Carbon Paste Electrode Incorporating 1-[4-(Ferrocenyl Ethynyl) Phenyl]-1-Ethanone for Voltammetric Determination of *D*-Penicillamine

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Electrocatalytic oxidation of *D*-penicillamine (*D*-PA) at the surface of 1-[4-(ferrocenyl-ethynyl) phenyl]-1-ethanone modified carbon paste electrode (4-FEPEMCPE) was thoroughly investigated using cyclic voltammetry (CV) and double step potential chronoamperometry methods in aqueous solution with various pH. It has been found that under optimum condition (pH 7.00) in cyclic voltammetry, the oxidation of *D*-PA occurred at a potential about 300 mV less positive than that unmodified carbon paste electrode. The electrocatalytic oxidation peak current of *D*-PA showed a linear dependent on the *D*-PA concentration and linear calibration curves were obtained in the ranges of 6.0×10^{-5} M – 1.6×10^{-3} M and 7.0×10^{-6} M – 2.3×10^{-4} M of *D*-PA concentration with cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods respectively. The detection limits (3σ) were determined as 4.6×10^{-5} M and 3.9×10^{-6} M. The proposed method was applied to determine *D*-PA in pharmaceutical preparation (capsules) and synthetic human serum by standard addition and recovery methods, respectively. The results obtained in the recovery study were comparable to those labelled.

1. INTRODUCTION

The presence of a thiol, amino and carboxyl group in penicillamine compound provides a number of ways of interacting with organic [1-3] and inorganic [4-8] species or both [9, 10], in a variety of complex formation and redox reactions that yield some spectrophotometrically active product. It is recognized that these endogenous thiol can also be oxidized to disulfides in the presence of transition metals such as copper and iron [11-13].

Sulfhydryl compounds (thiols, R-SH) are known to play many roles withinphysislogical systems. D-Penicillamine is a highly potent therapeutic agent used for many years in the treatment of various illnesses. It is the drug of first choice for patients with Wilson's disease [14], an autosomal recessive disorder of copper transport [15]. Increasing the amount of *D*-penicillamine can cause rashes early in treatment. It can also cause loss of appetite, nausea, abdominal pain, loss of the sense of taste, bone marrow suppression and serious kidney disease [16-20]. All patients who take penicillamine require regular blood and urine tests for monitoring [21].

Different direct and indirect methods based on spectrophotometry [22-24], chromatography [25], and titrimetry [26] have been proposed for the analysis of PA in pharmaceutical preparations. Among these methods, the electrochemical detection has been very successful for application in pharmaceutical and clinical preparations [27, 28]. The chemical modification of electrodes using electron transfer mediators is an interesting field in analytical chemistry. One of the most important effects of any mediator is a reduction of the overpotential required for electrochemical reaction and enhancement of the sensitivity (current) and selectivity of the method [29, 30]. Electrochemical detection using carbon-based electrodes has been used for the determination of thiols. Many types of carbon-based electrodes such as glassy carbon [31, 32], carbon composites [33, 34], carbon nanotubes [35, 36] and carbon pastes [37-41] are used as modified electrodes. In the United States pharmacopoeia (USP) PA tablets are assayed by an ion-pairing HPLC method [42].

Among the transition metal compounds, such as metallophthalocyanines [43-46], hexacyanometallates [47], aquacobalamin [48], cobalt salophen [49], ferrocene and some of its derivatives [37-41] have used the most applications as electrocatalysts in the voltammetric detection of thiols.

To the best of our knowledge, no study with 1-[4-(ferrocenyl-ethynyl) phenyl]-1-ethanone modified carbon paste electrode for voltammetric determination of *D*-PA has been reported. The present work aims at the study of the electrochemical behavior of *D*-penicillamine at carbon paste electrodes modified with 1-[4-(ferrocenyl-ethynyl) phenyl]-1-ethanone. Detailed studies were performed using the cyclic voltammetry, differential pulse voltammetry and double step potential chronoamperometry. This electrode has been successfully applied to determination of *D*-PA in pharmaceutical preparation (capsules) and synthetic human serum by standard addition and recovery methods, respectively.

