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ELECTROCATALYTIC OXIDATION OF CYSTEINE BY INDIGO CARMINE MODIFIED GLASSY CARBON ELECTRODE

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Abstract

The electrocatalytic oxidation of L-Cysteine (L-Cys) has been studied by Indigo carmine (IND) at the glassy carbon electrode (GCE). Cyclic voltammetry and chronoamperometry techniques were used to investigate the suitability of IND as a mediator in aqueous solution with various pHs. It has been found that under the optimum conditions (pH 8.0) the oxidation of L-Cys occurs at a potential of about 290 mV less positive than that of an unmodified GCE. The heterogeneous electron transfer rate constant between IND and the GCE and the diffusion coefficient of IND were estimated to be about 2.62×10^{-3} cm s^{-1} and 4.10×10^{-6} cm^2 s^{-1}, respectively. The diffusion coefficient of L-Cys (D= 1.8×10^{-6} cm^2 s^{-1}), and its kinetic parameters such as electron transfer coefficient, ($\alpha = 0.41$) and the catalytic reaction rate constant, ($k=5.9\times10^4$ $M^{-1}s^{-1}$) were also determined using electrochemical approaches. The voltammetric response of the modified electrode was linear against the concentration of L-Cys in the ranges of 1.0×10^{-4} M- 1.5×10^{-2} M with cyclic voltammetry method. The detection limit (3σ) was determined as 5.1×10^{-5}.

Keywords: L-cysteine; glassy carbon electrode; electrocatalysis; indigo carmine

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Resumen

Se ha estudiado la oxidación electrocalítica de L-Cisteína (L-Cys) por índigo carmín (IND) sobre un electrodo de carbono vítreo (GCE). Se emplearon las técnicas de voltamperometría cíclica y cronoamperometría para investigar la utilidad del IND como mediador en solución acuosa a varios pHs. Se ha encontrado que bajo condiciones optimas (pH 8.0) la oxidación de L-Cys ocurre a un potencial de cerca de 290 mV menos positivo que sobre un GCE no modificado. La velocidad de transferencia electrónica heterogénea entre IND y el GCE y el coeficiente de difusión de IND fueron estimados en 2.62×10^{-3} cm s^{-1} y 4.10×10^{-6} cm^2 s^{-1}, respectivamente. El coeficiente de difusión de la L-Cys (D= 1.8×10^{-6} cm^2 s^{-1}) y sus parámetros cinéticos tales como el coeficiente de transferncia electrónica ($\alpha = 0.41$) y constante de reacción catalítica ($k=5.9\times10^4$ $M^{-1}s^{-1}$), se determinaron usando criterios electroquímicas. La respuesta voltamétrica del electrodo modificado fue lineal con la concentración de L-Cys en el rango de 1.0×10^{-4} M- 1.5×10^{-2} M con el método de voltamperometría cíclica. El límite de detección (3σ) fue establecido como 5.1×10^{-5}.

Palabras clave: L-cysteine; electrodo de carbono vítreo; electrocatálisis; índigo carmín

Introduction

L-Cys (2-amino-3-mercaptopropanoic acid) is one of the most important amino acids. It plays an important role in biological systems and has been widely used in medicine and food chemistry [1,2] It is also present in various cosmetic and pharmaceutical preparations, for example, used in some antibiotics and treatment of skin damage [3], as well as it participates on a great number of biochemical processes [4]. Studies focusing in the L-Cys determination have attracted considerable attention in nowadays [5, 6].

In this sense, several methods for its detection and quantification have been reported including, spectrofluorimetry [7], high performance liquid chromatography [8] and electrochemical methods [9–12]. However, most of them experienced difficulties with sample preparation, necessity of molecules derivatization or lack of sufficient sensitivity, which limit their practical utility [13]. Electrochemical methods present the advantages of simplicity and high sensitivity. However, the major problem related to the direct electrochemical detection of thiols is the high overpotential required for their oxidation on the most conventional electrodes. In this context, the use of bare electrodes for their detection has a great number of limitations, such as low selectivity, repeatability and the slow electron transfer reaction. Thus, chemically modified electrodes (CMEs) have been widely used as sensitive and selective analytical methods for detection and quantification of thiols at lower potentials [14], since the direct oxidation of thiols on the solid electrodes is slow and requires high overpotentials [15].

