes [36-40], in this study, we prepared 2, 7-bis-(ferrocenyl ethynyl)-fluorene-9-one (Scheme 1) and applied it for the modification of carbon paste electrode. Then, we described the electrochemical behavior and suitability of 2, 7-BFEFO as a new electrocatalyst in the electrocatalysis and determination of AA in an aqueous buffer solution by cyclic voltammetry, double potential step chronoamperometry and differential pulse voltammetry. The linear range and sensitivity for the present modified electrode were greatly improved compared with those obtained for a bare electrode. The electrode was also used for the determination of AA in some pharmaceutical preparations.



Scheme 1

2. EXPERIMENTAL PART

2.1. Regents and Material

High viscous paraffin oil (density=0.88 g cm^{-3}), graphite powder (particle diameter =0.1 mm), AA, and all other chemicals were received from Fluka and used without further purification. Twice distilled water was used for preparing all of the solutions and throughout the experiments. AA solutions were freshly prepared in buffer solutions. The buffer solutions were 0.1 M of phosphate and were prepared from ortho phosphoric acid and its salts in the pH ranges 2.00-11.00.

2.2. Synthesis of 2, 7-Bis (ferrocenyl ethynyl) fluoren-9-one

Ethynylferrocene was coupled with 2, 7-dibromofluoren-9-one through Sonogashira reaction to give 2, 7-bis (ferrocenyl ethynyl) fluoren-9-one which upon treatment with H_2 in the presence of palladium on active carbon was converted to 2, 7-bis (ferrocenyl ethyl) fluoren-9-one in almost quantitative yield [36], as described in the following procedure:

In a flask were placed 2.03 g (6 mmol) 2, 7-dibromofluoren-9-one, 210 mg (0.3 mmol) bis (triphenylphosphine) palladium dichloride, and 57 mg (0.3 mmol) copper (I) iodide under an argon atmosphere. To this mixture were added 100 ml triethylamine, 30 ml DMF, and 3.8 g (18 mmol) ethynylferrocene, and the resulting mixture was heated to reflux for 4 h. Excess triethylamine was evaporated under reduced pressure, and the residue was added to 1 litre water. After filtration, the row product was redissolved in dichloromethane and subsequently filtered through a short silica gel column, and then the filtrate was concentrated. Addition of 100 ml petrolether afforded 3.4 g (5.7 mmol, 95%) 2, 7-bis (ferrocenyl ethynyl) fluoren-9-one as brown solid: mp 240-242 °C; UV (CH_2Cl_2): λ_{max} (lg ε) = 221 nm (4.59), 289 (4.56), 348 (4.31), 489 (3.58); IR (KBr): $\tilde{\nu}$ = 3092 cm^{-1} , 2208 (C≡C), 1715 (C=O), 1601, 1485, 1422, 1194, 1106, 917, 821, 786; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.76 (d, *J* = 1.34 Hz, 2 H), 7.59 (dd, *J* = 7.76, 1.35 Hz, 2 H), 7.46 (d, *J* = 7.76 Hz, 2 H), 4.52 (pseudo t, 4 H), 4.27 (pseudo t, 4 H), 4.25 (s, 10 H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 192.55 (C=O), 142.74 (C), 137.43 (CH), 134.50 (C), 127.23 (CH), 125.14 (C), 120.42 (CH), 90.92 (C), 85.12 (C), 71.57 (CH), 70.06 (CH), 69.12 (CH), 64.65 (C); MS (70 eV): m/z (%) = 596 (100.0) [M^+], 475 (4.5),

298 (24.2), 186 (6.5), 121 (7.2); high resolution MS: calcd. 596.0526; found 596.0516; Found: C 72.32, H 3.52; Calcd. For $C_{37}H_{24}Fe_2O \cdot 1/6CHCl_3$ (616.19): C 72.45, H 3.95 [41].

2.3. Pharmaceutical Preparations

The following commercial AA formulations available from local sources were subjected to the described analytical procedure: (1) Tablets containing sodium saccharin combined with AA (effervescent tablets); (2) Ampoules containing AA as the single component; and (3) Multivitamin syrup containing vitamins A, B_1, B_2, B_6, B_{12}, D_3, E, and C and nicotinamide.

2.4. Modified Electrode Preparation

The general procedure to prepare the modified carbon paste electrode was to mix graphite powder with 2, 7-BFEFO (0.5% w/w) and paraffin oil (1:1 (w/w). After thorough hand mixing in a mortar and pestle to obtain a very fine paste, a portion of the composite mixture was packed into the end of a glass tube (ca. 3.4 mm i.d. and 15 cm long). Electrical contact was made by pushing a copper wire down the glass tube into the back of the mixture. Unmodified electrodes were prepared in the same way without addition of 2, 7-BFEFO. The working surface of the electrode was polished using a soft polishing tissue to obtain a shiny surface.

2.5. Instrumentation

Cyclic voltammetric studies were carried out with a Potentiostat/Galvanostat (BHP 2061-C Electrochemical Analysis System, Behpajooh, Iran) coupled with a Pentium IV personal computer connected to a HP laser jet 6L printer. A conventional three-electrode cell was employed incorporating with a carbon-paste working electrode (with or without 2, 7-BFEFO), an $Ag|AgCl|KCl_{sat}$ reference electrode and a Pt-wire counter electrode. Also, a pH-meter (Ion Analyzer 250, Corning) was used to read the pH of the buffered solution.