2. EXPERIMENTAL PART

2.1. Regents and Material

The solvent used for the electrochemical studied was twice distilled water. Buffer solutions were prepared from orthophosphoric acid and its salts in the pH ranges 3.00–10.00. High viscosity paraffin (density = 0.88 g cm^{-3}) from Fluka was used as the pasting liquid for the carbon paste electrode. Graphite powder (particle diameter = 0.1 mm) from Merck was used as the working electrode (WE) substrate. Potassium chloride from Fluka was used as the supporting electrolyte. The 1-[4-(ferrocenyl-ethynyl) phenyl]-1-ethanone was prepared by a reported procedure [50]. Stock

solution of *D*-PA was freshly prepared as required in 0.1 M phosphate buffer at the desired pH (3.00 – 10.00). *D*, *L*-alanine, *L*-arginine, *L*-aspartic acid, glycine, *L*-histidine, *L*-lysine, *D*, *L*-methionine, glutathione, *L*-phenylalanine, *L*-serine, *D*, *L*-tryptophan, *L*-cysteine and citric acid for preparation of synthetic human serum were purchased from Merck. *All* other reagents were of analytical grade.

2.2. Working Electrode

A 1 % (w/w) 1-[4-(ferrocenyl-ethynyl) phenyl]-1-ethanone spiked carbon powder was made by dissolving the given quantity of 1-[4-(ferrocenyl-ethynyl) phenyl]-1-ethanone in diethyl ether and hand mixing with 99 times its weight of graphite powder with a mortar and pestle. The solvent was evaporated by stirring a mixture of 1% 1-[4-(ferrocenyl-ethynyl) phenyl]-1-ethanone spiked carbon powder and paraffin was blended by hand–mixing and the resulting paste was inserted in the bottom of a glass tube (with internal radius 1.5 mm). The electrical connection was implemented by a copper wire lead fitted into a glass tube. A carbon paste electrode without 1-[4-(ferrocenyl-ethynyl) phenyl]-1-ethanone was used as a blank to determine the background current.

2.3. Instrumentation

The electrochemical experiments were performed using an Autolab Potentiostat/Galvanostat (Netherlands) coupled with a Pentium IV personal computer with a standard three electrode configuration. A platinum wire was used as the auxiliary electrode. The 4-FEPEMCPE was used as the working electrode. The reference electrode was Ag|AgCl|KCl$_{3M}$ (Metrohm). Also, a pH–meter (Ion Analyzer 250, Corning) was used to read a pH of the buffered solution.

3. RESULTS AND DISCUSSION

### 3.1. Electrochemical Behavior of 4-FEPEMCPE

We have previously shown that a carbon paste electrode spiked with 1-[4-(ferrocenyl-ethynyl) phenyl]-1-ethanone is constructed by the incorporation of 1-[4-(ferrocenyl-ethynyl) phenyl]-1-ethanone in a graphite powder paraffin oil matrix. Its cyclic voltammograms (Fig. 1) exhibits one anodic and cathodic peak corresponding with redox system Fc / Fc$^+$, which show a quasi-reversible behavior in an aqueous medium [51]. Also, the obtained result shows that the redox process of Fc/Fc$^+$ in 1-[4-(ferrocenyl-ethynyl) phenyl]-1-ethanone is independent on the pH of aqueous solution.

Table 1. Cyclic voltammetric data obtained for constructed 4-FEPEMCPEs in 0.1 M phosphate buffer solution (pH 7.00) at 20 mV s^{-1}.

E_{pa} (V) [a]	E_{pc} (V) [a]	$E_{1/2}$ (V) [a]	ΔE_p (V) [a]	I_{pa} (μA)	I_{pc} (μA)
0.62	0.50	0.56	0.12	3.16	-0.96
(1.1) [b]	(0.57) [b]	(3.8) [b]	(2.5) [b]	(4.1) [b]	(2.8) [b]

