

Differential Pulse Voltammetry Determination of L-Cysteine with Ferrocene-Modified Carbon Paste Electrode

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The preparation of ferrocene-modified carbon paste electrode by incorporation of a mediator into a graphite powder–paraffin oil matrix is described. The suitability of this chemically modified electrode for electrocatalytic oxidation of L-cysteine was studied in buffered solution by cyclic voltammetry and double potential step chronoamperometry. It has been found that, under optimum conditions (pH = 7.00), the oxidation of L-cysteine at the surface of such an electrode occurs at a potential about 500 mV less positive than on an unmodified carbon paste electrode. The kinetic parameters such as electron transfer coefficient α and catalytic reaction rate constant K'_h were determined using various electrochemical approaches. The electrocatalytic oxidation peak current of L-cysteine at the surface of this modified electrode was used for determination of L-cysteine by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Linear analytical curves were obtained in the ranges of 10^{-5} M (= mol dm^{-3})–10^{-3} M and 8.9×10^{-6} M–2×10^{-4} M of L-cysteine concentration with CV and DPV methods, respectively. The detection limits were determined as 6.5×10^{-6} M and 4.7×10^{-6} M by CV and DPV methods. This method was also examined for determination of L-cysteine in samples, such as Soya protein powder, serum and plasma of human blood, using recovery and standard addition methods.

L-Cysteine, a naturally occurring amino acid with a thiol group, participates in a number of biochemical processes that depend directly on the particular reactivity of thiols. Its oxidized derivatives have additional metabolic functions.[1] Also, L-cysteine is widely used in the food industry as an antioxidant and in the pharmaceutical industry in drug formulation and as a biomarker.[2] L-Cysteine exhibits irreversible oxidation, requiring positive overpotential at most conventional electrode surfaces.[3] Therefore, its electrochemical detection require a facilitating electron-transfer agent.

The modification of electrode surfaces with redox mediators that facilitate electron transfer processes occurring in the homogeneous phase has been used in electrocatalysis,[4–6] molecular recognition,[7,8] electrochemical sensors,[9,10] and bioelectrocatalysis.[11–13] It is well documented that functionalization of an electrode surface can offer significant analytical advantages in voltammetric experiments. For example, the detection of irreversibly oxidized species such as L-cysteine has been shown to be facilitated by electrode modification with substituted and unsubstituted transition metal phthalocyanines adsorbed or immobilized onto a graphite electrode.[14–17] This is also true when a glassy carbon electrode is coated with conductive polymer films,[18] prussian blue or related metal hexacyanoferrates encapsulated is silica,[19] low molecular weight charge transfer salts,[20] and fullerene-C_{60}.[3] Such electrode modifications have the objective of increasing the stability of the electrode response, decreasing the overpotential associated with the electrode process, and/or increasing the oxidative current of the sulfuryl compound. These chemically modified electrode electrocatalytic systems also are used to minimize the problems with poor selectivity and sensitivity that are commonly associated with the use of solid electrodes.[2]

Electrochemically well-behaved ferrocene (FC) and its derivatives are widely used in electrochemistry, because of their good stability in solution and rapid response to many electroactive substances. However, it has been found that FC and its derivatives are not adsorbed strongly on the electrode surface.[21] Therefore, incorporation of this compound into an electrode matrix such as carbon paste electrode can be the best procedure for preparation of chemically modified electrodes with ferrocene derivatives.[5,6,22] Thus, the ease and speed of preparation for obtaining a new reproducible surface, the low residual current, the porous surface, the control of the amount of modifier, and the low cost are some advantages of chemically modified carbon paste electrodes.[23,24]

In this paper, we describe the use of ferrocene as a mediator for the electrooxidation of L-cysteine in aqueous media. In addition, the suitability of this modified electrode in the electrocatalysis and also the determination of L-cysteine by cyclic voltammetry, double potential step chronoamperometry, and differential pulse voltammetry are discussed.

Experimental

Reagents and Materials. The solvent used for the electrochemical studies was twice distilled water. Buffer solutions were prepared from orthophosphoric acid and its salts in the pH range 2.00–9.00. 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. Ferrocene and L-cysteine (from Fluka) were used as received. All other reagents were of analytical grade.

Working Electrode. A 1% (w/w) ferrocene spiked carbon powder was made by dissolving the given quantity of ferrocene

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in diethyl ether and hand mixing with 99 times its weight of graphite powder using a mortar and pestle. The solvent was evaporated by stirring. A 1:1 (w/w) mixture of 1% ferrocene-spiked carbon powder and paraffin oil was blended by hand-mixing; the resulting paste was inserted in the bottom of a glass tube. The electrical connection was implemented by a copper wire lead fitted into a glass tube. The internal radius of this tube was 3.0 mm. A carbon paste electrode without ferrocene was used as a blank to determine the 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 II personal computer connected to a HP LaserJet 6L printer; experiments were performed in a three compartment cell. A platinum wire was used as the auxiliary electrode. The ferrocene-modified carbon paste electrode (FMCPE) was used as the working electrode. The reference electrode, was a double junction $Ag|AgCl|KCl_{sat}$ (Metrohm). Also, a pH-meter (Ion Analyzer 250, Corning) was used to read the pH value of the buffered solution.