In this context, the surface modification of conventional electrodes is an important development in electroanalysis. A variety of efficient catalysts has been developed to induce the reduction or oxidation of some organic compounds [16,17], since these catalysts promote a faster electron transfer between the electrode surface and electroactive species in solution. Thus, a wide variety of compounds has been used as electron transfer mediators for electrooxidation of L-Cys [18-29].

In the present work, we found that an indigocarmin modified glassy carbon electrode (INDMGCE) possesses good electrocatalytic activity toward the electrocatalytic oxidation of L-Cys. Cyclic voltammetry and chronoamperometry were used to characterize the electrochemical properties of the electrode and to investigate its electrocatalytic effect on L-Cys oxidation.

Experimental

Apparatus and chemicals

A potentiostat/galvanostat (SAMA 500, electroanalyzer system, I. R. Iran) was used for carrying out the electrochemical experiments. A conventional three electrode cell was used at 25 ± 1°C. A saturated calomel electrode (SCE), platinum wire, and INDMGCE were used as reference, auxiliary and working electrodes, respectively. A Metrohm model 691 pH / mV meter was also used for pH measurements.

All solutions were freshly prepared with double-distilled water. IND, L-Cys and other reagents were analytical grade (Merck). Buffer solutions were prepared from ortho phosphoric acid, and its salts in the pH range of 2.0–12.0.

The GCE surface was polished mechanically with 0.05 μm alumina powders, using a polishing cloth until the electrode surfaces had a mirror-finish and then rinsed thoroughly with double-distilled water, successively.

Results and Discussion

Electrochemical properties of INDM at GCE

The IND is soluble in aqueous media; therefore we used GCE and studied its electrochemical properties in a buffer aqueous solution (pH 8.0) by cyclic voltammetry. Cyclic voltammograms of IND in the buffer solution (pH 8.0) exhibited an anodic and corresponding cathodic peak. Experimental results (Figure 1) show well-defined and reproducible anodic and cathodic peaks related to the IND redox system (with E_{pa}= 0.307 V, E_{pc}= 0.250 V vs. SCE and ΔE = 0.057 V). 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 redox couple in IND shows a quasireversible behavior in an aqueous medium at the GCE.

In addition, the effect of the scan rate of the potential on the electrochemical properties of the redox couple in IND was studied in an aqueous solution by cyclic voltammetry (Figure 1). The plots of the anodic and cathodic peak currents were linearly dependent on square root of the sweep rate ($\nu^{1/2}$) at all scan rates (ν= 100-1000 mV s^{-1}) (Figure 1A). This behavior indicates that the nature of redox process is diffusion controlled.

One of the methods to calculate the heterogeneous electron transfer rate constant was given by Nicholson [30]. This method was based on cyclic voltammetry procedure and potential difference between peaks (ΔE_p). They presented a working curve that $n\Delta E_p$ vs. ψ was defined. Also ψ was as follow:

$$\psi = k_s (RT)^{1/2} / (nFD \pi v)^{1/2} \qquad (1)$$

From this working curve for scan rates of 100, 200 and 300 mV s^{-1} the values of ψ can be calculated as 0.263, 0.186 and 0.155. By diffusion coefficient of IND in solution determining by chronoamperometry (D =4.10×10^{-6} cm^2 s^{-1}) and related equation, the mean k_s value was calculated as 2.62×10^{-3} cm s^{-1}.

Effect of pH on the IND at GCE

The electrochemical response of the IND molecule is generally pH dependent. Thus, the electrochemical behavior of the IND at GCE was studied at different pHs using cyclic voltammetry. Anodic and cathodic peak potentials of the IND were shifted to less positive values with increases in pH. A potential- pH diagram was constructed by plotting the peak potential values as a function of pH (Figure 1B). This diagram is composed of a straight line with slope = 44 mV/pH such behavior suggests that it obeys the Nernst equation for a two electron and proton transfer reaction [31].