[a] Versus Ag | AgCl | KCl$_{3M}$ as reference electrode.
[b] The values in parentheses indicate the calculated RSD (%).
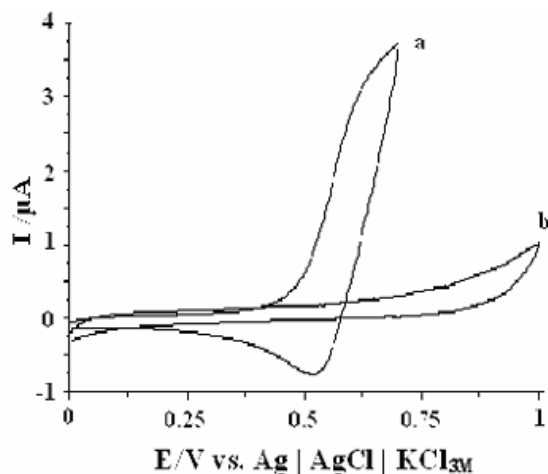
Figure 1. The cyclic voltammograms of (a) 4-FEPEMCPE and (b) bare CPE in 0.1 M phosphate buffer solution (pH 7.00) containing KCl 0.1 M as supporting electrolyte at a scan rate 20 mV s^{-1}.

The summary of electrochemical data for the mediator obtained at the carbon paste electrode is presented in Table 1. The calculated RSD for various parameters accepted as the criteria for a satisfactory surface reproducibility.

3.2. pH Optimization of the Solution

It well known, the electrochemical behavior of *D*-PA is dependent on pH value of the aqueous solution [49], whereas the electrochemical properties of Fc/Fc$^+$ redox couple are independent pH [41]. Therefore, we studied the electrochemical behavior of *D*-PA in 0.1 M phosphate buffered solution with various pH (2.00<pH<11.00) at the surface of 4-FEPEMCPE by cyclic voltammetry. Result shows that the catalytic current increases as pH increasing.
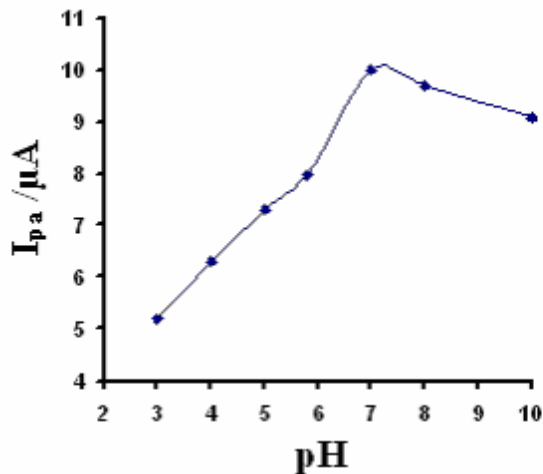

Figure 2. Current-pH curve for electrooxidation of 1mM *D*-PA in 0.1 M phosphate buffer solution with various pH values: (a) 3.00, (b) 4.00, (c)5.00, (d) 6.00, (e) 7.00,(f) 8.00 and (g) 10.00 at the surface of 4-FEPEMCPE at scan rate 20 mV s^{-1}.

Therefore, pH has effect on the kinetic of the catalytic reaction. Figure 2 shows the variation of I_{pa} versus the variation of pH. As can be seen, that maximum electrocatalytic current was obtained in pH 7.00. Therefore, pH 7.00 was chosen as the optimum pH for electrocatalysis of *D*-PA oxidation at the surface of 4-FEPEMCPE. Hence, all electrochemical experiments were done at this pH.

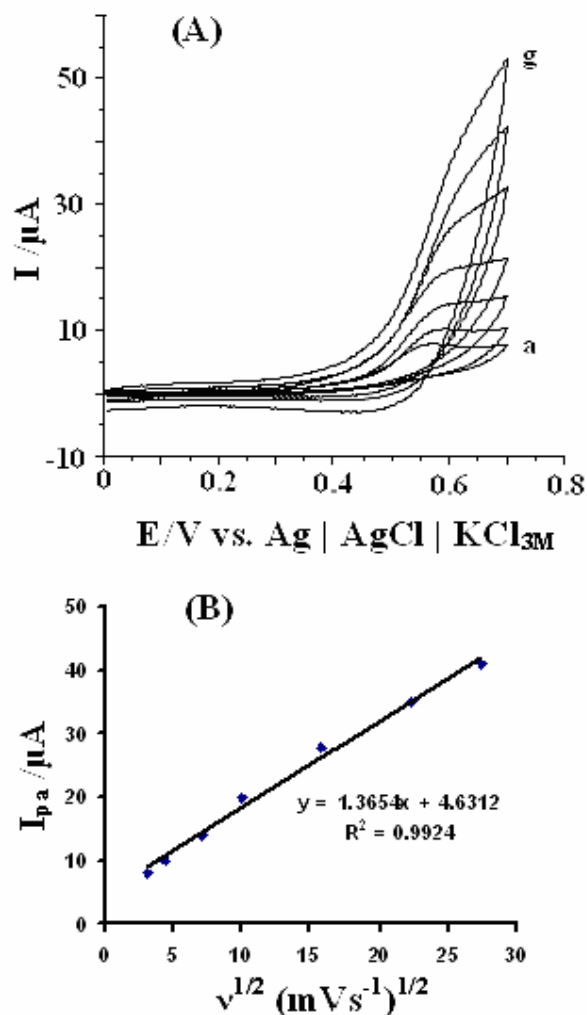### 3.3. Electrooxidation of D-PA at the surface of 4-FEPEMCPE