Procedures of Samples Preparation. Soya Protein Solution: Soya protein powder (produced by Trader Joey's, America) has a low concentration of L-cysteine, but it was not detectable by voltammetry. Therefore, a solution of this sample was prepared by dissolving a known quantity (0.01 g) of the Soya protein powder in 10 mL phosphate buffer solution (pH = 7.00). Determination and recovery experiments of L-cysteine at the various concentration levels of L-cysteine in the sample were carried out by addition of determined solution into Soya protein solutions where the concentration of L-cysteine in the sample was 0.2 mM, 0.7 mM, or 1.0 mM. Then the cyclic voltammetry of each prepared sample was carried out.

Plasma and Serum of Human Blood: The plasma samples were obtained from the blood of volunteers. Every 10 mL of human blood was taken and collected in a syringe containing heparin and centrifuged at 2000 × *g* for 6 min. The plasma was separated and stored at −80 °C until analysis. 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 then centrifuging it.

Results and Discussion

Electrochemistry of FMCPE. The electrochemical properties of FMCPE were studied by cyclic voltammetry in pure buffered aqueous solution. The cyclic voltammogram exhibits an anodic peak at a forward scan of the potential related to the oxidation of ferrocene (Fc) to Fc^+, whereas at a reverse scan of the potential, a cathodic peak appears related to the reduction of Fc^+ (Fig. 1a). The cyclic voltammograms of bare CPE in pure supporting electrolyte shows no anodic and cathodic peaks (Fig. 1b). The experimental results show that well-defined and reproducible anodic and cathodic peaks related to oxidation and reduction of Fc/Fc^+ redox couple with quasi-reversible behavior. Because of the peak separation potential, ($\Delta E_p = E_{pa} - E_{pc}$) is greater than the $59/n$ mV expected for a reversible system. The results also show that the variation in the pH has no effect on the half-wave potential of the Fc/Fc^+ redox system. Therefore, the redox process of Fc/Fc^+ is not dependent on the pH.

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 FMCPEs (Table 1). The calculated r.s.d for various parameters such as peak currents, surface coverages, and peak potentials, accepted as the criteria for a satisfactory surface reproducibility, vary between 1–4%. This degree of reproducibility is virtually the same as that expected for the renewed or ordinary carbon paste surface;[25,26] however, we regenerated the surface of FMCPE before each experiment.

Optimization of the Solution pH. It is well known, that L-cysteine molecules will dissociate to produce more RS^- ($pK_{a1} = 1.92$, $pK_{a2} = 8.37$, $pK_{a3} = 10.70$).[3] Therefore, the electrochemical properties of L-cysteine are dependent on the pH value of the aqueous solution, whereas the electrochemical behavior of Fc/Fc^+ redox couple is independent of pH. We studied the electrochemical behavior of L-cysteine in buffered solutions with different pH values ($1.00 < pH < 9.00$) at the surface of FMCPE by cyclic voltammetry. Cyclic voltammograms of L-cysteine in acidic media at the surface of FMCPE show that the oxidation of L-cysteine in this condition cannot be catalyzed at the surface of FMCPE (Figs. 2a, b). However,

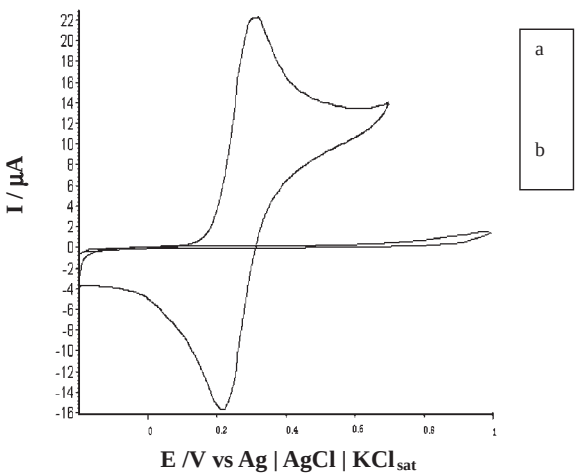

Fig. 1. (a) Cyclic voltammograms of FMCPE in 0.1 M phosphate buffer solution (pH 7.00) at a scan rate of 50 mV/s; (b) as (a) at the surface of a bare carbon paste electrode.

Table 1. Cyclic Voltammetric Data Obtained from Five Separately Constructed FMCPEs in 0.1 M Phosphate Buffer Solution (pH = 7.00) at 50 mV/s

E_{pa}/V[a)]	E_{pc}/V[a)]	ΔE_P/V	I_{pa}/μA	I_{PC}/μA	Γ_a/mol cm^{-2}	Γ_c/mol cm^{-2}
0.320	0.214	0.970	22.62	−20	4.7×10^{-9}	4.24×10^{-9}
(0.7)[b)]	(0.7)[b)]		(2.5)[b)]	(3.6)[b)]	(2.4)[b)]	(3.6)[b)]

a) Versus $Ag|AgCl|KCl_{sat}$ as reference electrode. b) The values in parentheses indicate the calculated r.s.d. (%) values.