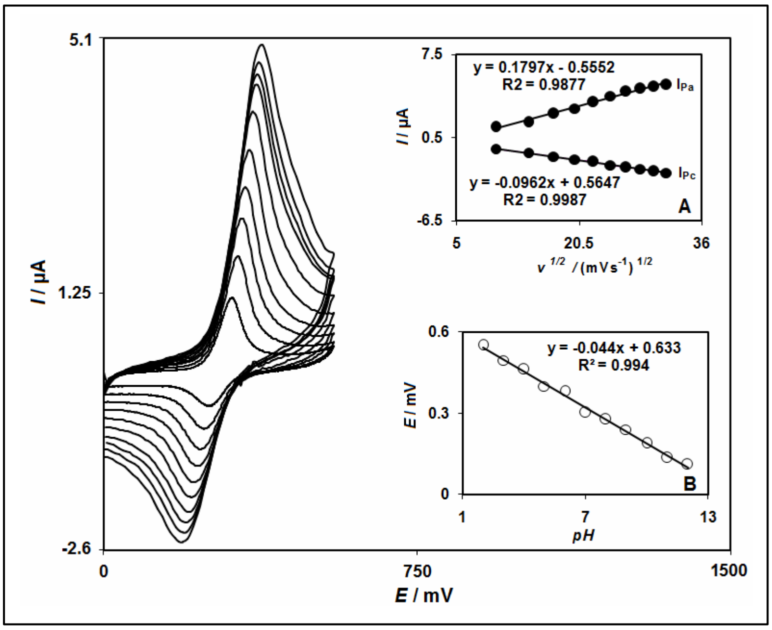

Figure 1. Cyclic voltammograms of GCE in 0.1M phosphate buffer (pH 8.0) containing 0.1 mM IND at various scan rates: 100, 200, 300, 400, 400 500, 600, 700, 800, 900 and 1000 mVs^{-1}. Insets: (A) variation of I_p vs. square root of scan rate, and (B) Effect of pH (2.0-12.0) of 0.1 M phosphate buffer on the peak potential of 0.1 mM IND; where scan rate is 100 mVs^{-1} and the potential range from 0.04 to 0.65 V vs. SCE.

Effect of IND concentration

The influence of IND concentration on the peak currents was studied for the range of 50.0 to 500.0 µM, in the solutions containing 0.1 mM of L-Cys at pH 8.0. The results showed that by increasing the IND concentration up to 100.0 µM, the peak current increased, whereas higher concentration of IND caused a slight decrease in the magnitude of peak current, probably due to the formation of IND aggregates.

Electro-catalytic oxidation of L-Cys

The utility of the modified electrode for oxidation of L-Cys was evaluated by cyclic voltammetry. The cyclic voltammetric responses of an unmodified GCE in 0.1 M phosphate buffer (pH 8.0), without and with 1.0 mM L-Cys in solution, are shown in Figure 2 (curves a and b respectively). INDMGCE cyclic voltammetric responses in 0.1 M phosphate buffer (pH 8.0), without and with 1.0 mM L-Cys in solution, are shown in Fig. 2 (curves c and d respectively). INDMGCE in 0.1 M phosphate buffer (pH 8.0), with 1.0 mM L-Cys in solution showed a large anodic peak (without a cathodic counterpart). That the current observed is associated with L-Cys oxidation and not with the oxidation of IND is demonstrated by comparing the current in Figure 2, curve d with those in Figure 2, curve c, which shows the cyclic voltammetric behavior of an electrode modified with IND in an L-Cys -free electrolyte (0.1 M phosphate buffer with pH 8.0). It is apparent that the anodic current associated with the IND is significantly less than that obtained in the solution containing L-Cys. As can be seen, electrocatalytic activity toward L-Cys on the modified electrode was significant (Figure 2, curves d), with a defined peak potential, around 310 mV vs. SCE. Thus, a decrease in over-potential and enhancement of peak current for L-Cys oxidation are achieved with the modified electrodes. The comparison of curves c and d (Figure 2) shows that the anodic peak current of the mediator was greatly increased in the presence of L-Cys over that ordinarily observed just for the IND redox couple, while the corresponding cathodic peak disappears on the reverse scan of the potential. The results show that L-Cys oxidation at an unmodified GCE occurred irreversibly with a peak potential of 660 mV vs. SCE (pH 8.0), while its oxidation potential at the INDMGCE appeared at 310 mV vs. SCE. The oxidation of L-Cys at the surface of the INDMGCE occurs in neutral pH (pH 8.0) at a potential about 350 mV less positive than that at an unmodified GCE.