2.6. Procedures for Pharmaceutical Preparations

Injection: A 1 mL ampoule volume was transferred to a 100 mL flask and diluted to volume with bidistilled water. A 1 mL portion of the solution was diluted in a voltammetric cell to 10 mL of 0.1 M phosphate buffer (pH 7.00) and 0.1 M $LiClO_4$, and the voltammogram was recorded.

Effervescent C tablets: A 100 mg portion of a finely powdered sample was dissolved in a 50 mL flask with bidistilled water. A 1 mL portion of the solution was diluted in a voltammetric cell to 10 mL of 0.1 M phosphate buffer (pH 7.00) and 0.1 M $LiClO_4$, and the voltammogram was recorded.

Multivitamin syrup: A 5 mL Multivitamin syrup volume was transferred to a 50 mL flask and diluted to volume with bidistilled water. A 1 mL portion of the solution was diluted in a voltammetric cell to 10 mL of 0.1 M phosphate buffer (pH 7.00) and 0.1 M $LiClO_4$, and the voltammogram was recorded.

Iodine titration method: A 0.05 M iodone solution was standardized in the usual way with a primary standard of As_2O_3 or titrisol thiosulfate solution. For a pharmaceutical analysis, an iodometric procedure, described in the US pharmacopeia (USP), was used [42].

3. RESULTS AND DISCUSSION

3.1. Electrochemical Properties of 2, 7-BFEFO

According to our knowledge, there is no report on a study of the electrochemical properties and especially the electrocatalytic activity of 2, 7-bis (ferrocenyl ethynyl) fluoren-9-one in aqueous media. Because this compound is insoluble in aqueous media, we prepared 2, 7-BFEFO modified carbon paste electrode (2, 7-BFEFOMCPE). Therefore, we studied the electrochemical behavior of 2, 7-BFEFOMCPE in pure buffered aqueous solution by cyclic voltammetry. Figure 1a shows cyclic voltammograms of 2, 7-BFEFOMCPE in a 0.1 M phosphate buffered aqueous solution (pH 7.00) with 0.1 M $LiClO_4$ as the supporting electrolyte. As can be seen, the cyclic voltammogram exhibits an anodic peak at a forward scan of the potential related to the oxidation of the ferrocene nucleus (Fc) to Fc^+, whereas at a reverse scan of the potential, a cathodic peak appears related to the reduction of Fc^+ to Fc. The cyclic voltammogram of bare CPE in pure supporting electrolyte shows no anodic or cathodic peaks (Fig. 1b). The experimental results show that well-defined and reproducible anodic and cathodic peaks related to Fc/ Fc^+ redox system (with E_{pa} = 480 mV, E_{pc} = 280 mV, $E_{1/2}$ = 380 mV vs. Ag | AgCl | KCl_{sat} and ΔE_p =200 mV). As can be seen, the peak separation potential, ΔE_p (=E_{pa}-E_{pc}), is greater than the (59/n) mV expected for a reversible system. This result suggests that the Fc/ Fc^+ redox couple in 2, 7-BFEFOMCPE shows a quasi reversible behavior in an aqueous medium.

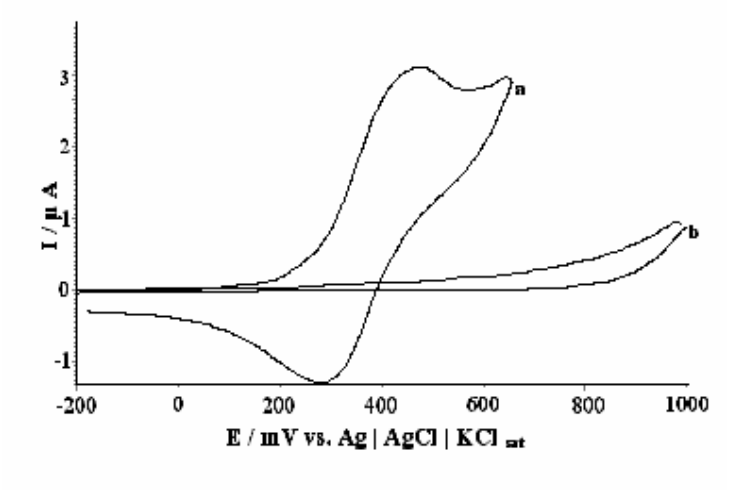


Figure 1. Cyclic voltammograms of 2, 7-BFEFOMCPE (a) and bare carbon paste electrode (b) in 0.1 M phosphate buffer solution (pH 7.00) at 100 mV s^{-1}.

In addition, the effect of the scan rate of potential (v =10-1000 mV s^{-1}) on the electrochemical properties of the Fc/ Fc^+ redox couple in 2, 7-BFEFOMCPE was studied in an aqueous solution by cyclic voltammetry. The plots of the anodic and cathodic peak currents were linearly dependent on square root of the sweep rate ($v^{1/2}$) with a correlation coefficient of 0.9988, at all scan rates (Fig. 2). This behavior indicates that the nature of redox process is diffusion controlled.

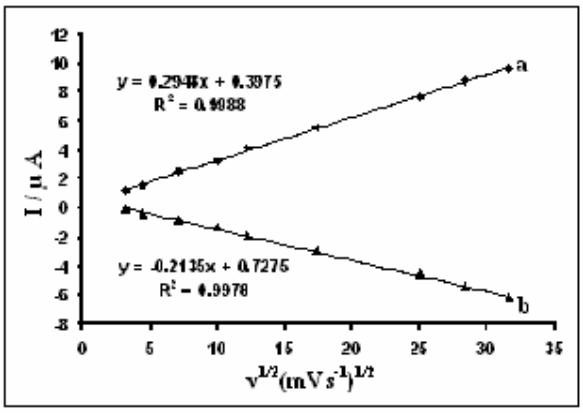


Figure 2. The plot of anodic (a) and cathodic(b) peak currents of 2,7-BFEFOMCPE vs. $v^{1/2}$ from the cyclic voltammograms of 2,7- BFEFOMCPE in 0.1 M phosphate buffer (pH 7.00), at various scan rates (data points from left to right): 10, 20, 50, 100, 150, 300, 625, 800, and 1000 mV s^{-1}.