The cyclic voltammograms of 4-FEPEMCPE and CPE in phosphate buffered solutions (pH 7.00) in the absence and the presence of 1 mM *D*-PA are presented in Fig. 3. In the absence of *D*-PA, a pair of well-defined redox peaks of 4-FEPEMCPE can be observed (Fig. 3, curve c). Upon the addition of 1 mM *D*-PA, there was a drastic enhancement of the anodic peak current, and in addition, no cathodic current was observed in the reverse scan of potential (Fig. 3, curve d). This behaviour is consistent with a very strong electrocatalytic effect. Under the same experimental condition, at the bare CPE, *D*-PA oxidation occurs irreversibly with a peak potential of nearly 850 mV versus Ag|AgCl|KCl$_{3M}$ (Fig. 3b), in the absence of *D*-PA no peaks appears (Fig. 3a). Therefore the oxidation of *D*-PA at surface of 4-FEPEMCPE occurs at a potential about 300 mV less positive than at a bare carbon paste electrode. The above results show the oxidation of *D*-PA is facilitated and catalyzed by presence of 1-[4-(ferrocenyl-ethynyl) phenyl]-1-ethanone spiked into carbon paste electrode. The presence of 1-[4-(ferrocenyl-ethynyl) phenyl]-1-ethanone as the mediator on the surface electrode provides an alternative reaction site to carbon paste for electron transfer process of *D*-PA. Therefore, current due to the oxidation of *D*-PA is increased when a 4-FEPEMCPE was used.
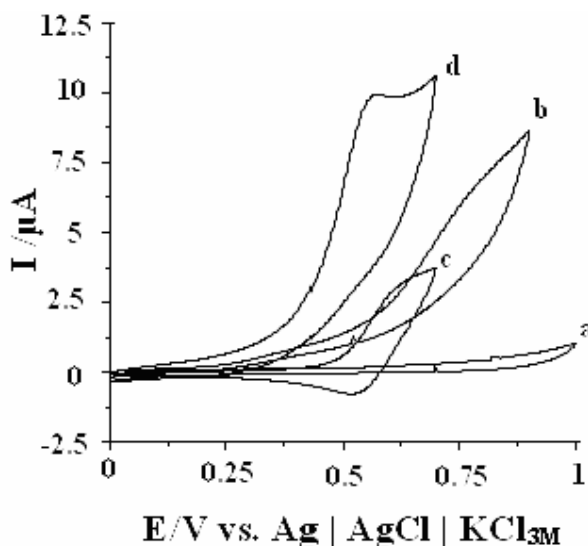
Figure 3. Cyclic voltammograms of (a) CPE in 0.1 M phosphate buffer solution (pH 7.00) at scan rate 20 mV s^{-1} and (b) as (a) in the presence of 1 mM *D*-PA, (c) as (a) and (d) as (b) at the surface of 4FEPEMCPE.

The effect of the potential scan rate on the electrocatalytic property of 4-FEPEMCPE toward *D*-PA was studied by cyclic voltammetry. Figure 4A shows the cyclic voltammograms of the 4-

FEPEMCPE at various scan rates (10-750 mV/s). These results show that the catalytic effect of the mediator appeared at scan rates up to 200 mV/s and the cathodic current would be increased with increasing scan rate, because, in short time–scale experiments, there is not enough time for the catalytic reaction to take place completely. However, the oxidation current of *D*-PA increased linearly with the square root of the scan rate of potentials (Fig. 4B), which demonstrate a diffusion controlled electrochemical process.