The reaction scheme would probably via following mechanistic steps, which L-Cys can be oxidized by oxidized form of IND produced at GCE:

$$(\mathrm{IND})_{\mathrm{red}} \rightarrow (\mathrm{IND})_{\mathrm{ox}} + 2\mathrm{H}^{+} + 2\mathrm{e}^{-} \qquad \mathrm{E} \qquad (2)$$

$$(\mathrm{IND})_{\mathrm{ox}} + (2\text{L-Cys})_{\mathrm{red}} \rightarrow (\mathrm{IND})_{\mathrm{red}} + (2\text{L-Cys})_{\mathrm{ox}} \qquad \mathrm{C}' \qquad (3)$$

The effect of scan rate on the electrocatalytic oxidation of L-Cys at the INDMGCE was investigated by cyclic voltammetry. The oxidation peak potential shifted with increasing scan rates towards a more positive potential, confirming the kinetic limitation of the electrochemical reaction. Also, a plot of peak height (I_p) versus the square root of scan rate ($\upsilon^{1/2}$), in the range of 100-900 mV s^{-1} was constructed (Figure 3A). This plot was found to be linear, suggesting that, at sufficient overpotential, the process was diffusion rather than surface controlled.

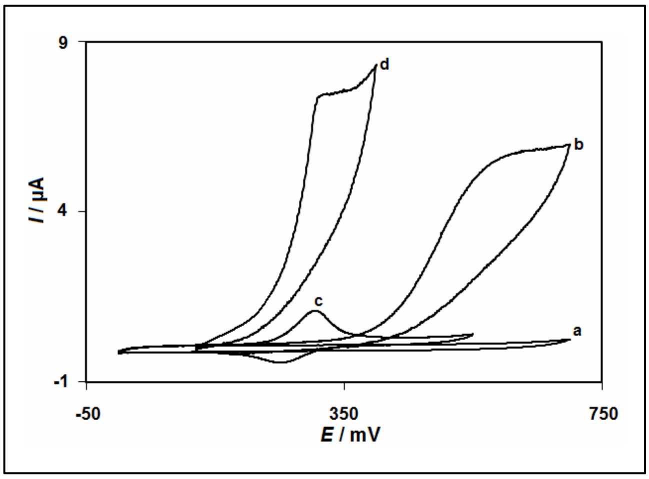

Figure 2. a) Cyclic voltammograms of bare GCE in 0.1 M phosphate buffer solution (pH 8.0) at scan rate 100 mV s^{-1}, b) Cyclic voltammograms of bare GCE in 0.1 M phosphate buffer solution (pH 8.0) with 1.0 mM L-Cys at scan rate 100 mV s^{-1}, c) Cyclic voltammograms in 0.1 M phosphate buffer solution (pH 8.0) at scan rate 100 mV s^{-1} at the INDMGCE and d) Cyclic voltammograms in 0.1 M phosphate buffer solution (pH 8.0) with 1.0 mM L-Cys, at scan rate 100 mV s^{-1} at the INDMGCE.

To obtain information about the rate-determining step, the Tafel plot was drawn (Figure 3B), as derived from points in the Tafel region of the cyclic voltammogram in scan rate of 10 mV s^{-1}. Results of polarization studies for the electrooxidation of L-Cys at a INDMGCE show that a one-electron process was involved in the rate-determining step, assuming an average charge transfer coefficient of $\alpha = 0.41$

The number of electrons in the overall reaction can also be obtained from the slope of the I_p versus $\upsilon^{1/2}$ plot (Figure 3A). Using the slope of this plot and according to the following equation for a totally irreversible diffusion-controlled process [31]:

$$I_p = 2.99\times10^5 \, n \, [(1-\alpha)n_\alpha]^{1/2} \, A \, C^* \, D^{1/2} \, \nu^{1/2} \qquad (4)$$

and considering $(1-\alpha)n_\alpha = 0.59$ it is estimated that the total number of electrons involved in the anodic oxidation of L-Cys is $n = 1.24$.

