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VOLTAMMETRIC DETERMINATION OF L-CYSTEIC ACID ON A 1-[4-(FERROCENYL-ETHYNYL)PHENYL]-1-ETHANONE MODIFIED CARBON PASTE ELECTRODE

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ABSTRACT. The electrochemical behaviour of L-cysteic acid was studied at the surface of 1-[4-(ferrocenyl-ethynyl)phenyl]-1-ethanone modified carbon paste electrode (4FEPEMCPE) in aqueous media using cyclic voltammetry and double step potential chronoamperometry. It has been found, that under optimum condition (pH 7.00) in cyclic voltammetry, the oxidation of L-cysteic acid at the surface of 4FEPEMCPE is occurred at a potential about 220 mV less positive than that an unmodified carbon paste electrode. The kinetic parameters such as electron transfer coefficient, α, and catalytic reaction rate constant, K'_h, were also determined using electrochemical approaches. The electrocatalytic oxidation peak current of L-cysteic acid showed a linear dependent on the L-cysteic acid concentration and linear calibration curves were obtained in the ranges of 9×10^{-5} - 6.2×10^{-3} M and 2.0×10^{-7} - 1.17×10^{-5} M of L-cysteic acid concentration with cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods, respectively. The detection limits (3σ) were determined equal to 2.3×10^{-5} M and 8.7×10^{-8} M by CV and DPV methods. This method was also examined as a selective, simple and precise new method for voltammetric determination of L-cysteic acid in serum of patient's blood with migraine disease.

KEY WORDS: 1-[4-(Ferrocenyl-ethynyl)phenyl]-1-ethanone, L-Cysteic acid, Electrocatalysis, Cyclic voltammetry, Differential pulse voltammetry, Chronoamperometry

INTRODUCTION

The detection of thiol-derivated substances is an important field of research as they can be present as contaminants in fuels [1] and act as physiological indicators in biological fluids [2]. Also they use as markers of food deterioration [3]. One of the methods which applied nowadays for measurement of this material, use of chemically-modified electrodes (CMEs), that attracted scholarly attentions in last decade [4]. Various methods have been used for the immobilization of electroactive materials in the fabrication of CMEs. These methods include adsorption [5], covalent bonding [6], immobilization of electroactive materials as a dopant in a conducting polymer matrix [7], and so on.

L-cysteic acid (3-sulfoalanine; β-sulfoalanine) is a sulfur containing amino acids which occur normally in the outer part of sheep's fleece, where the wool is exposed to light and weather. During migraine and alzaymer total plasma thiols, L-cysteic acid was found to be high [8, 9]. To date, several methods such as gas chromatography [10] and HPLC [11] have been used for determination of L-cysteic acid in various samples.

On the other hand, although Fawcett *et al.* have been reported the electrooxidation mechanism of chemisorbed L-cysteic acid on a polycrystalline gold electrode [9] and we reported the electrocatalytic oxidation and voltammetric determination of L-cysteic acid at the surface of *p*-bromanil modified carbon paste electrode [12]. Our literature survey shows that, there is not another report about the electrocatalytic determination of L-cysteic acid.

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Therefore, in continuation of our studies concerning the preparations and applications of some ferrocene derivatives carbon paste modified electrodes [13-16], in this study, the application of 1-[4-(ferrocenyl-ethynyl)phenyl]-1-ethanone was discussed as a mediator for the electrooxidation of L-cysteic acid (as a thiol) in aqueous media. Also, the suitability of the 1-[4-(ferrocenyl-ethynyl)phenyl]-1-ethanone modified carbon paste electrode in the electrocatalysis and also determination of L-cysteic acid are discussed by cyclic voltammetry, double potential step chronoamperometry and differential pulse voltammetry.

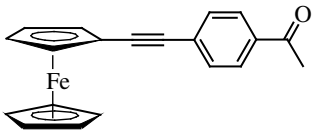
EXPERIMENTAL

Regents

The solvent used for the electrochemical studies was twice distilled water. Buffer solutions were prepared from orthophosphoric acid and its salts in the pH ranges of 3.00 - 9.00. High viscosity paraffin (density = 0.88 gcm^{-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. L-cysteic acid was from Fluka and was used as received. All other reagents were of analytical grade.