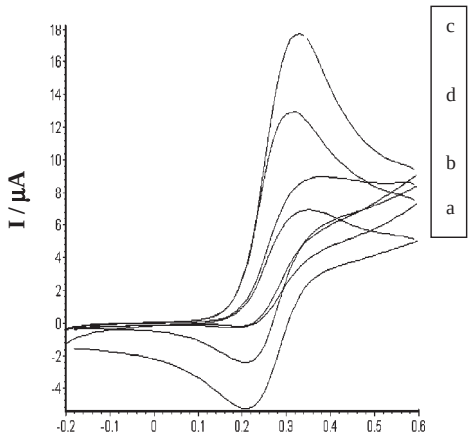

Fig. 2. Cyclic voltammograms of 1 mM L-cysteine at the surface of FMCPE at various pH values: (a) 2.00, (b) 5.00, (c) 7.00, and (d) 9.00 in 0.1 M phosphate buffer solution at a scan rate of 10 mV/s.

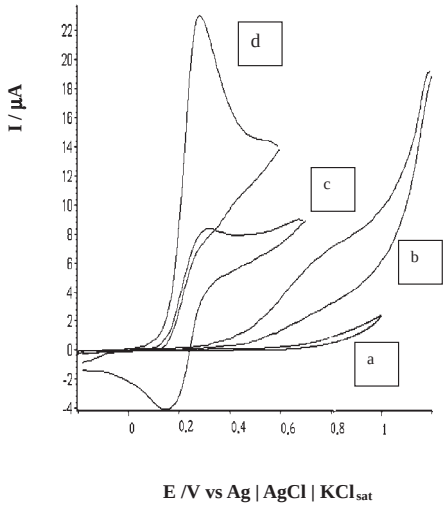

Fig. 3. Cyclic voltammograms of (a) CPE in 0.1 M phosphate buffer solution (pH 7.00) at a scan rate of 10 mV/s and (b) as (a) + 1 mM L-cysteine. (c) as (a) and (d) as (b) at the surface of FMCPE.

Table 2. Comparison of the Efficiency of Some Modified Electrodes Used in the Electrocatalysis of L-Cysteine

Substrate	Modifier	pH	E_p (bar) /mV[a)]	E_p (mod) /mV[b)]	Peak potential shift/mV s^{-1}	Scan rate /mV s^{-1}	Reference
Carbon electrode	fullerene-C60	7.00 (KH_2PO_4 buffer)	—	—[c)]	100	100	3
Glassy carbon electrode	10-ethylphenothiazine	($NaClO_4$ ethanol + Water)	—	600[d)]	—	50	34
Ordinary pyrolytic graphite disk (OPG) electrode	vitamin B12, coenzyme B12	7.00 (KH_2PO_4 buffer)	800[e)]	700[e)]	100	100	35
Sol–gel carbon ceramic electrode	Ru-complex	2.00 (phosphate buffer)	1200[f)]	800[f)]	400	10	36
Carbon paste electrode	ferrocene	7.00 (phosphate buffer)	800[d)]	300[d)]	500	10	this work

a) L-Cysteine peak potential at the surface of unmodified electrode. b) L-Cysteine peak potential at the surface of FMCPE. c) vs Ag|AgCl (3 M NaCl). d) vs Ag|AgCl|KCl_{sat}. e) vs saturated calomel. f) vs Ag|AgCl|KCl_{sat}/3 M KCl.

the oxidation potential of L-cysteine is pH-dependent and shifts toward less positive potential by increasing the solution pH. So, the thermodynamic driving force for the catalysis will vary with pH, making the peak currents and the shapes of the cyclic voltammograms different at various pH values. Consequently, in buffered solutions of pH > 5, the conditions are favored so that the catalyzed oxidation of L-cysteine is started by mediation of Fc^+ trapped at the surface of carbon paste electrodes. This appears as a gradual growth in the anodic peak current and a simultaneous decrease in the cathodic peak current in the FMCPE voltammograms.

According to the results obtained from cyclic voltammograms at various pH values, pH 7.00 was chosen as the optimum pH for electrocatalysis of L-cysteine at the surface of the FMCPE (Fig. 2c). As can be seen in Figs. 2c, d, the catalytic current was decreased at high pH values. Therefore, cyclic voltammograms obtained for an unmodified carbon paste electrode and for FMCPE in a phosphate buffer solution (0.1 M, pH = 7.00) in the presence (10^{-3} M) and absence of L-cysteine are illustrated in Fig. 3. At an unmodified surface, the L-cysteine oxidation occurs irreversibly at the potential of nearly 800 mV versus Ag|AgCl|KCl_{sat} (curve b), whereas in the absence of L-cysteine no peaks appear (curve a). In 10^{-3} M of L-cysteine solution, the anodic peaks that are observed for FMCPE in the absence of L-cysteine increase greatly, while the corresponding cathodic waves disappear on the reverse scan (curves c and d). This behavior is typical of that expected for electrocatalysis at chemically modified electrodes. The L-cysteine oxidation occurs at 350 mV vs Ag|AgCl|KCl_{sat} at FMCPE surface; therefore, it is shifted about 500 mV toward less positive potential. Note that this value is comparable with other values reported by other research groups for catalytic ox-