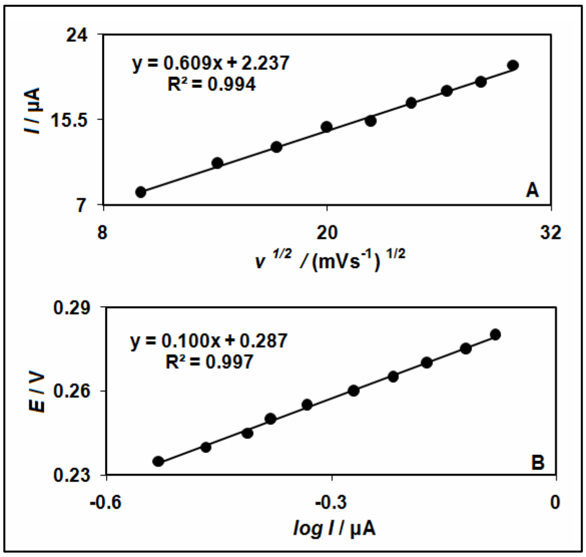

Figure 3. (A) Variation of the anodic peak currents versus $v^{1/2}$ in the presence of 1.0 mM L-Cys. (B) Tafel plot derived from the rising part of the voltammogram recorded at a scan rate of 10 mVs^{-1}

Chronoamperometric measurements

Double potential step chronoamperometry was employed for investigation the electrochemical processes of INDMGCE. The results show very symmetrical chronoamprograms with an equal charge consumed for the oxidation and reduction of the redox couple in IND. The plot of net electrolysis I vs. $t^{-1/2}$ shows a straight line, which extrapolates close to the origin. The slope of the linear region of the I vs. $t^{-1/2}$ plot produces the diffusion coefficient, D, of the IND by using the Cottrell equation [31]:

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2} \qquad (5)$$

Where C_b is the known concentration of IND and D is the diffusion coefficient of IND. Therefore, we calculated $D = 4.1\times10^{-6}$ cm^2 s^{-1} for IND.

The catalytic oxidation of L-Cys by an INDMGCE was also studied by chronoamperometry. Chronoamperometric measurements of different concentrations of L-Cys at INDMGCE were done. The experimental plots of I versus $t^{-1/2}$ with the best fits for different concentrations of L-Cys were employed. The slopes of the resulting straight lines were then plotted versus the L-Cys concentrations (Figure 4A), from whose slope and using the Cottrell equation [32] we calculated a diffusion coefficient of 1.8×10^{-6} cm^2 s^{-1} for L-Cys.

We have also used the chronoamperometric method of Galus to evaluate the catalytic rate constant, k / M^{-1}s^{-1}, for the reaction between L-Cys and the INDMGCE [32]:

$$I_C / I_L = \gamma^{1/2}[\pi^{1/2} \operatorname{erf}(\gamma^{1/2}) + \exp(-\gamma) / \gamma^{1/2}] \qquad (6)$$

Where I_C is the catalytic current of L-Cys at the INDMGCE, I_L the limited current in the absence of L-Cys and $\gamma = kC_bt$ (C_b is the bulk concentration of L-Cys) 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:

$$I_C / I_L = \pi^{1/2} \gamma^{1/2} = \pi^{1/2} (kC_bt)^{1/2} \qquad (7)$$

Where t is the time elapsed. The above equation can be used to calculate the rate constant (k) of the catalytic process. Based on the slope of the I_C / I_L versus $t^{1/2}$ plot (Figure 4 B); k can be obtained for a given L-Cys concentration. Such plots obtained from the Chronoamperograms. From the value of the slope for 0.2 mM L-Cys, the value of k was found to be $k = 5.9 \times 10^{4}$ M^{-1}s^{-1}. The value of k explains as well as the sharp feature of the catalytic peak observed for catalytic oxidation of L-Cys at the INDMGCE.

