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The Study of Redox Electrode Processes of Sulfur-Containing Compounds by Voltammetry

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Abstract

In this work the general case of quasi-reversible redox electrode processes of thiol compounds proceeding on a mercury-film electrode via a CE (chemical-electrochemical) mechanism has been revealed and investigated by voltammetry. The quantitative criteria for correlation between theory and experiment corresponding to a CE mechanism and new approach for estimating the kinetic parameters of the preceding chemical reaction have been suggested.

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Keywords: compounds; redox chemistry; oxidative stress; methods of experiment design; voltammetry.

1. Introduction

Biologically active sulfur-containing compounds such as glutathione, cysteine, methionine, lipoic acid, containing thiol groups play an important role in the physiological and biochemical processes in living organisms. Determination of sulfur-containing compounds in blood and tissues has a diagnostic value in medicine. It has been found that the concentration of sulfhydryl groups in the serum of patients with pathology of the central nervous system is dependent on the type of diseases (tumors, inflammation) and its activity. The activity of the pathological process with liver diseases (cirrhosis) directly corresponds to a reduction of sulfur-containing compounds in the serum.

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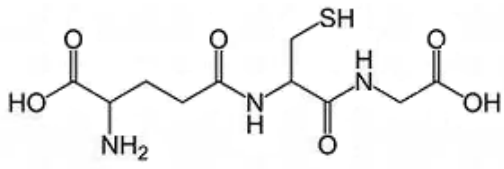
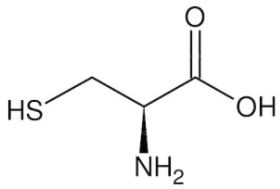
There are many methods for determining the thiol compounds in biological systems. Traditional methods include optical methods, such as colorimetric and fluorimetric[1,2], HPLC[3,4] and some electrochemical methods, such as amperometric and potentiometric titration[5-7].

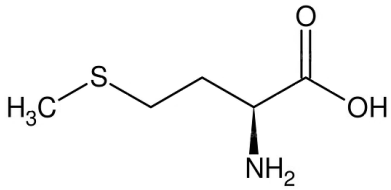
Voltammetry is a convenient approach to the detection and determination of concentrations of biologically active substances. In order to develop the method of determining the total contents of thiol compounds in blood serum, it is necessary to study redox electrode processes of sulfur-containing compounds such as glutathione, cysteine, methionine, lipoic acid (Figure 1). There are a lot of works about mechanisms of electrochemical properties of thiol compounds, including glutathione on a mercury dropping electrode[8-9]. However, they contain large amounts of controversy regarding the mechanism of oxidation of thiol groups on a mercury-containing electrode. First of all, the question concerns the existence of a previous chemical stage (formation of a chemical compound between mercury and thiol groups), identification of the limiting stage and the number of electrons involved in the electrode process. For these aims an effective and convenient voltammetric approach has been applied. High specificity and sensitivity, at low costs, allows voltammetry to compete with traditional methods.



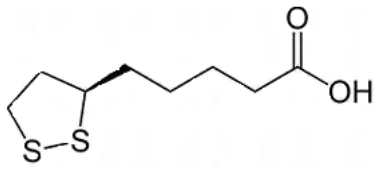
glutathione



cysteine



methionine



lipoic acid

Fig. 1. Scheme of the thiol compounds structures

2. Materials and methods

2.1. Chemicals and reagents

Glutathione was supplied by Sigma (Germany). Investigation solution of 0.28 mmol/dm^3 glutathione was prepared in water. Borate buffer (0.01 mol/dm^3 $Na_2B_4O_7$, pH 9.18) was used as a supporting electrolyte. Nanopure water was used for making solutions. The supporting electrolyte being analyzed had been deoxygenated by passing nitrogen for 10 min prior measurements.

2.2. Instrumentations

A voltammetric analyzer TA–2 (Tomanalyt, Tomsk, Russia) in connection with PC was used in this work. Voltammetric curves were recorded at a room temperature in a three-electrode electrochemical cell connecting to the analyzer. A working mercury-film electrode, silver-silver chloride electrodes with KCl saturated ($Ag|AgCl|KCl_{sat}$) were used as reference and counter electrodes. The thermostatic cell was maintained at 25.0 ± 0.5°C. pH was measured using a digital pH- meter model M64 (Belorussia).