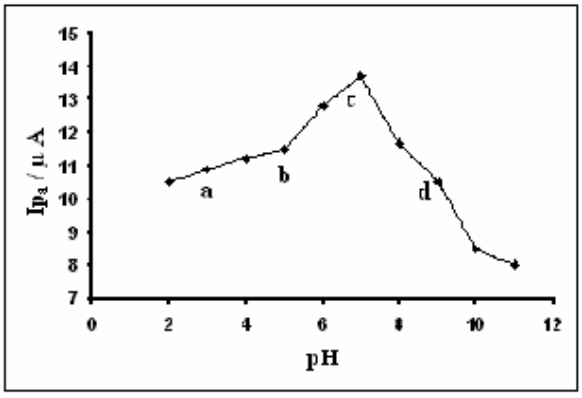


Figure 3. Current-pH curve for electrooxidation of 1.0 mM AA at the surface of 2, 7-BFEFOMCPE at various pH values: a) 3.00, b) 5.00, c) 7.00, and d) 9.00 in 0.1 M phosphate buffered solution at a scan rate of 10 mV s^{-1}.

The electrode capability for the generation of a reproducible surface was examined by cyclic voltammetric data obtained in optimum solution pH from five separately prepared 2, 7-BFEFOs. The calculated RSD for various parameters accepted as the criteria for a satisfactory surface reproducibility (1- 4%). This degree of reproducibility is virtually the same as that expected for the renewal or ordinary carbon paste surface [43, 44]. However we regenerated the surface of 2, 7-BFEFOMCPE before each experiment.

3.2. pH Optimization of the Solution

The electrochemical behavior of AA (pK_{a1} =4.17, pK_{a2} =11.5) [25] is dependent on the pH value of the aqueous solution, whereas the electrochemical properties of Fc/ Fc^+ redox couple is

independent on pH. Therefore, pH optimization of the solution seems to be necessary in order to obtain the electrocatalytic oxidation of AA. Thus we studied the electrochemical behavior of AA in 0.1 M phosphate buffered solutions in different pH values ($2 \leq pH \leq 11$) at the surface of 2, 7-BFEFOMCPE by cyclic voltammetry. It was found that the electrocatalytic oxidation of AA at the surface of 2, 7-BFEFOMCPE was more favored under neutral conditions than in acidic or basic medium. This appears as a gradual growth in the anodic peak current and a simultaneous decrease in the cathodic peak current in the cyclic voltammograms drawn at the surface of 2,7-BFEFOMCPE. The results show the anodic peak potential of AA at the surface of 2, 7-BFEFOMCPE was shifted to a less-positive potential. The variation of I_{pa} versus the variation of pH was shown in figure 3. As can be seen, the anodic peak current and the shifted potential value for electrooxidation of AA are high at a biological pH (pH 7.00). Thus, the pH 7.00 was chosen as the optimum pH for electrocatalysis of AA oxidation at the surface of 2, 7-BFEFOMCPE.

3.3. Electrochemistry of AA at 2, 7-BFEFOMCPE

In order to test the electrocatalytic activity of the 2,7-BFEFOMCPE for electrooxidation of AA, its cyclic voltammetric responses at 10 mV s^{-1} were obtained in the phosphate buffer solution (pH 7.00) in the absence and in the presence of 1.00 mM AA, and the data are presented in Figure 4. In the absence of AA, a pair of well-defined redox peaks of 2, 7-BFEFO can be observed (Fig. 4, curve a). Upon the addition of 1.00 mM AA, there was a drastic enhancement of the anodic peak current, and in addition, no cathodic current was observed in the reverse scan (Fig. 4, curve b).

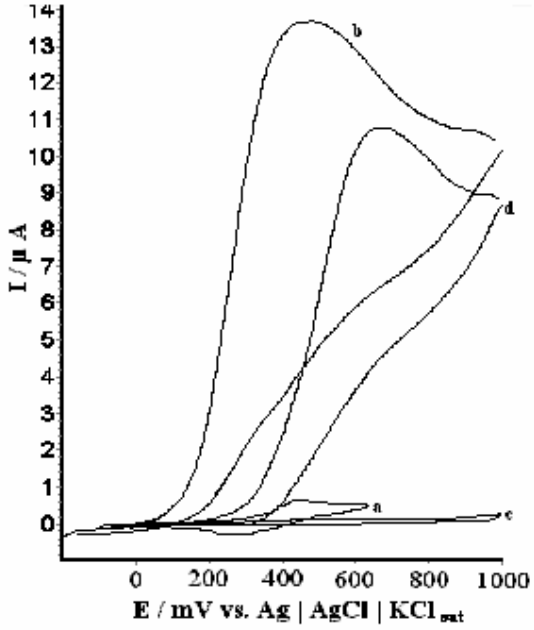


Figure 4. Cyclic voltammograms of (a) 2,7-BFEFOMCPE in 0.1 M phosphate buffer (pH 7.00) solution, (b) as (a) in the presence of 1.0 mM AA (c) as (a) and (d) as (b) for an unmodified CPE. In all cases the scan rate was 10 mV s^{-1}.