Figure 4. (A) Cyclic voltammograms of 1 mM *D*-PA acid at various scan rates: (a) 10, (b) 20, (c) 50, (d) 100, (e) 250, (f) 500 and (g) 750 mV s^{-1} in 0.1 M phosphate buffer solution (pH 7.00). (B) Plot of I_{pa} versus $\upsilon^{1/2}$ for the oxidation of *D*-PA acid at the surface of 4-FEPEMCPE

In order to get the information on the rate determining step, a Tafel slop (b) determinates using the following equation for a totally irreversible diffusion controlled process [52]:

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

Based on Equation 1, the slope of E_p versus log υ is b/2, where b indicates the Tafel slope that was found to be 0.066 V (Fig. 5). This slop value indicated an electron transfer process.

The values of αn_α (where α is the transfer coefficient and n_α is the number of electrons involved in the rate determining step) were calculated for the oxidation of *D*-PA at pH 7.00 at both modified and unmodified CPEs according to the following equation [53]:

$$\alpha n_\alpha = 0.048/(E_P - E_{P/2}) \qquad (2)$$

Where, $E_{P/2}$ is the potential corresponding to $I_{P/2}$. The values for αn_α were found to be 0.48 and 0.37 for the oxidation of *D*-PA at the surface of the 4-FEPEMCPE and CPE, respectively. These values clearly show that not only the over potential for *D*-PA oxidation is reduced at the surface of 4-FEPEMCPE, but also the rate of the electron transfer process is greatly enhanced, this phenomenon is thus confirmed by the larger I_{pa} values recorded during cyclic voltammetry at 4-FEPEMCPE.
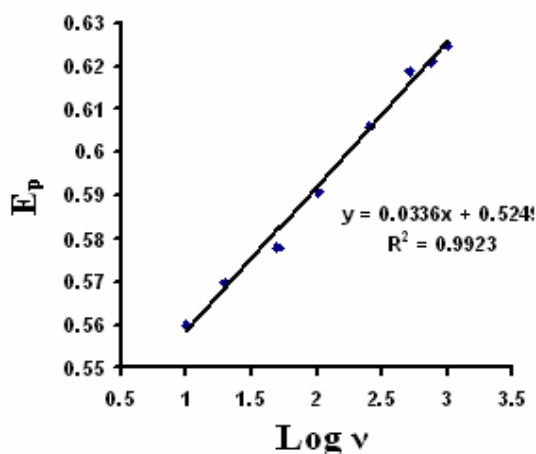

Figure 5. The peak potential, E_p dependence on log ν for the oxidation of *D*-PA acid at the surface of 4-FEPEMCPE.

3.3. Chronoamperometry Studies

Double potential step chronoamperometry, as well as other electrochemical methods was employed for the investigation of electrode processes at chemically modified electrodes. Figure 6A shows the current–time curves of 4-FEPEMCPE obtained by setting the working electrode potential at the 0.7 V (first potential step) and 0.3 V (second potential step) vs. Ag|AgCl|KCl$_{3M}$ for various concentration of *D*-PA in buffered aqueous solutions (pH 7.00). As can be seen, there is not any net cathodic current corresponding to the reduction of Fc$^+$ to Fc in the presence of *D*-PA, when the second potential step is employed. The oxidation current value increases with increasing of the *D*-PA concentration at first potential step. 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 *D*-PA the charge value associated with forward chronoamperometry is significantly greater than that observed for backward chronoamperometry (Fig. 6B, curve b´). Figure 6C shows plots of currents sampled at fixed time as a