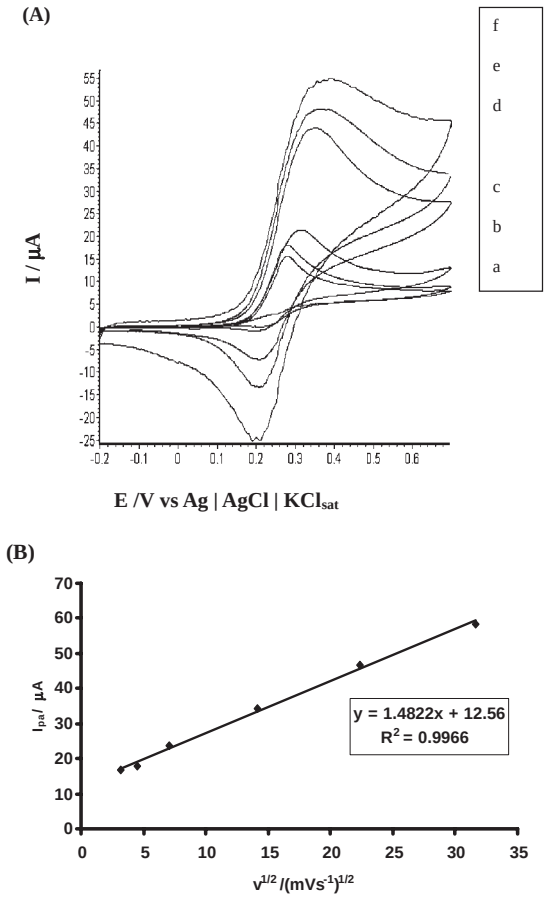

Fig. 4. (A) Cyclic voltammograms of 0.8 mM L-cysteine at various scan rates: (a) 5, (b) 10, (c) 20, (d) 50, (e) 100, and (f) 500 mV/s in 0.1 M phosphate buffer solution (pH 7.00). (B) Plot of I_{pa} versus of $v^{1/2}$ for the oxidation of L-cysteine at the surface of FMCPE.

idation of L-cysteine at the surface of chemically modified electrodes by other mediators (see Table 2).

The effect of the potential scan rate on the electrocatalytic properties of FMCPE toward L-cysteine was studied by cyclic voltammetry. Figure 4A shows the cyclic voltammograms of the FMCPE at various scan rates (5–1000 mV/s). These results show that the catalytic effect of the mediator appeared at scan rates up to 10 mV/s. It can also be noted from Fig. 4A that, with an increasing scan rate, the peak potential for the electrooxidation of L-cysteine shifts to more positive potentials, suggesting a kinetic limitation in the reaction between the redox sites of FMCPE and L-cysteine. In addition, the cathodic current would increase with increasing scan rate, because in short time-scale experiments, there is not enough time for the catalytic reaction to finish. However, the oxidation current of L-cysteine increased linearly with the square root of the scan rate of potentials (Fig. 4B), suggesting that at sufficient overpotential the reaction is mass transport controlled. These results show that the overall electrochemical oxidation of L-cysteine at a modified electrode might be controlled by the cross-exchange process between L-cysteine and the redox site of the FMCPE and the diffusion of L-cysteine.

In order to get the information on the rate determining step, one can calculate a Tafel slope, (b), using the following equation for a totally irreversible diffusion controlled process:[27]

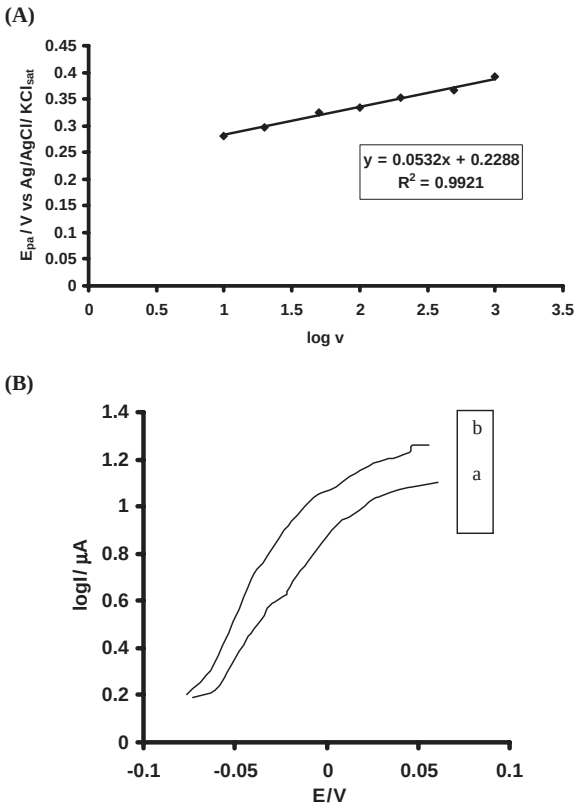

Fig. 5. (A) The peak potential E_p dependence on log(v) for the oxidation of L-cysteine at the surface of FMCPE obtained from data on Fig. 4B and (B) the Tafel plot driven from a current–potential curve obtained on the FMCPE in the presence of different L-cysteine concentrations: (a) 0.5, (b) 1.0 mM at scan rate of 10 mV/s.