This behavior is consistent with a very strong electrocatalytic effect [26, 36, 37]. Under the same experimental conditions, the direct oxidation of AA at an unmodified carbon paste electrode shows an irreversible wave at more positive potentials (Fig. 4, curve d). The reaction scheme would be probably via following mechanistic steps, which ascorbic acid (AH_2 in the following C´ reaction) can be oxidized by 2, 7-bis (ferricenium ethynyl) fluoren-9-one ion produced in the surface of electrode:

$$Fc \rightarrow Fc^{+} + e^{-} \qquad : E$$

$$Fc^{+} + AH_2 \rightarrow Fc + A + 2H^{+} \qquad : C´$$

The catalytic peak potential of AA at the surface of this modified electrode is found to be about 480 mV vs. $Ag \mid AgCl \mid KCl_{sat}$, whereas, that at bar carbon paste electrode is about 685 mV versus $Ag \mid AgCl \mid KCl_{sat}$. Thus, a decrease in the over voltage of approximately 205 mV and an enhancement of the peak current is also achieved with the modified electrode. This value is comparable with the values reported by other research groups for electrocatalytic oxidation of AA at the surface of chemically modified electrodes by other mediators (see Table 1).

Table 1 Comparison of the efficiency of some modified electrodes used in the electrocatalysis of ascorbic acid.

Substrate	Modifier	pH	E_p (bar) (mV)	E_p (mod) (mV)	Peak potential shift (mV)	Scan rate (mV/s)	Ref.
Carbon paste electrode	cobalt(II)-4-methylsalophen	5.00 (acetate buffer)	402[c]	302[c]	100	100	50
Graphite-epoxy composite electrode	Cobalt phthalocyanine	5.00 (phosphate buffer)	320	170 [d]	150	20	11
Microdisk gold electrode	3,4-Dihydroxy benzoic acid	7.00 (phosphate buffer)	380 [d]	180 [d]	200	10	14
Glassy carbon electrode	Ni (II)-complex	7.00 (Tris-HCl)	-------	-------	200	100	18
Glassy carbon electrode	Ferrocene methanol	4.00 (glysine buffer)	500 [d]	200 [d]	300	5	25
Carbon paste electrode	Ferrocene carboxylic acid	5.00 (phosphate buffer)	682[c]	434[c]	248	10	26
Glassy carbon electrode	Ppy/FCN	4.00 (glysine buffer)	500 [d]	200 [d]	300	5	51
Carbon paste electrode	2,7-Bis (ferrocenyl ethynyl) fluoren-9-one	7.00 (phosphate buffer)	685[c]	480[c]	205	10	Present work

[a] Ascorbic acid oxidation peak potential at the surface of unmodified electrodes.
[b] Ascorbic acid oxidation peak potential at the surface of chemically modified electrodes.
[c] vs. $Ag \mid AgCl \mid KCl_{sat}$
[d] vs. saturated calomel electrode.

The effect of the potential scan rate on the electrocatalytic properties of 2, 7-BFEFO in 0.1 M phosphate buffered solution containing 1 mM ascorbic acid was studied. Figure 5A shows the cyclic

voltammograms of the 2, 7-BFEFOMCPE at various potential scan rates (v =10-1000 mV s^{-1}). Figure 5B shows a plot of the catalytic peak current versus the square root of the sweep rate, which is linear. This result suggests that at sufficient overpotential, the reaction is diffusion limited. It can be noted from figure 5C that, with an increase in the scan rate, the peak potential for the catalytic oxidation of AA shifts to the more positive potentials, suggesting a kinetic limitation in the reaction between the redox sites of 2,7-BFEFOMCPE and AA [36, 37, 40].