Synthesis of 1-[4-(ferrocenyl-ethynyl) phenyl]-1-ethanone

4-Bromoacetophenone (4.6 g, 23 mmol), ethynylferrocene (6.28 g, 29.9 mmol), triethylamine (100 mL) and dimethylformamide (40 mL) were mixed under argon with catalytic amounts of bis(triphenylphosphine) palladiumdichloride (420 mg 0.6 mmol) and copper(I) iodide (114 mg, 0.6 mmol). The mixture was allowed to reflux for a period of 3 h, after which excess triethylamine was evaporated under reduced pressure. The residue was added to 1 litre water and the resulting precipitate was filtered. The row product was redissolved in dichloromethane and chromatographed on silica gel column (petroleum ether/CH_2Cl_2 1:0-01) to give pure 1-[4-(ferrocenyl-ethynyl) phenyl]-1-ethanone (6.9 g, 91 % yield) (Scheme 1). M.p. 147 °C; IR (KBr): ν (cm^{-1}) = 3272 (≡C-H), 3077, 2207 (C≡C), 1678 (C=O), 1600, 1404, 1265, 1162, 828, 813; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.91 (d, *J* = 8.30 Hz, 2 H), 7.55 (d, *J* = 8.30 Hz, 2 H), 4.53 (pseudo t, 2 H), 4.28 (pseudo t, 2 H), 4.25 (s, 5 H), 2.60 (s, 3 H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 197.30 (C=O), 135.62 (C), 131.36 (CH), 129.00 (C), 128.27 (CH), 92.58 (C), 85.27 (C), 71.62 (CH), 70.06 (CH), 69.24 (CH), 64.40 (C), 26.59 (CH_3).



Scheme 1

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 1:1 (w/w) mixture of 1 % 1-[4-(ferrocenyl-ethynyl)phenyl]-1-ethanone spiked carbon powder and paraffin oil was blended by hand-mixing and the resulting

paste was inserted in the bottom of a glass tube (with internal radius 3.0 mm). The electrical connection was implemented by a copper wire lead fitted into a glass tube. A carbon paste electrode (CPE) without 1-[4-(ferrocenyl-ethynyl)phenyl]-1-ethanone was used as a blank to determine background current.

Instrumentation

The electrochemical experiments were carried out using a Potentiostat/Galvanostat (BHP 2061-C Electrochemical Analysis System, Behpajooh, Iran) coupled with a Pentium III personal computer connected to a HP laser jet 6 L printer, and experiments were performed in a three compartment cell. A platinum wire was used as the auxiliary electrode. The 1-[4-(ferrocenyl-ethynyl)phenyl]-1-ethanone modified carbon paste electrode (4FEPEMCPE) and Ag|AgCl|KCl_{sat} (from Metrohm) were used as the working and reference electrodes, respectively. A pH-meter (Ion Analyzer 250, Corning) was used to read the pH of the buffered solutions.

Procedure of serum plasma of patient blood preparation

For preparation of serum, 10 mL of blood was taken and collected in a sample tube. The serum of blood was separated after putting the sample in an incubator at 37 °C for 30 min and centrifuging it.

RESULTS AND DISCUSSION

Electrochemical behavior of (4FEPEMCPE)

We have recently constructed 4FEPEMCPE by incorporation of 1-[4-(ferrocenyl-ethynyl) phenyl]-1-ethanone into carbon paste matrix and studied its electrochemical properties in buffered aqueous solution by cyclic voltammetry. Its cyclic voltammograms exhibits an anodic (E_{pa} = 0.60 V) and corresponding cathodic peaks with E_{pc}= 0.42 V vs. Ag|AgCl| KCl_{sat} (Figure l, curve a) related to Fc/Fc^+ redox couple with quasi-reversible behavior (Table 1) [17]. The cyclic voltammogram of bare CPE in pure supporting electrolyte shows no anodic or cathodic peaks ((Figure l, curve b). 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.