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

Based on Eq. 1, the slope of E_p versus $\log v$ is $b/2$, where b indicates the Tafel slope. The slope of E_p versus $\log v$ plot was found to be 0.053 V in this work (Fig. 5A), thus, $b = 2 \times 0.053 = 0.106$ V. This slope value indicated an electron transfer process, which is the rate-limiting step by assumption of a transfer coefficient (α) equal to 0.52.

The number of electrons involved in the rate-determining step can be obtained by using another method. Tafel plots were drawn by using the data obtained from the rising part of the current–voltage curve at a scan rate of 10 mV/s in two different concentration of L-cysteine (0.5 mM and 1 mM) (Fig. 5B). A mean slope of 8.46 (V per decade)$^{-1}$ indicates that an electron transfer process has occurred that was rate-limiting, by assumption of a transfer coefficient of $\alpha = 0.50$.[27–29] The results obtained from these two different methods are in good agreement.

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-cysteine at pH = 7.00 at both modified and unmodified CPEs, according to the following equation:[30]

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

Here, $E_{p/2}$ is the potential corresponding to $I_{p/2}$. The values for αn_α, were found to be 0.51 and 0.24 for the oxidation of L-cysteine at the surface of the FMCPE and CPE, respectively. These values clearly show that not only is the overpotential for L-cysteine oxidation reduced at the surface of FMCPE, 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 FMCPE.

Chronoamperometric Studies. Double step potential chronoamperometry as well as other electrochemical methods were employed for investigation of electrochemical processes at chemically modified electrodes.[5,26,27,31] Figure 6A shows the current–time curves of the FMCPE obtained by setting the working electrode potential at 0.35 V (at the first potential step) and 0.00 V (at the second potential step) vs $Ag|AgCl|KCl_{sat}$ for various concentration of L-cysteine in a buffered aqueous solution (pH = 7.00). As can be seen, there is no net cathodic current corresponding to the reduction of mediator in the presence of L-cysteine, while the forward and backward potential step chronoamperometry results on the modified electrode in the blank buffered solution show very symmetrical chronoamperograms with an equal charge consumed for the oxidation and reduction of the redox couple in the CPE (Fig. 6A(a)). However, in the presence of L-cysteine the charge value associated with forward chronoamperometry is significantly greater than that observed for backward chronoamperometry (Fig. 6B(c′)). Figure 6C shows plots of currents sampled at fixed times as a function of the L-cysteine concentration added to a blank solution (pH = 7.00) at different times after the application of a potential step. Comparing 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-cysteine concentration, but that the slope of the calibration graph is increased with a decrease in the time elapsed after a potential-step application.

For an electroactive material with a diffusion coefficient (D), the current corresponding to the electrochemical reaction (under diffusion control) is described by Cottrell's law:[32]

$$I = nFA_gD^{1/2}C_O\Pi^{-1/2}t^{-1/2}. \quad (3)$$

Here D is the diffusion coefficient, and C_O is the bulk concentration. A_g is the geometric area of the modified carbon paste electrode. The plot of I versus $t^{-1/2}$ would be linear; from its slope, the value of D can be obtained. Chronoamperometry of the modified electrode in the presence of L-cysteine represents a typical I–t curve, which indicates that the observed current must be controlled by L-cysteine diffusion in the solution. A plot of I versus $t^{-1/2}$ for a FMCPE in the presence of L-cysteine gives a straight line; the slopes of such lines can be used to estimate the diffusion coefficient of L-cysteine (D) in the range (0.5–1 mM). The mean value of the D was found to be 4.26×10^{-5} $cm^2\,s^{-1}$. Therefore, the results show that the oxidation of L-cysteine can be catalyzed by mediator at the surface of FMCPE.

The rate constant for the chemical reaction between L-cysteine and redox sites in FMCPE, K'_h can be evaluated by chronoamperometry according to the method described in Ref. 33:

$$I_C/I_L = \gamma^{1/2}[\Pi^{1/2}\,\mathrm{erf}(\gamma^{1/2}) + \exp(-\gamma)/\gamma^{1/2}]. \quad (4)$$