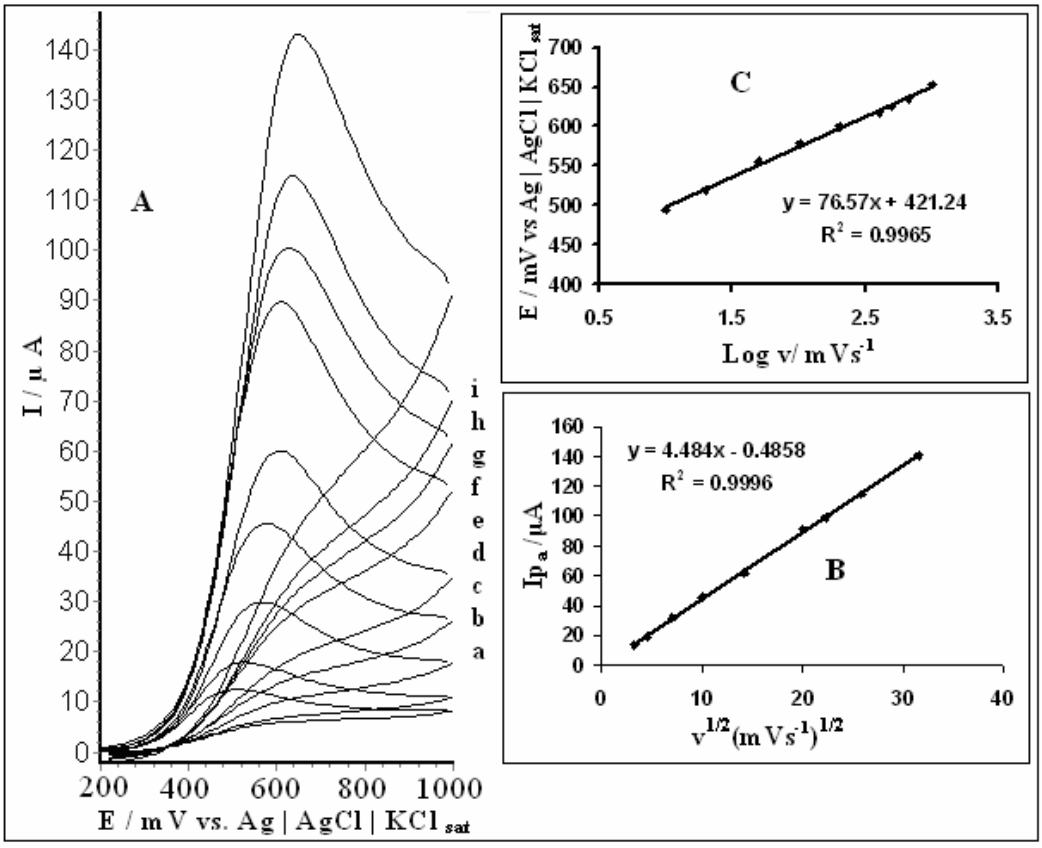


Figure 5. (A) Cyclic voltammograms of the 2, 7-BFEFOMCPE in the presence of 1.0 mM AA at various scan rates (data points from **a** to **i**): 10, 20, 50, 100, 200, 400,500, 666 and 1000 mV s^{-1} in 0.1 M phosphate buffer solution (pH 7.00). B) The variation of the anodic peak currents vs. $v^{1/2}$ obtained from data of (A). (C) Dependence of the peak potential, E_p, on log v for the oxidation of AA at the surface of 2, 7-BFEFOMCPE obtained from data of Fig. 5A.

In order to obtain information on the rate-determining step a Tafel slope, b, was determined using the following equation for a totally irreversible diffusion controlled process [34]:

$$E_p = (b/ 2) \log v + \text{constant} \qquad (1)$$

Based on Equation 1, the slope of E_p vs. log v plot is (b/ 2), where b indicates the Tafel slope. The slope of E_p vs. log v plot was found to be 76.57 mV in this work, thus b = 2×76.57=153.14 mV. This

slope value indicates an electron transfer process, which is the rate limiting step by assumption of a transfer coefficient α equal to 0.47.

The values of αn_α (where n_α is the number of electrons involved in the rate determining step) were calculated for the oxidation of AA at pH of 7.00 at both modified and unmodified carbon paste electrodes, according to the following equation [45]:

$$\alpha n_\alpha = 0.048/ (E_p - E_{p/2}) \qquad (2)$$

where, $E_{p/2}$ is the potential corresponding to $I_{p/2}$. The values for αn_α were found to be 0.46 and 0.21 for the oxidation of AA at the surface of modified and unmodified electrodes, respectively. These values clearly show that not only overpotential for AA oxidation is reduced at the surface of 2, 7-BFEFOMCPE, but also the rate of electron transfer process is greatly enhanced. This phenomenon is thus confirmed by large I_{pa} values recorded during the cyclic voltammetry at 2, 7-BFEFOMCPE.

3.4. Chronoamperometric studies

The catalytic oxidation of AA at the surface of 2, 7-BFEFOMCPE was also studied by double potential step chronoamperometry. Chronoamperograms obtained by setting the working electrode potential at 0.85 V (at the first potential step) and at 0.0 V (at second potential step) vs. $Ag|AgCl|KCl_{sat}$ for the various concentration of AA in buffered aqueous solutions (pH 7.00) are depicted in Figure 6A. As can be seen, there is not any net cathodic current corresponding to the reduction of mediator in the presence of AA, when the potential is stepped from 0.85 to 0.0 V vs. $Ag|AgCl|KCl_{sat,}$ while the forward and backward potential step chronoamperometry on the modified electrode in the blank buffered solution show very symmetrical chronoamperograms with an equal charge consumed for the oxidation and reduction of Fc/Fc^+ redox system in the CPE (Fig. 6B, curve a´). However, in the presence of AA, the charge value associated with forward chronoamperometry is significant greater than that of observed for backward chronoamperometry (Fig. 6B, curves b´ and c´).

For an electroactive material (AA in this case) with an apparent diffusion coefficient, D_{app}, the current for the electrochemical reaction (at a mass transport limited rate) is described by the Cottrell equation [46]**:**

$$I = n F A D_{app}^{1/2} C_b \pi^{-1/2} t^{-1/2} \qquad (3)$$

where D_{app} and C_b are the apparent diffusion coefficient ($cm^2\ s^{-1}$) and the bulk concentration (mol cm^{-3}), respectively. Under diffusion control, a plot of I versus $t^{-1/2}$ will be linear, and from the slope the value of D can be obtained. Figure 6C shows the experimental plots with the best fits for different concentration of AA (0.00, 0.8257 and 1.66 mM). We calculated $D_{app}=8.65\times10^{-6}\ cm^2\ s^{-1}$ from the slopes of the resulting straight lines. We designate the obtained value as an apparent diffusion coefficient, since we believe that in the experimental conditions the diffusion of AA from solution bulk to electrode surface can be affected to some extent by the rate of electron transfer between substrate

and modifier. However, the calculated value of the diffusion coefficient is in good agreement with those previously reported [26, 40, 47].