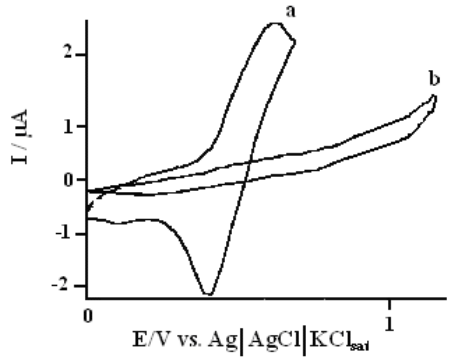


Figure 1. Cyclic voltammograms of a) 4FEPEMCPE and b) bare CPE in 0.1 M phosphate buffer solution (pH 7.00) at scan rate 10 mV s^{-1}.

Table 1. Cyclic voltammetric data obtained for constructed 4FEPEMCPE in 0.1 M phosphate buffer solution (pH 7.00) at the scan rate of 10 mVs^{-1}.

E_{pa} (V) [a]	E_{pc} (V) [a]	I_{pa} (µA)	I_{pc} (µA)	Γ_a (mol cm^{-2})	Γ_c (mol cm^{-2})
0.60 (0.75)	0.42 (2.1)	2.00 (1.7)	-1.2 (2.2)	$2.9\,5 \times 10^{-9}$ (3.1)	1.35×10^{-9} (2.5)

[a] Versus Ag|AgCl|KCl_{sat} as reference electrode. The values in parentheses indicate the calculated RSD %.

Electrooxidation of L-cysteic acid at the surface of 4FEPEMCPE

It is well known that the electrochemical behavior of L-cysteic acid is dependent on the pH value of the aqueous solution, whereas the electrochemical properties of Fc/Fc^+ redox couple are independent pH. Therefore, pH optimization of the solution seems to be necessary in order to obtain the electrocatalytic oxidation of L-cysteic acid. Thus we studied the electrochemical behavior of L-cysteic acid in 0.1 M phosphate buffered solutions in different pH values ($3.00 < pH < 9.00$) at the surface of 4FEPEMCPE by cyclic voltammetry. Results showed that the catalytic current increases with increasing pH. Figure 2 shows the variation of I_{pa} versus the variation of pH values. As can be seen, the maximum electrocatalytic current was obtained in the pH 7.00. Therefore, pH 7.00 was chosen as the optimum pH for electrocatalytic oxidation of L-cysteic acid at the surface of 4FEPEMCPE. Hence, all electrochemical experiments were done at this pH.

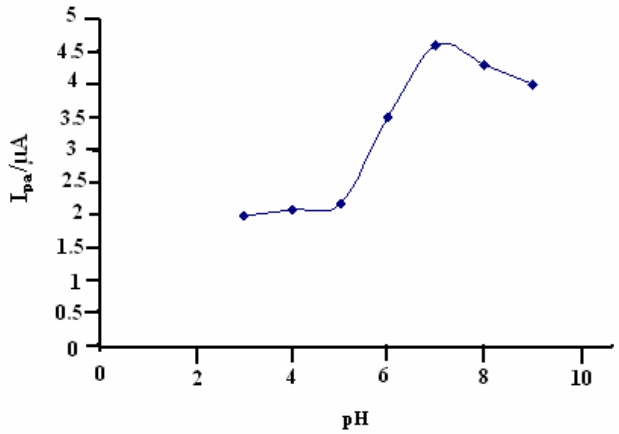


Figure 2. Anodic peak current-pH curve for electrooxidation of 0.5 mM L-cysteic acid in 0.1 M phosphate buffer solution at the surface of 4FEPEMCPE. Scan rate was 10 mV s^{-1}.