function of the *D*-PA concentration, added to a blank solution (pH 7.00) at different times after the application of a potential step. Comparison of graphs (a), (b), (c) and (d) in this figure suggests that in all cases, there is a similar connection in between the currents measured at fixed time and the *D*-PA concentration, but the slope of the calibration graph increases with decreasing of the time elapsed after potential step application.
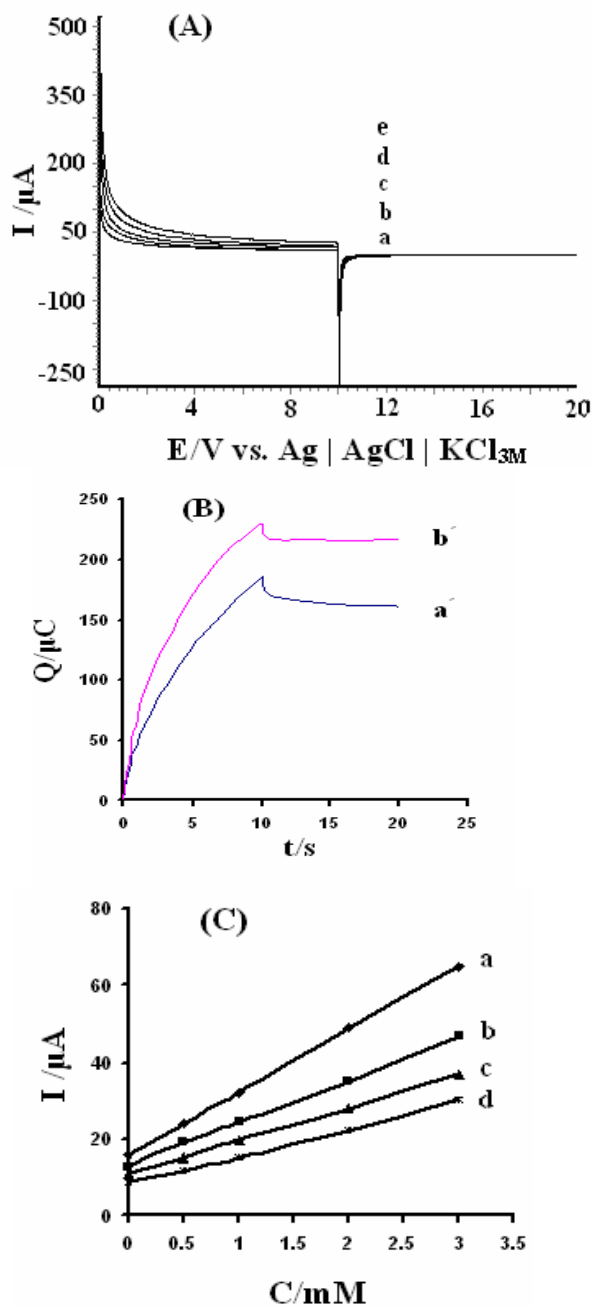
Figure 6. (A) Double step potential chronoamperograms obtained at the 4-FEPEMCPE in the (a) absence and presence of (b) 0.5, (c) 1.0, (d) 2.0 and (e) 3 mM of *D*-PA in 0.1 M phosphate buffer solution (pH 7.00). First and second potential steps were 0.70 and 0.30 V vs. Ag|AgCl|KCl$_{3M}$. (B) The charge–time curves: (a′) for curve (a) and (b′) for curve (b). (C) Dependence of the fixed time current observed [for (a) 2, (b) 4, (c) 6 and (d) 8 s after the first potential step] vs. *D*-PA concentration derived for data of the (A).

The rate constant for the chemical reaction between *D*-PA and redox sites in 4-FEPEMCPE, K_h can be evaluated by chronoamperometry according to the method described in [54]:

$$I_C/I_L = \gamma^{1/2} [\pi^{1/2} \, erf \, (\gamma^{1/2}) + exp \, (-\gamma) / \gamma^{1/2}] \qquad (3)$$

Where, I_C is the catalytic current of 4-FEPEMCPE in the presence of *D*-PA and I_L is the limited current in the absence of *D*-PA and $\gamma = k_h C_b t$ (C_b is the bulk concentration of *D*-PA, mol cm^{-3}) is the argument of 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:

$$I_C/I_L = \pi^{1/2} \gamma^{1/2} = \pi^{1/2} (k_h C_b t)^{1/2} \qquad (4)$$

Where, k_h and t are the catalytic rate constant (cm^3 mol^{-1} s^{-1}) and time elapsed (s) respectively. The above equation (4) can be used to calculate the rate constant of catalytic process, k_h. From the slope of I_C/I_L versus $t^{1/2}$ plot the value of k_h can be simply calculated for a given concentration of substrate. The calculated value of k_h is 2.66×10^2 cm^3 mol^{-1} s^{-1} using the slope of I_C/I_L- $t^{1/2}$ plot. This value of k_h explains as well as the sharp feature of the catalytic peak observed for catalytic oxidation of *D*-PA at the surface of 4-FEPEMCPE.
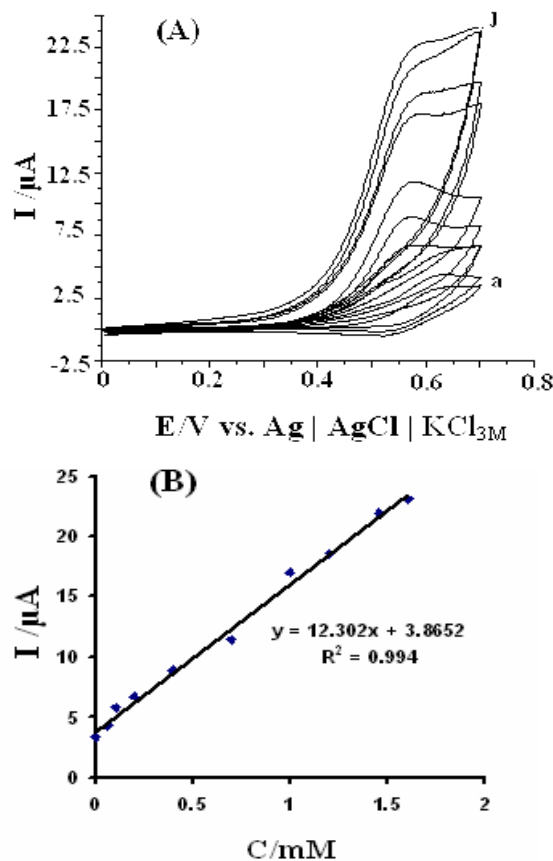
Figure 7. (A) Cyclic voltammograms of *D*-PA at various concentrations: (a) absence and (b) presence of 0.06, (c) 0.1, (d) 0.2, (e) 0.4, (f) 0.7, (g) 1.0, (h) 1.2, (i) 1.45 and (j) 1.6 mM at the surface of 4-FEPEMCPE in 0.1 M phosphate buffer solution (pH 7.00) at a scan rate of 20 mV s^{-1}. (B) Plot of electrocatalytic peak currents [from CV of (A)] vs. the *D*-PA concentrations.

3.4. Electrocatalytic Determination of D-PA

The electrocatalytic peak current of D-PA oxidation at the surface of 4-FEPEMCPE was used for determination of D-PA in solution. Therefore, cyclic voltammetry (Fig. 7A) and differential pulse voltammetry experiments (Fig. 8A) were performed using 4-FEPEMCPE in phosphate buffer solution containing various concentration of D-PA. The results show the electrocatalytic peak current of D-PA oxidation at the surface of 4-FEPEMCPE was linearly dependent on the D-PA concentration and the range of this linearity depends on the amount of mediator in the electrode matrix. The mediated oxidation peak current of D-PA at the surface of a 1% 4-FEPEMCPE was proportional to the concentration of substrate with the linear range of 6.0×10^{-5} M $- 1.6 \times 10^{-3}$ M (with a correlation coefficient of 0.9940) and 7.0×10^{-6} M $- 2.3 \times 10^{-4}$ M (with the correlation coefficient of 0.9947) in the cyclic voltammetry and differential pulse voltammetry, respectively (Figs. 7B, 8B). The detection limits (3σ) were 4.6×10^{-5} M and 3.9×10^{-6} M with CV and DPV, respectively. Thus, the catalytic oxidation of D-PA can readily be applied for the determination of D-PA.
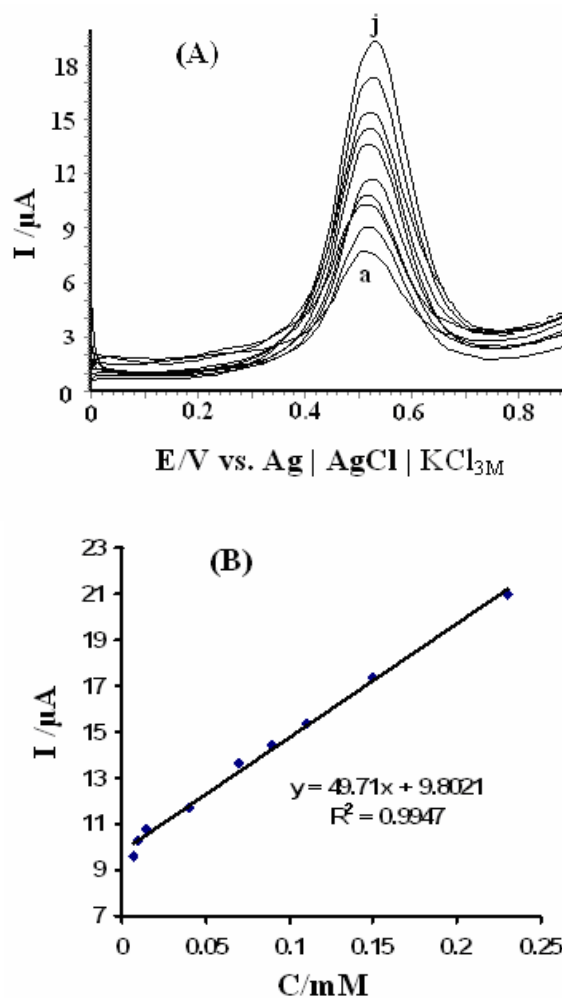
Figure 8. (A) Differential pulse voltammograms of the D-PA at various concentrations: (a) 0.00, (b) 0.07, (c) 0.01, (d) 0.015, (e) 0.04, (f) 0.07, (g) 0.09, (h) 0.11, (i) 0.15 and (j) 0.23 mM at the surface of 4-FEPEMCPE in 0.1 M phosphate buffer solution (pH 7.00) at a scan rate of 20 mV s^{-1}. (B) Plot of electrocatalytic peak currents [from DPV of (A)] vs. the D-PA concentrations.