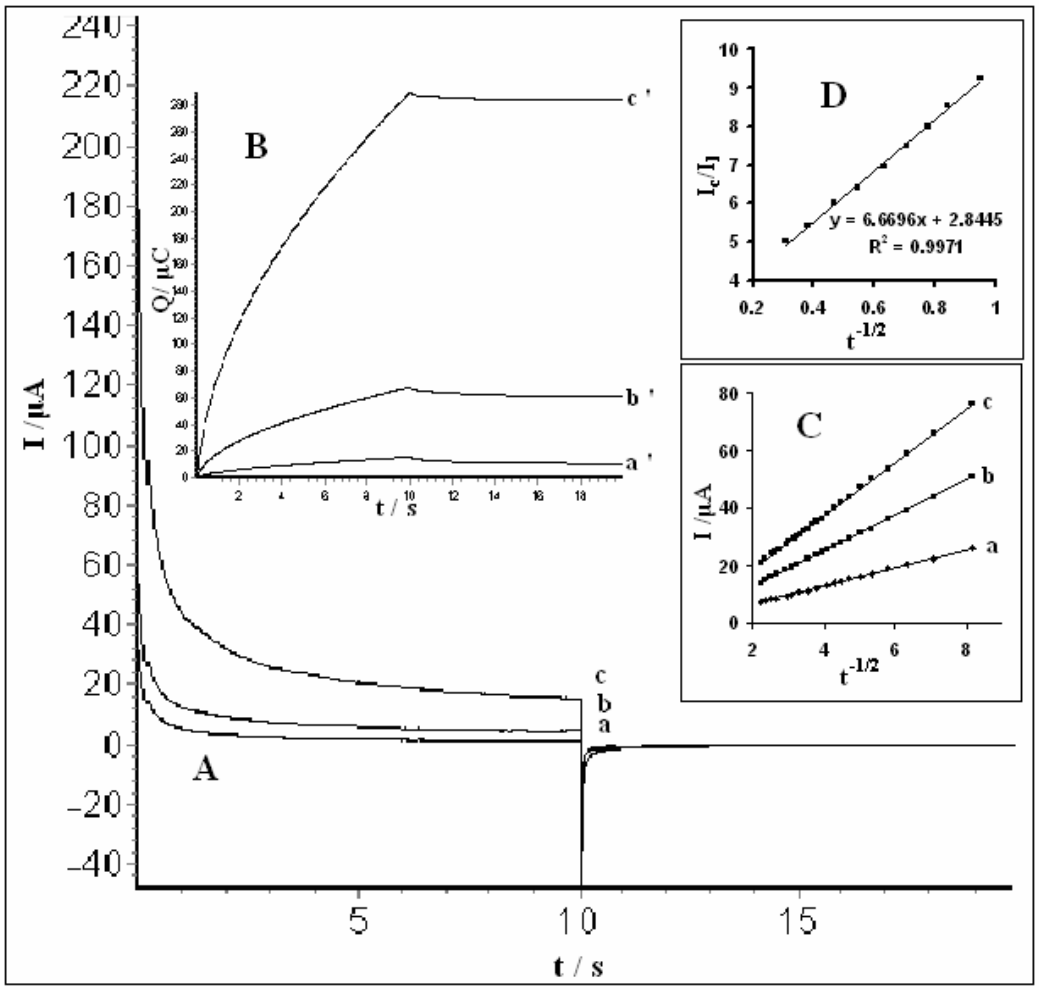


Figure 6. (A) Chronoamperograms obtained at 2, 7-BFEFOMCPE in the absence (a) and presence of (b) 0.825 and (c) 1.66 mM of AA in 0.1 M phosphate buffer + 0.1 M $LiClO_4$, the first and the second potential steps were 0.85 V and 0.0 V vs. Ag | AgCl | KCl_{sat} respectively. (B) Shows the charge-time curves (a′), (b′) and (c′) for curves (a), (b) and (c). (C) Plot of I versus $t^{-1/2}$ obtained from chronoamperograms of Fig. 6A and (D) Dependence of I_C/I_L on the $t^{1/2}$ driven from the of figure 6A.

Chronoamperometry can also be employed to evaluate the catalytic rate constant, k, for the reaction between AA and the 2, 7-BFEFO according to the method of Galus [48]:

$$I_C / I_L = \gamma^{1/2} [\pi^{1/2} \text{ erf } (\gamma^{1/2}) + \text{pxp } (-\gamma) / \gamma^{1/2}] \qquad (4)$$

where I_C is the catalytic current of AA at the 2, 7-BFEFOMCPE, I_L the limited current in the absence of AA and $\gamma = k C_b t$ (C_b is the bulk concentration of AA) is the argument of the error function. In the

cases where γ exceeds 2 the error function is almost equal to 1 and therefore the above equation can be reduced to:

$$I_C / I_L = \pi^{1/2} \gamma^{1/2} = \pi^{1/2} (k C_b t)^{1/2} \qquad (5)$$

where t is the time elapsed (s). The above equation can be used to calculate the rate constant of the catalytic process k. Based on the slope of the I_C / I_L versus $t^{1/2}$ plot; k can be obtained for a given AA concentration.