The cyclic voltammograms of 4FEPEMCPE and CPE in phosphate buffer solutions (pH 7.00) in the presence of 0.5 mM L-cysteic acid are presented in Figure 3. The comparison of Figures 1 and 3 shows that, in the absence of L-cysteic acid, a pair of well-defined redox peaks of 4FEPEMCPE can be observed (Figure 1, curve a). Upon the addition of 0.5 mM L-cysteic acid, there was a drastic enhancement of the anodic peak current, and in addition, no cathodic current was observed in the reverse scan of potential (Figure 3, curve b). This behaviour is consistent with a very strong electrocatalytic effect. Under the same experimental condition, the direct oxidation of L-cysteic acid at the surface of CPE shows an irreversible peak at more positive potential (Figure 3, curve a). Therefore, the catalytic oxidation peak potential is found to be about 610 mV, whereas that of the uncatalyzed oxidation peak is about 830 mV. Thus, a decrease in the overvoltage of approximately 220 mV and an enhancement of the peak current is

also achieved by the modified electrode. The above results show the oxidation of L-cysteic acid 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 of electrode provides an alternative reaction site to carbon paste for electron transfer process of L-cysteic acid. Therefore, current due to the oxidation of L-cysteic acid is increased when a 4FEPEMCPE was used.

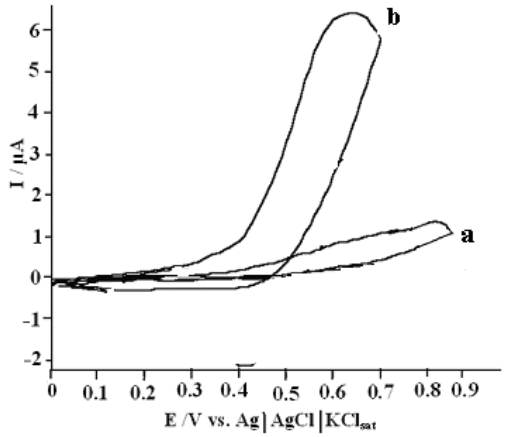


Figure 3. Cyclic voltammograms of a) bare CPE and b) 4FEPEMCPE in the presence of 0.5 mM L-cysteic acid in 0.1 M phosphate buffer solution (pH 7.00) at scan rate 10 mV s^{-1}.

The effect of the potential scan rate on the electrocatalytic property of 4FEPEMCPE toward the L-cysteic acid was studied by cyclic voltammetry. Figure 4 shows the cyclic voltammograms of the 4FEPEMCPE at various scan rates (5-300 mV s^{-1}). These results show that the catalytic effect of the mediator can be appeared at scan rates smaller than 25 mV s^{-1}. It can also be noted from Figure 4 that with an increasing scan rate, the peak potential for the electrooxidation of L-cysteic acid shifts to more positive potentials, suggesting a kinetic limitation in the reaction between the redox sites of 4FEPEMCPE and L-cysteic acid. In addition the cathodic current would increase with increasing scan rate, because in short time–scale experiments, there is no enough time for catalytic reaction to take place completely. However, the oxidation current of L-cysteic acid increased linearly with the square root of the scan rate of potentials (with linear equation: $y = 0.39x + 4.0106$, $R^2 = 0.9992$), which demonstrates a diffusion controlled electrochemical process.

In order to get the information on the rate determining step, Tafel slop (b) determinates using the following equation for a totally irreversible diffusion controlled process [18]:

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

Based on Equation 1, the slope of E_{pa} versus log ν is b/2, where b indicates the Tafel slope. The slope of E_p versus log ν plot was found to be 0.049 V (with linear equation: $y = 0.0494x + 0.5303$, $R^2 = 0.9989$) thus, b = 2 x 0.0494 = 0.098 V. This slope value indicated an electron transfer process, which is the rate limiting step by assumption of a transfer coefficient (α) equal to 0.6. Also, 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 L-cysteic acid at pH 7.00 at both modified and unmodified CPEs according to the following equation [19]:

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

where, $E_{P/2}$ is the potential corresponding to $I_{P/2}$. The values for αn_{α} were found to be 0.6 and 0.2 for the oxidation of L-cysteic acid at the surface of the 4FEPEMCPE and CPE, respectively. These values clearly show that not only the overpotential for L-cysteic acid oxidation is reduced at the surface of 4FEPEMCPE, 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 the surface of 4FEPEMCPE.