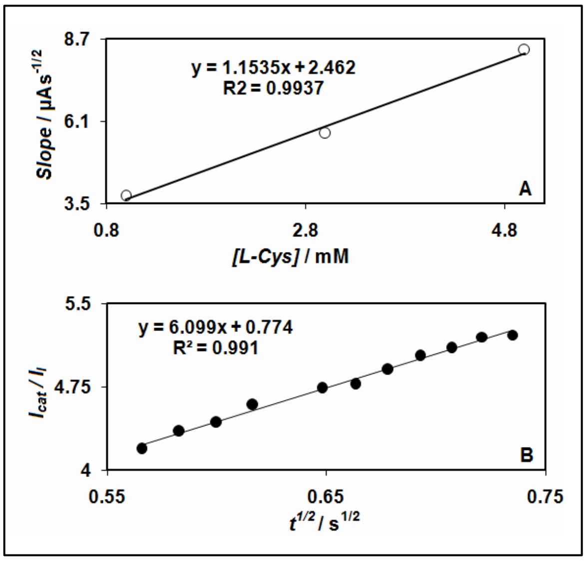

Figure 4. (A) Plot of the slope of the straight lines against the L-Cys concentration obtained from Chronoamperograms in the presence of 1.0, 3.0 and 5.0 mM of L-Cys. (B) Dependence of I_{cat}/I_l on $t^{1/2}$ / $s^{1/2}$ derived from the data of chronoamperograms.

Calibration plot and limit of detection

Cyclic voltammetry was used to determine the concentration of L-Cys (Figure 5). Voltammograms clearly show that the plot of peak current versus L-Cys concentration is constituted of two linear segments with different slopes (slope: 7.826 µA.mM^{-1} for first linear segment and 2.958 µA.mM^{-1} for second linear segment), corresponding to two different ranges of substrate concentration, 0.1- 0.7 mM for first linear segment and 0.7-15.0 mM for second linear segment. The decrease of sensitivity (slope) in the second linear range is likely to be due to kinetic limitation [31].

The detection limit (3σ) for L-Cys in the lower range region was found to be 5.1×10^{-5} M. As can be seen linear correlations don't go through the origin of the plots, because mediator itself has a current. Then, linear correlations don't go through the origin of the plots.

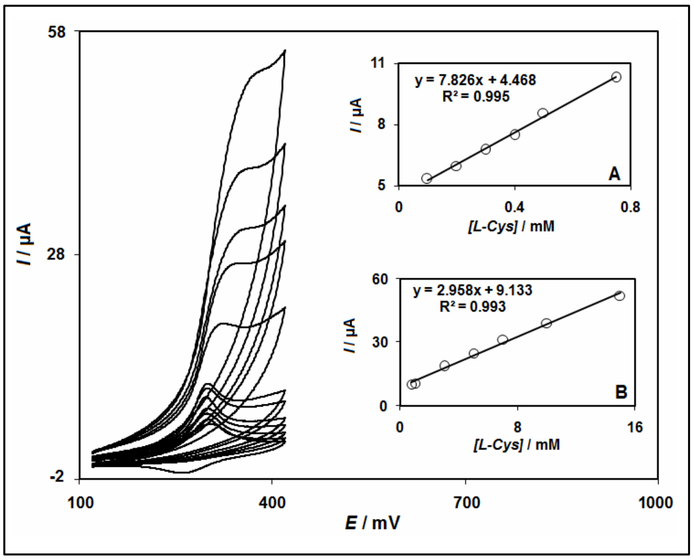

Figure 5. Cyclic voltammograms of INDMGCE in 0.1M phosphate buffer solution (pH 8.0) containing different concentrations of L-Cys (from inner to outer) 0.1, 0.2, 0.3, 0.4, 0.5, 0.75, 1.0, 3.0, 5.0, 7.5, 10.0 and 15.0 mM in 0.1 mM IC respectively. Insets: (A) Plot of the electrocatalytic peak current as a function of L-Cys concentration in the range of 0.1- 0.75 mM and (B) Plot of the electrocatalytic peak current as a function of L-Cys concentration in the range 0.75-15.0 mM.

Conclusions

In the present study, Indigocarmine modified glassy carbon electrode (INDMGCE) was used for the determination of L-Cys. The CV investigations showed effective electrocatalytic activity in lowering the anodic overpotential for L-Cys oxidation. The results show that the oxidation of L-Cys is catalyzed at pH 8.0, whereas the peak potential of L-Cys is shifted by 350 mV to a less positive potential at INDMGCE. The kinetic parameters of the electrocatalytic process and the diffusion coefficients of L-Cys in an aqueous solution were determined. Finally, the electrocatalytic oxidation currents of L-Cys at INDMGCE were linear to concentration of L-Cys.

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