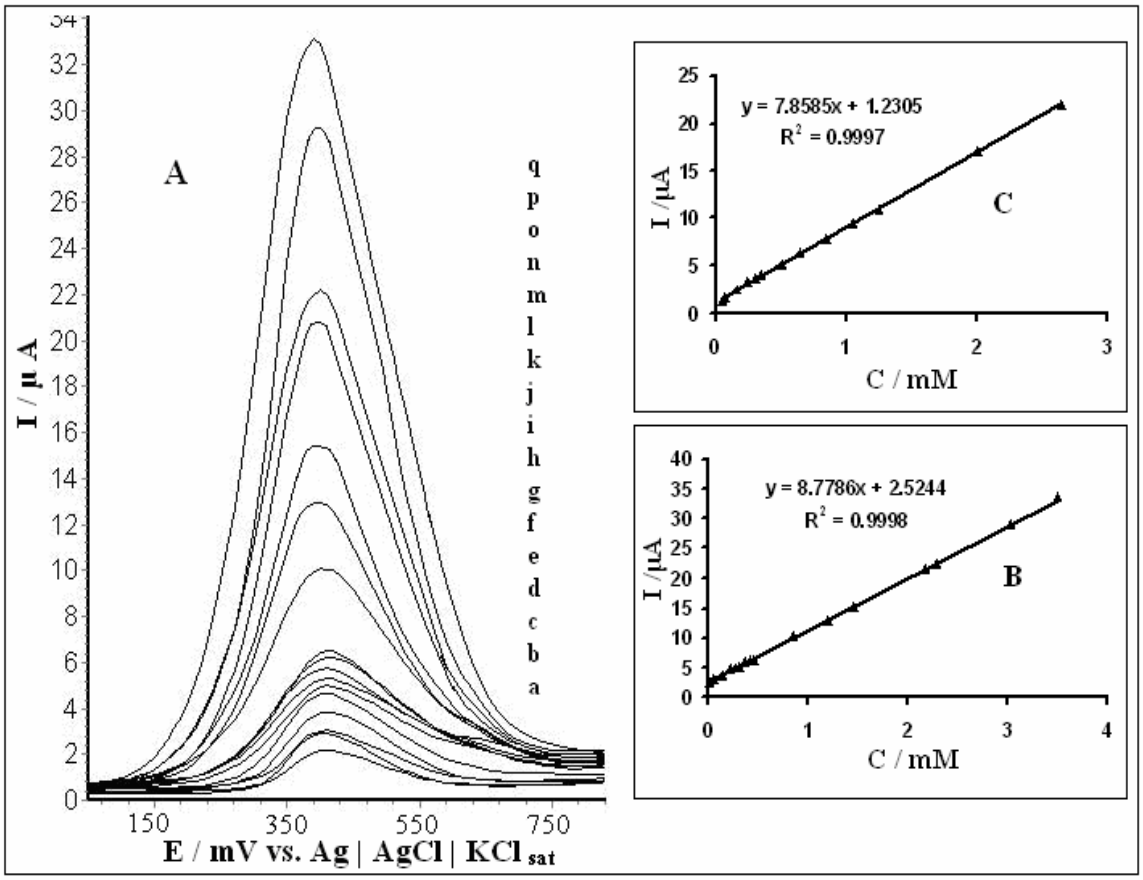


Figure 7. (A) Differential pulse voltammograms at the 2, 7-BFEFOMCPE in the presence of (a) 0.009 (b) 0.01; (c) 0.05; (d) 0.14; (e) 0.22; (f) 0.27; (g) 0.31; (h) 0.37; (i) 0.42; (j) 0.46; (k) 0.86; (l) 1.20; (m) 1.46; (n) 2.17; (o) 2.29; (p) 3.02 and (q) 3.50 mM of AA in 0.1 M phosphate buffer solution (pH 7.00). (B) Plot of electrocatalytic peak currents [from DPV (A)] vs. AA concentration. (C) The Plot of electrocatalytic peak currents from cyclic voltammograms of AA at various concentrations (data points from left to right): 0.05, 0.07, 0.16, 0.24, 0.3, 0.34, 0.50, 0.64, 0.85, 1.05, 1.25, 2.00 and 2.65 mM.

Such plots obtained from the chronoamperograms in Figure 6A are shown in Figure 6D. From the values of the slopes an average value of k was found to be $K=8.55\times10^3$ M^{-1} s^{-1}. Also this value is comparable with those previously reported for the electrocatalytic oxidation of AA at electrodes modified with other mediators [38, 49].

3.5. Electrocatalytic Determination of AA

The electrocatalytic peak current of AA oxidation at the surface of 2, 7-BFEFOMCPE can be used for determination of AA in solution. Therefore, cyclic voltammetry and differential pulse voltammetry experiments were performed using 2, 7-BFEFOMCPE in a phosphate buffer solution containing various concentrations of AA. The results show that the electrocatalytic peak current of AA oxidation at the surface of 2, 7- BFEFOMCPE was linearly dependent on AA concentration, and also the range of this linearity is dependent on the amount of the mediator in the electrode matrix.

The mediated oxidation peak currents of AA at the surface of a 0.5% 2, 7-BFEFOMCPE were proportional to the concentration of AA within the ranges of 5.00×10^{-5} M - 2.65×10^{-3} M and 9.0×10^{-6} M-3.5×10^{-3} M (with the correlation coefficients of 0.9997 and 0.9998) in the cyclic voltammetry and differential pulse voltammetry respectively (Fig. 7). The detection limits (2δ) were 1.8×10^{-5} M and 4.2×10^{-6} M in the CV and DPV methods, respectively**.**

3.6. Sample analysis

Effervescent tablets, vitamin C ampoules and multivitamin syrup purchased from local sources without interference from recipients and other drugs were selected as real samples for analysis by the proposed method using the standard addition method.