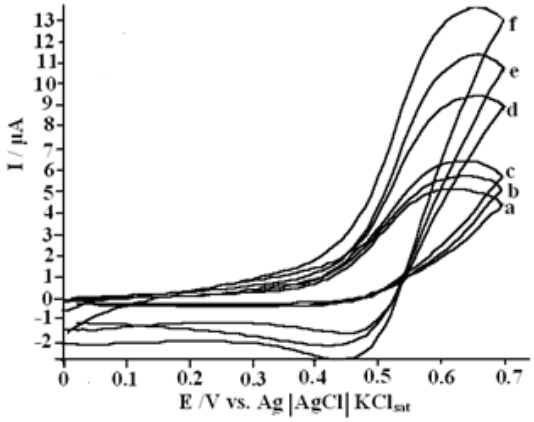


Figure 4. Cyclic voltammograms of 0.3 mM L-cysteic acid at various scan rates: a) 5; b) 10; c) 25; d) 100; e) 200 and f) 300 mV s^{-1} in 0.1 M phosphate buffer solution (pH 7.00).

Chronoamperometric studies

Double step potential chronoamperometry was also employed to the investigation of electrochemical behavior of aqueous buffered solution (pH 7.00) containing of various concentration of L-cysteic acid at 4FEPEMCPE by setting the working electrode potential at 0.70 V (at the first potential step) and 0.35 V (at the second potential step) vs. Ag|AgCl|KCl $_{sat}$ (Figure 5A). As can be seen, there is not any net cathodic current corresponding to the reduction of mediator in the presence of L-cysteic acid, when the potential is stepped from 0.70 V to 0.35 V vs. Ag|AgCl|KCl $_{sat}$. However, in the presence of L-cysteic acid the charge value associated with forward chronoamperometry is signification greater than that observed for backward chronoamperometry (Figure 5B(d)). Figure 5C shows plots of currents sampled at fixed time as a function of the L-cysteic acid concentration, added to a blank solution (pH 7.00) at different times after the application of potential step. Comparing of graphs (a), (b), (c), and (d) in this figure suggests that in all cases there is a similar connection between the currents measured at a fixed time and the L-cysteic acid concentration, but the slope of the calibration graph is increased with a decrease in the time elapsed after a potential-step application. The linearity of electrocatalytic current vs. $\nu^{1/2}$ shows this current is controlled by diffusion of L-cysteic acid from bulk solution toward surface of electrode that caused to near-Cottrellian behavior. Therefore, the slope of linear region of Cottrell's plot can be used to estimate the diffusion coefficient of L-cysteic acid. A plot of I versus $t^{-1/2}$ for a 4FEPEMCPE in the presence of L-cysteic acid gives a straight line, the slope of such lines can be used to estimate the diffusion coefficient of L-cysteic acid (D) in the ranges of 1.0-3.0 mM. The mean value of the D found to be 4.53 x 10^{-5} cm^2s^{-1}.