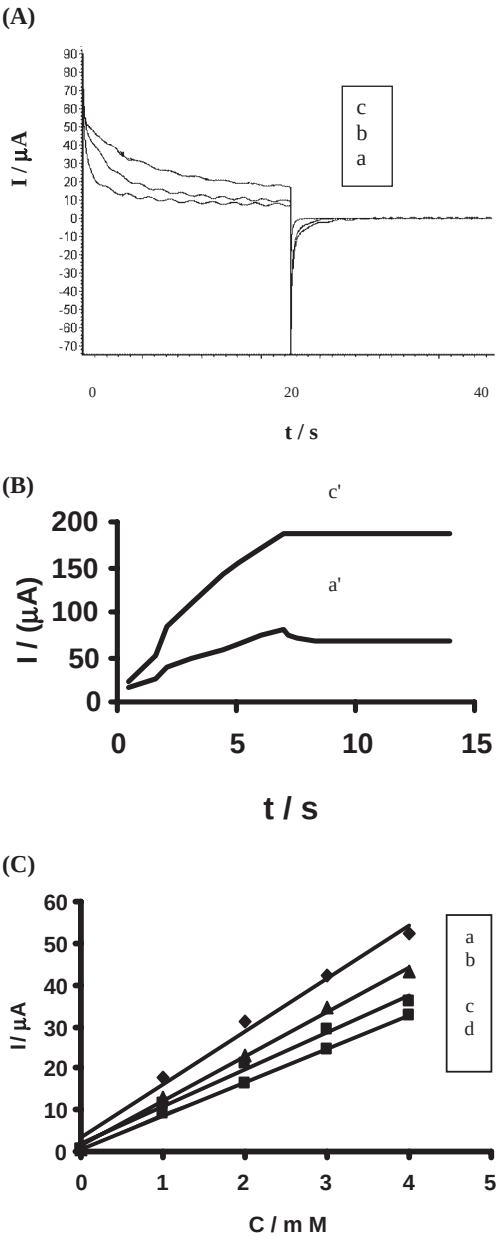

Fig. 6. (A) Chronoamperograms obtained at the FMCPE in the absence of (a) and in the presence of (b) 1.0; (c) 2.0 mM of L-cysteine in 0.1 M phosphate buffer solution (pH 7.00). First and second potential steps were 350 and 0.0 mV vs $Ag|AgCl|KCl_{sat}$. (B) shows the charge–time curves: (a′) for curve (a) and (c′) for curve (c). (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 L-cysteine concentration (1) 0.00, (2) 1.00, (3) 2.00, (4) 3.00, (5) 4.00 mM.

Here I_C is the catalytic current of FMCPE in the presence of L-cysteine and I_L is the limiting current in the absence of L-cysteine; $\gamma = k_hC_bt$ (C_b is the bulk concentration of L-cysteine, $mol\,cm^{-3}$) is the argument of the error function. In the cases where γ exceeds 2, the error function is almost equal to 1 and the above equation can be reduced to:

$$I_C/I_L = \Pi^{1/2}\gamma^{1/2} = \Pi^{1/2}(k_hC_bt)^{1/2}. \quad (5)$$

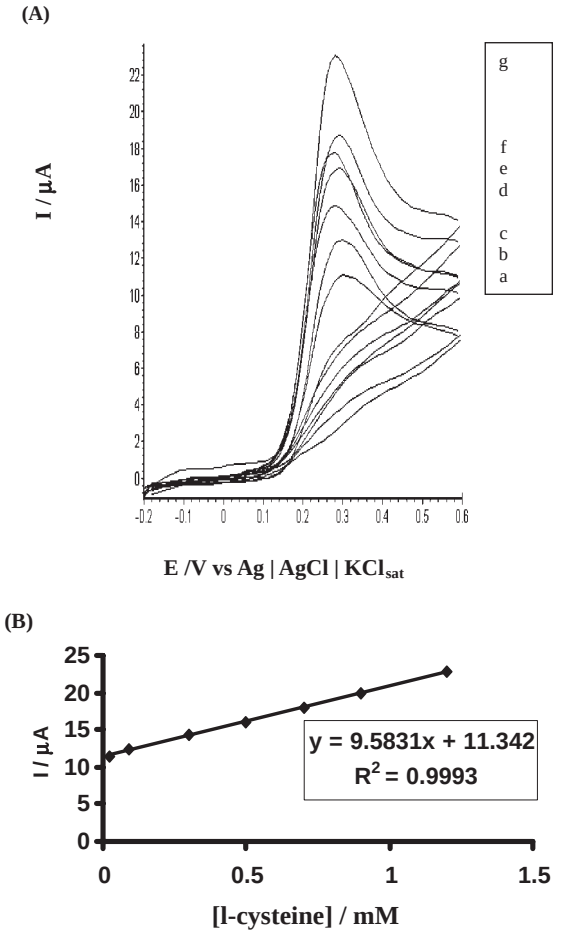

Fig. 7. (A) Cyclic voltammograms of L-cysteine at various concentrations: (a) 0.00, (b) 0.02, (c) 0.09, (d) 0.3, (e) 0.5, (f) 0.7, (g) 0.9, and (h) 1.2 mM at the surface of FMCPE in 0.1 M phosphate buffer solution (pH 7.00) at a scan rate of 10 mV/s. (B) Plot of electrocatalytic peak currents (from CV of (A)) vs the L-cysteine concentration.