All samples were diluted with phosphate buffer (pH 7.00) and then appropriate amounts of these diluted samples were transferred to the electrochemical cell for the determination of each species using CV. Figure 8A shows typical cyclic voltammograms recorded for a diluted vitamin C ampoule solution (curve (a)). As can be seen in this figure, adding a standard concentration solution of AA to this solution caused an increase in the oxidation peak height (curves (b) to (f)). Thus, the peak was attributed to AA oxidation. The determination of AA in pharmaceutical preparations was carried out by the standard addition method in order to prevent of any matrix effect. Figure 8B shows a typical linear plot of I_{pa} versus the AA concentration for a vitamin C ampoule sample. The evaluation of the AA concentration was found to be more suitable with the aid of these plots. The results for the analysis of these pharmaceutical preparations with this method compared favorably with those obtained by the USP standard method (Table 2).

Table 2. Determination of ascorbic acid in dosage forms.

Pharmaceutical preparation	Claimed (mg)	Proposed method [a] (mg), (%RSD)	Iodine method [a] (mg), (%RSD)	$F_{exp.}$	$T_{exp.}$
Ampoule	500 per 5 mL	499 (0.5)	501 (0.6)	1.14	1.19
Multivitamine syrup	60 per 5mL	59.8 (1.5)	60.2 (1.7)	2.2	1.3
Effervescent tablet	1000 per tablet	961.2 (0.3)	965.1 (0.5)	1.9	0.9

[a] Result based on five replicate determinations per samples. Theoretical values for t=2.31 and F=6.39 (p=0.05).

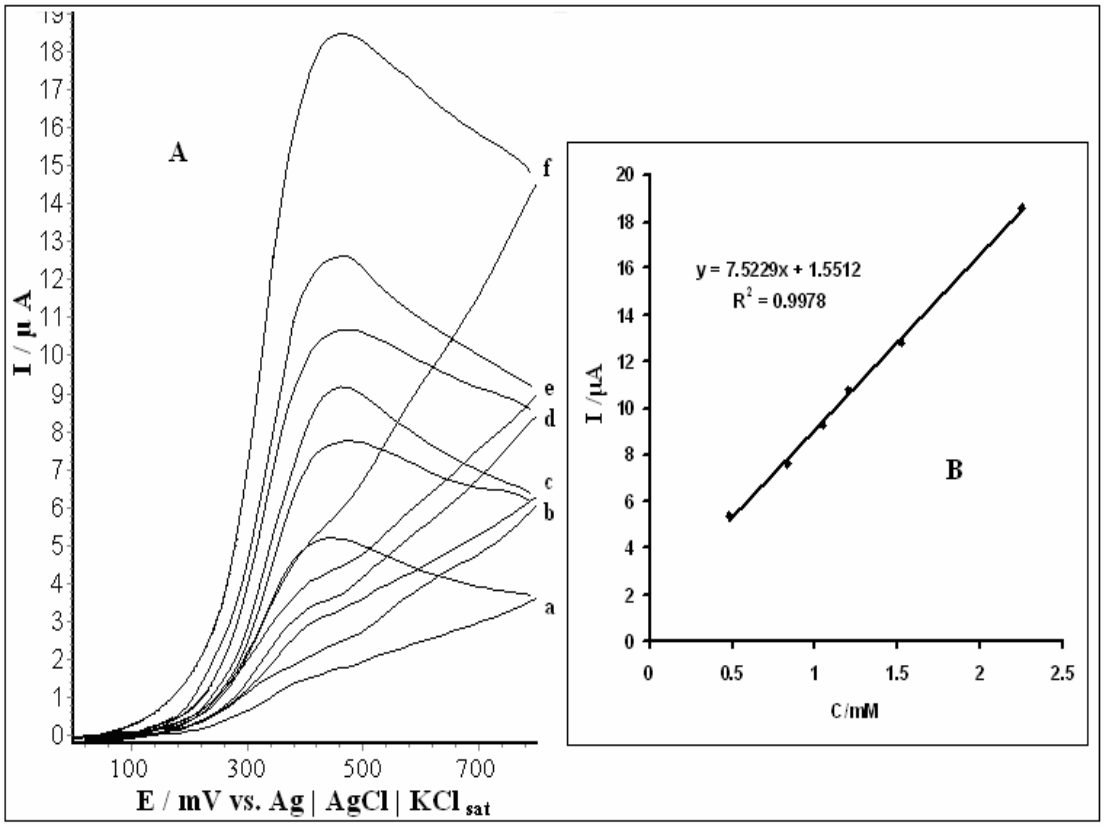


Figure 8. (A) Cyclic voltammograms of (a) 1000 order diluted solution of vitamin C ampoule (500 mg) in 0.1 M phosphate buffer (pH 7.00) and 0.1 M $LiClO_4$. (b) to (f) as (a) after adding 0.35, 0.57, 0.72, 1.04 and 1.77 mM AA, respectively; scan rate was 10 mV s^{-1}. (B) Plot of I_{pa} as a function of added AA concentration to an ampoule sample.

4. CONCLUSIONS

This work shows the ability of 2, 7-Bis (ferrocenyl ethynyl) fluoren-9-one as a modifier in carbon paste electrode for electrocatalysis of AA oxidation. The results demonstrated that the electrooxidation of AA at the surface of 2, 7-BFEFOMCPE occurs at a potential about 200 mV less positive than bare carbon paste electrode. The kinetic parameters of the electrocatalytic process and the diffusion coefficients of AA in an aqueous solution were determined. Finally, the electrocatalytic oxidation currents of AA at the surface of 2, 7-BFEFOMCPE was linear to concentration of AA. This method was also used for the determination of AA in some pharmaceutical preparations, such as effervescent tablets, multivitamin syrup and ampoules containing AA by using a standard addition method.

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