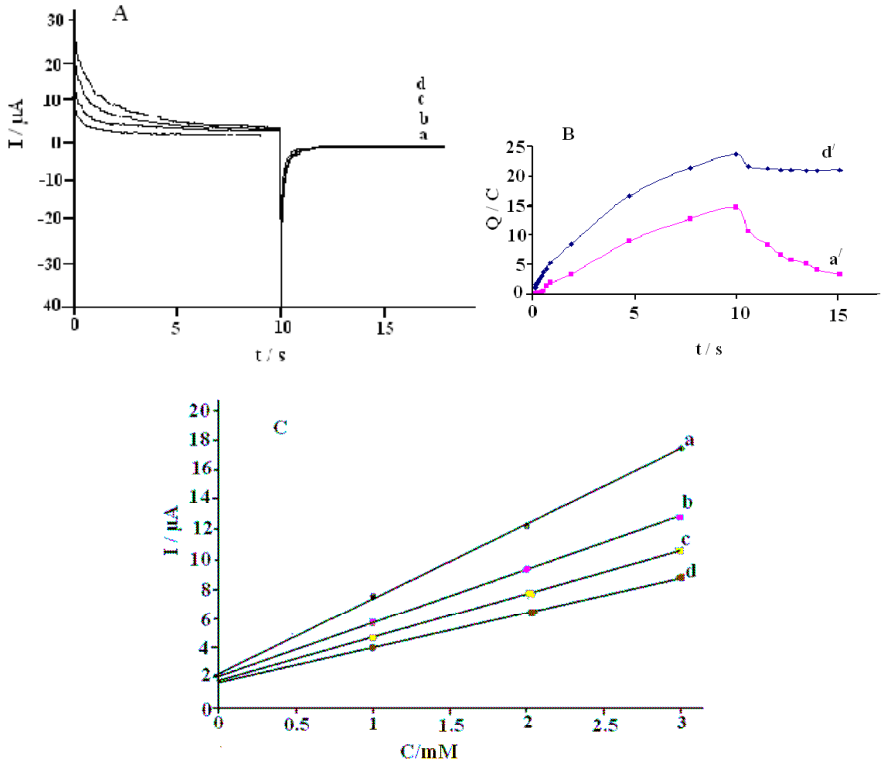


Figure 5. (A) Chronoamperograms obtained at the 4FEPEMCPE in the absence a) and presence of b) 1.0; c) 2.0 and d) 3.0 mM of L-cysteic acid in 0.1 M phosphate buffer solution (pH 7.00). First and second potential steps were 0.70 V and 0.35 V vs. Ag|AgCl|KCl $_{sat.}$ (B) This part shows the charge-time curves: a') for curve a) and d') for curve d). (C) Dependence of the fixed–time current observed for a) 0.5; b) 1.0; c) 1.5; and d) 2 s after the first potential step vs. L-cysteic acid concentration derived for data of the (A).

Therefore, the results show that mediator at the surface of 4FEPEMCPE can catalyze the oxidation of L-cysteic acid.

The rate constant for the chemical reaction between L-cysteic acid and redox sites in 4FEPEMCPE, k_h can be evaluated by chronoamperometry according to the method described in [20]:

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

where I_C is the catalytic current of 4FEPEMCPE in the presence of L-cysteic acid and I_L is the limited current in the absence of L-cysteic acid and $\gamma = k_h C_b t$ (C_b is the bulk concentration of L-cysteic acid, mol/L) 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:

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

where k_h and t are the catalytic rate constant (L 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. Having

measured the catalytic current, i.e. I_C, it is possible to carry out the electrode process in identical condition, but in the absence of L-cysteic acid, in order to determine I_L. 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 1.02 x 10^4 L $mol^{-1}s^{-1}$ using the slope of I_C/I_L-$t^{1/2}$ plot (Figure 6). This value of k_h explains as well as the sharp feature of the catalytic peak observed for catalytic oxidation of L-cysteic acid at the surface of 4FEPEMCPE. On the other hand, the surface coverage (Γ) of a modified electrode prepared at optimum condition was obtained from the equation Γ = Q/nFA, where Q is the charge obtained by integration the anodic peak under the background correction, A is the geometric area of electrode; and other symbols have their usual meanings. The calculated value of Γ was 7.2 x 10^{-8} $molcm^{-2}$ at pH 7.00. Using this value of coverage, the heterogeneous rate constant of catalytic reaction was calculated as K'_h = 2.1 x 10^{-3} cms^{-1}.

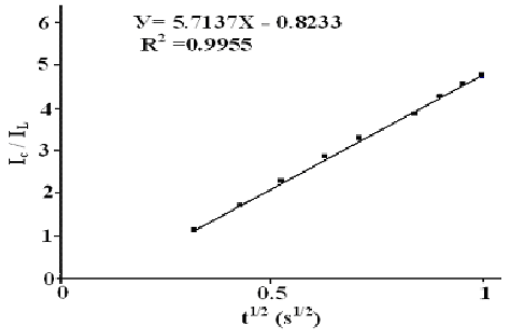


Figure 6. Dependence of I_C/I_L on the $t^{1/2}$.