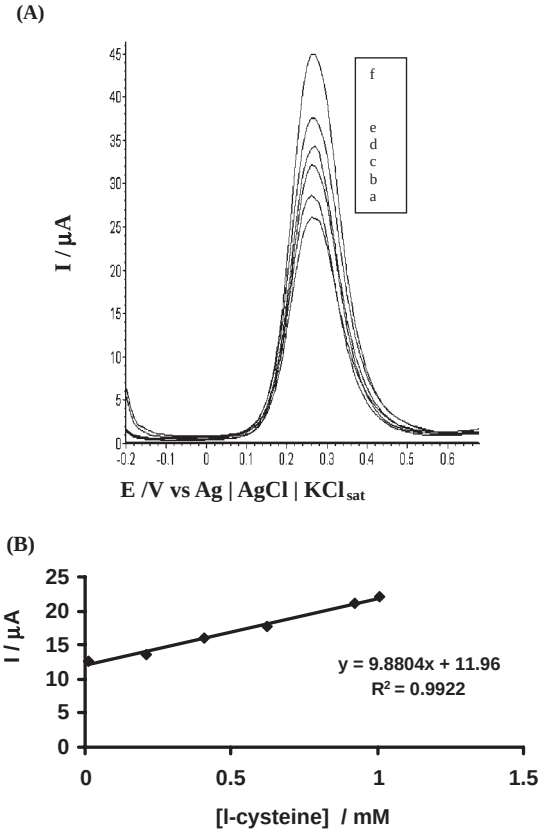

Fig. 8. (A) Differential pulse voltammograms at the FMCPE in the absence of (a) and in the presence of (b) 0.0089, (c) 0.21, (d) 0.41, (e) 0.92, and (f) 1.01 mM in 0.1 M phosphate buffer solution (pH 7.00). (B) Plot of electrocatalytic peak currents (from DPV of (A)) vs the L-cysteine concentrations.

Where k_h and t are the catalytic rate constant and the time elapsed. The above Eq. 5 can be used to calculate the rate constant of the catalytic process, k_h. Having measured the catalytic current, i.e. I_C, one can carry out the electrode process under identical conditions, but in the absence of L-cysteine, in order to determine I_L. From the slope of the 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 4.48×10^3 $M^{-1}\,s^{-1}$ using the slope of I_C/I_L–$t^{1/2}$ plot. This value of k_h explains also the sharp feature of the catalytic peak observed for catalytic oxidation of L-cysteine at the surface of FMCPE. On the other hand, the surface coverage (Γ) of a modified electrode prepared at optimum conditions was obtained from the equation $\Gamma = Q/nFA$, where Q is the charge obtained by integrating the anodic peak under the background correction and A is the geometric area of the electrode; an other symbols have their usual meanings. The calculated value of Γ was 4.45×10^{-8} mol cm^{-2} at pH 7.00. Using this value of coverage, we calculated the heterogeneous rate constant of catalytic reaction (k'_h) as $k'_h = 1.99 \times 10^{-1}$ cm s^{-1}.

Electrocatalytic Determination of L-Cysteine. The electrocatalytic peak current of L-cysteine oxidation at the surface of the FMCPE can be used for determination of L-cysteine in solution. Therefore, cyclic voltammetry and differential pulse voltammetry experiments were performed using FMCPE in phosphate buffer solution containing various concentration of L-cysteine. The results show that the electrocatalytic peak current of L-cysteine oxidation at the surface of FMCPE was linearly dependent on the L-cysteine concentrations, and that the range of this linearity depended on the amount of mediator in the electrode matrix. The mediated oxidation peak currents of L-cysteine at the surface of a FMCPE were proportional to the concentration of the L-cysteine within the ranges 10^{-5} M–10^{-3} M (with a correlation coefficient of 0.9993) and 8.9×10^{-6} M–2×10^{-4} M (with the correlation coefficient of 0.9922) in the cyclic voltammetry and differential pulse voltammetry, respectively (Figs. 7A, B and 8A, B). The detection limits (2σ) were 6.5×10^{-6} M and 4.7×10^{-6} M, the sensitivity expressed as the slopes of the linear regions of the calibration curves are 9.583 mA M^{-1} and 9.880 mA M^{-1} for CV and DPV respectively. These values are comparable with the values obtained by other research groups. Thus, the catalytic oxidation of L-cysteine can readily be applied to the determination of L-cysteine.

Determination of L-Cysteine in Samples. In order to demonstrate the catalytic oxidation of L-cysteine in real sam-

ples, we examined this ability in the voltammetric determination of L-cysteine in Soya protein powder, serum and plasma of human blood. The determinations of L-cysteine in Soya protein powder and serum of human blood were achieved with direct use of a L-cysteine calibration curve in recovery experiments. In this area, we added a known amount of L-cysteine to buffer phosphate solution (pH = 7.00) containing a pre-determined amount of Soya protein powder or serum; then the concentrations of L-cysteine in each sample were determined by direct use of calibration curves. Therefore, the recovery data were obtained for L-cysteine added at specified concentrations to prepared samples (Table 3). Thus, recoveries of (104.4 (6.4%)), (101.5 (3.3%)), (99.4 (2.5%)), (100.2 (1.8%)), (102.2 (6.5%)), and (99.8 (1.7%)), were obtained after addition of 0.2, 0.7, and 1.0 mM of L-cysteine to Soya protein powder and serum buffer solution, respectively. As can be seen, the average recovery and standard deviation for the determination of L-cysteine added to Soya protein powder and serum of human blood samples are comparable to other results.[3]

The determination of L-cysteine in plasma of human blood was carried out by the standard addition method in order to prevent any matrix effect. Figure 9A shows typical cyclic voltammograms recorded for plasma samples. As can be seen in this figure, adding a known concentration solution of L-cysteine to the testing solution caused an increasing in the oxidation peak height (curve (b) to (f) of Fig. 9). Thus, the peak was attributed to L-cysteine oxidation. The concentration of L-cysteine in plasma of human blood was obtained as 0.56 mM. These experiments demonstrated the ability of FMCPE for voltammetric determination of L-cysteine with the high electrocatalytic effect and good reproducibility and recoveries.