3.5. Drug Analysis of D-PA capsules

In order to demonstrate the electrocatalytic oxidation of D-PA in pharmaceutical preparations, we examined this ability in the voltammetric determination of D-PA in the D-PA capsules (containing 250 mg D-PA) purchased from Laboratorios Rubio, S. A. Spain. The precision of the method was obtained on the basis of intra-assay using standard addition. The intra-assay data summarize in Table 2. In the United States Pharmacopoeia (USP), D-PA tablets are assayed by an ion-pairing HPLC method [4]. The data obtained for the analysis D-PA was compared favorably with that obtained by the USP standard method. There is not significant difference between the labeled contents and those obtained by the proposed method with the satisfactory recovery.

Results of the recoveries of the D-PA added to 10.0 ml of human synthetic serum are shown in Table 2. In these measurements, the composition of the synthetic serum was chosen near to its normal level in real human serum [55]. The study results are summarized in Table 2.

Table 2. Determination of D-PA in capsule and recovery data obtained for D-PA added at specified concentrations to buffer solution (pH 7.00) and synthetic human serum using a 4-FEPEMCPE.

Experiment	Sample preparation	Labeled value (mg/10 ml)	Amount found (mg/10 ml)	Mean recovery ± S.D. (%)		$T_{exp.}$	$F_{exp.}$
				Proposed-method [a]	USP-method [a]		
1	*D*-PA tablet	1	1.08	103.5 ± 1.2	102.6 ± 0.6	1.82	4.0
2	sample 1 + 1 mg D-PA	-	2.10	105 ± 2.1			
3	sample 1 + 1.7 mg D-PA	-	2.58	95.6 ± 0.7			
4	sample 1 + 3.5 mg D-PA	-	4.37	97.1± 3.1			
5	10 ml serum + 1 mg D-PA	-	1.06	106 ± 0.4			
6	10 ml serum + 2 mg D-PA	-	1.95	97.5 ± 1.5			
7	10 ml serum + 3 mg D-PA	-	2.97	99.0 ± 3.6			

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

4. CONCLUSIONS

This work demonstrates the construction of a chemically modified carbon paste electrode by the incorporation of 1-[4-(ferrocenyl-ethynyl) phenyl]-1-ethanone as a modifying specie. The electrochemical studies show an excellent electrocatalytic effect toward the oxidation of D-PA at pH 7.00, whereas, the oxidation of D-PA occurred at a potential about 300 mV less positive than that unmodified carbon paste electrode. Therefore, the electrocatalytic oxidation peak current of D-PA at the surface of 4-FEPEMCPE was used for voltammetric determination of D-PA in an aqueous solution. Thus, this method can be employed as a new and simple method for the voltammetric determination of D-PA trace amounts of D-PA (μM) in pharmaceutical preparations.

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