Electrocatalytic determination of L-cysteic acid

The electrocatalytic peak current of L-cysteic acid oxidation at the surface of the 4FEPEMCPE can be used for determination of L-cysteic acid in solution. Therefore, cyclic voltammetry and differential pulse voltammetry experiments were performed using 4FEPEMCPE in phosphate buffer solution containing various concentration of L-cysteic acid. The results show the electrocatalytic peak current of L-cysteic acid oxidation at the surface of 4FEPEMCPE was linearly dependent on the L-cysteic acid concentrations, and the range of this linearity depended on the amount of mediator in the electrode matrix. The mediated oxidation peak currents of L-cysteic acid at the surface of 4FEPEMCPE were proportional to the concentration of the L-cysteic acid within the ranges 9.0 x 10^{-5} M - 6.2 x 10^{-3} M (with linear equation y = 4.2599x + 2.3521, R^2 = 0.9946) and 2.0 x 10^{-7} M - 1.2 x 10^{-5} M (with linear equation y = 675.84x + 2.9657, R^2 = 0.9948) in the cyclic voltammetry and differential pulse voltammetry, respectively (Figure 7). The detection limits (3σ) were 2.3 x 10^{-5} M and 8.7 x 10^{-8} M for CV and DPV, respectively.

Determination of L-cysteic acid in real sample

The order to demonstrate the ability of 4FEPEMCPE for the catalytic oxidation of L-cysteic acid in real sample, we have examined this ability in the voltammetric determination of L-cysteic acid in serum of patient' s blood with migraine disease by using the standard addition method in order to prevent any matrix effect. Therefore, we studied the electrochemical behavior of 4FEPEMCPE in the presence of a known amount of L-cysteic acid to buffer phosphate solution

(pH 7.00) containing a deliberated amount of serum. The result shows the addition of a known concentration of L-cysteine to the solution caused as increase in the oxidation peak height. Thus, the anodic peak was attributed to L-cysteine oxidation and the other biological compounds in the serum of patient's blood with migraine disease do not interfere in the voltammetric determination of L-cysteic acid.

Therefore, the concentration of L-cysteic acid in serum of patient's blood with migraine disease was obtained as 0.075 mM. These experiments demonstrated the ability of 4FEPEMCPE for voltammetric determination of L-cysteic acid with the high electrocatalytic effect and good reproducibility.

In addition, recovery test of L-cysteic acid in the range from 0.08 mM to 0.4 mM were performed. The results are listed in Table 2. The recovery varied in the 98.5 to 102.5 %.

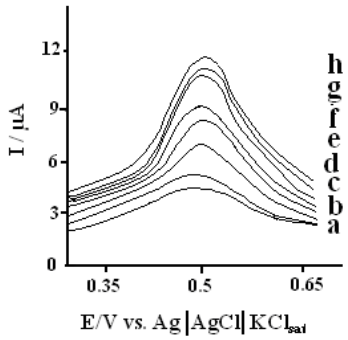


Figure 7. Differential pulse voltammograms of L-cysteic acid at various concentrations: a) 0.00; b) 0.0002; c) 0.0019; d) 0.0036; e) 0.0059; f) 0.0087; g) 0.0100; and h) 0.0120 mM at the surface of 4FEPEMCPE in 0.1 M buffer solution (pH 7.00) at a scan rate of 10 mV s^{-1}.

Table 2. Recovery of L-cysteic acid in 0.1 M phosphate buffer solution (pH 7.00) obtained using CV data at the surface of 4FEPEMCPE.

Added (mM)	Found (mM)	Recovery (%)
0.08	0.082	102.5
0.09	0.089	98.88
0.2	0.197	98.5
0.4	0.405	100.5

CONCLUSIONS

This work showed the ability of 1-[4-(ferrocenyl-ethynyl)phenyl]-1-ethanone as a modifier in carbon paste electrode for electrocatalysis of L-cysteic acid oxidation. The results demonstrated that the electrooxidation of L-cysteic acid at the surface 4FEPEMCPE occurs at the potential 220 mV less positive than at the bare carbon paste electrode. The kinetic parameter of the electrocatalytic process and the diffusion coefficients of L-cysteic acid in an aqueous solution were determined. Finally, the electrocatalytic oxidation currents of L-cysteic acid at the surface of 4FEPEMCPE were linear to concentration of L-cysteic acid. This modified electrode was also used for the determination of L-cysteic acid in patient-blood plasma.

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