Conclusion

This work demonstrates the ability of ferrocene as the modifying species in carbon paste electrodes. The chemically-

Table 3. Recovery Data Obtained for L-Cysteine Added at Specified Concentrations to Samples Prepared in Buffer Solution (pH = 7.00) Using a FMCPE

Sample	Recovered concentration of L-cysteine/mM	Recovery/%	Mean recovery/%	r.s.d/%
(a) Concentration of L-cysteine added to sample = 0.2 mM				
Soya protein powder				
1	0.312	104.0	104.4	6.4
2	0.342	114.0		
3	0.301	100.3		
4	0.298	99.3		
serum				
1	0.308	102.6	101.5	3.3
2	0.300	100.0		
3	0.294	98.0		
4	0.316	105.3		
(b) Concentration of L-cysteine added to sample = 0.7 mM				
Soya protein powder				
1	0.682	97.4	99.4	2.5
2	0.700	100.0		
3	0.691	98.7		
4	0.712	101.7		
serum				
1	0.699	99.8	100.2	1.8
2	0.689	98.4		
3	0.700	100.0		
4	0.720	102.8		
(c) Concentration of L-cysteine added to sample = 1.0 mM				
Soya protein powder				
1	1.00	100.0	102.2	6.5
2	1.12	112.0		
3	0.978	97.8		
4	0.989	98.9		
serum				
1	1.02	102.0	99.8	1.7
2	1.00	100.0		
3	0.992	99.2		
4	0.979	97.9		

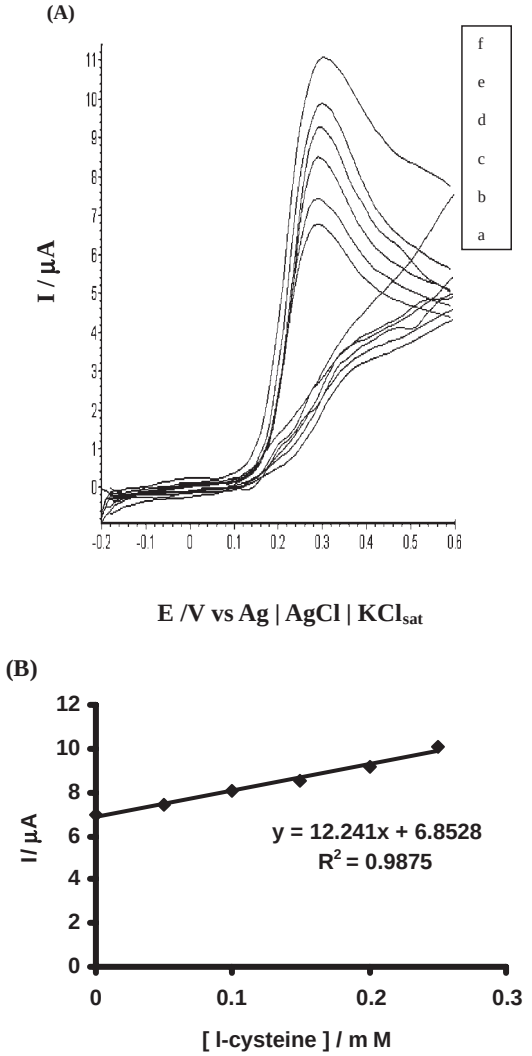

Fig. 9. (A) Cyclic voltammograms of FMCPE in 10 mL plasma of human blood after addition of (a) 0.00, (b) 0.05, (c) 0.10, (d) 0.15, (e) 0.20, and (f) 0.25 mM of L-cysteine standard solution at a scan rate of 10 mV/s. (B) Plot of electrocatalytic peak currents (from CV of (A)) vs the L-cysteine concentration.

modified carbon paste electrode was examined for electrocatalysis of L-cysteine oxidation. It has been shown by cyclic voltammetry, double step potential chronoamperometry, and differential pulse voltammetry that the electrocatalytic oxidation of L-cysteine at the surface of FMCPE occurs at a potential about 500 mV less positive than is the case for unmodified carbon paste electrodes. Moreover, from the results obtained, the kinetic parameters of the electrocatalytic process and the diffusion coefficient of L-cysteine in an aqueous solution was determined. In addition, the electrocatalytic oxidation peak current was used for determination of L-cysteine by cyclic voltammetry and differential pulse voltammetry. The usefulness of FMCPE for the determination of L-cysteine is verified by the values obtained for samples containing a known concentration of L-cysteine; excellent recovery data was obtained after L-cysteine was deliberately added to the samples.

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