2.3.Voltametric measurement

A volume of 10 cm^3 of borate buffer was placed in the electrochemical cell. The measurement involved the recording of cyclic voltammograms both with the investigated substance under the following conditions: glutathione in concentration of 2.8×10^{-4} mol/dm^3, potential range E = -0.4 ÷ +0.2 V, potential scan rate range of 30 ÷ 250 mV/s and without it. After the substance addition, the solution was stirred for about 20 s. After the stirring was stopped, the potential was scanned negatively.

3. Results and discussion

Voltammetry allows researching the redox process of tiol groups on a mercury film electrode. It has been suggested that the complex, which is oxidized or reduced on the mercury film electrode, previously adsorbed on the electrode surface and the common process of this interaction can be described as following:

$$A \underset{k_1}{\overset{k}{\rightleftharpoons}} Ox+ne \underset{k_{1S}}{\overset{k_S}{\rightleftharpoons}} Red \qquad (1)$$

The existence of a preceding chemical stage of the complex formation on the surface of the mercury-film electrode is supported by the following experimental data:

1) Potential of maximum reduction current of glutathione is shifted to more positive potentials while the potential scan rate (*W*) increasing;

2) Potential of maximum reduction current of glutathione is shifted to ΔE = 60/n (mV) while the potential scan rate (*W*) changing to ten times;

3) Potential of maximum reduction current of glutathione is linear at a logarithm square root of potential scan rate ($\log W^{1/2}$).

The dependence of the reduction peak current of glutathione on the square root of potential scan rate ($W^{1/2}$) on a mercury-film electrode is not linear at a potential scan rate range of 30 ÷ 150 mV/s. It indicates that the reduction of glutathione is a quasi-reversible process.

Thus, it is suggested that the redox process of glutathione on MFE is a quasi-reversible process with a preceding chemical stage of the complex $Hg(GS)_2$ formation. It can be described by the following CE mechanism of this interaction:

$$Hg^{2+} + 2\,GS^- \rightleftharpoons Hg(GS)_2(ad)$$

$$Hg(GS)_2(ad) + 2H^+ + 2e^- \rightleftharpoons Hg^0 + 2GSH$$

In the limiting case of semi-infinite diffusion the boundary problem for the scheme (1) on MFE can be described by the following equations:

$$\frac{\partial C_A(x,t)}{\partial t} = D_A \frac{\partial^2 C_A(x,t)}{\partial x^2} - kC_A(x,t), \qquad 1 < x < \infty$$

$$\frac{\partial C_{Ox}(x,t)}{\partial t} = D_{Ox} \frac{\partial^2 C_{Ox}(x,t)}{\partial x^2} + kC_A(x,t), \quad 1 < x < \infty$$

The boundary conditions can be estimated by the following equations:

$$\text{at } t=0, \quad x \geq 0, \quad \frac{C_{Ox}(x,0)}{C_A(x,0)} = \frac{k}{k_1} = K, \quad C_{Red}=0, \quad C_{Ox}(x,0) + C_A(x,0) = C^o,$$

$$\text{at } t>0, \quad x \to \infty, \quad C_{Ox}(x,t) + C_A(x,t) \to C^o \quad \frac{C_{Ox}(x,t)}{C_A(x,t)} = K = \frac{k}{k_1} \to \infty, \quad C_{Red} \to 0,$$

$$D_A\left[\frac{\partial C_A(x,t)}{\partial x}\right]_{x=l} = - D_{Ox}\left[\frac{\partial C_{Ox}(x,t)}{\partial x}\right]_{x=l},$$

$$\text{at } t>0, \quad x=l, \quad D_{Ox}\frac{\partial C_{Ox}}{\partial x} = kC_{Ox} = k_i C_{Ox}\exp(bt),$$

$$\text{where } b = \frac{an_aFV}{RT}, \quad k_i = k_s \exp\left[(-\frac{an_aF}{RT})(E_i - E^o)\right]$$

This problem has been solved by the method of numerical simulation using the programming language «Fortran 90» with the following assumptions:

1. The preceding chemical and electrochemical stages proceed in the forward direction
2. Equilibrium constant of the preceding chemical reaction corresponds to the irreversible process.
3. D_A and D_{Ox} are equal.
4. Oxidized substance (Ox) appears on the electrode surface only through preceding chemical stages, and not from the bulk solution.

Theoretical voltammograms of the oxidation process of glutathione on MFE at different potential scan rate (30 ÷ 100 mV/s) were obtained while solving the boundary problem. The theoretical voltammograms were compared with the experimental data (Figure 2).

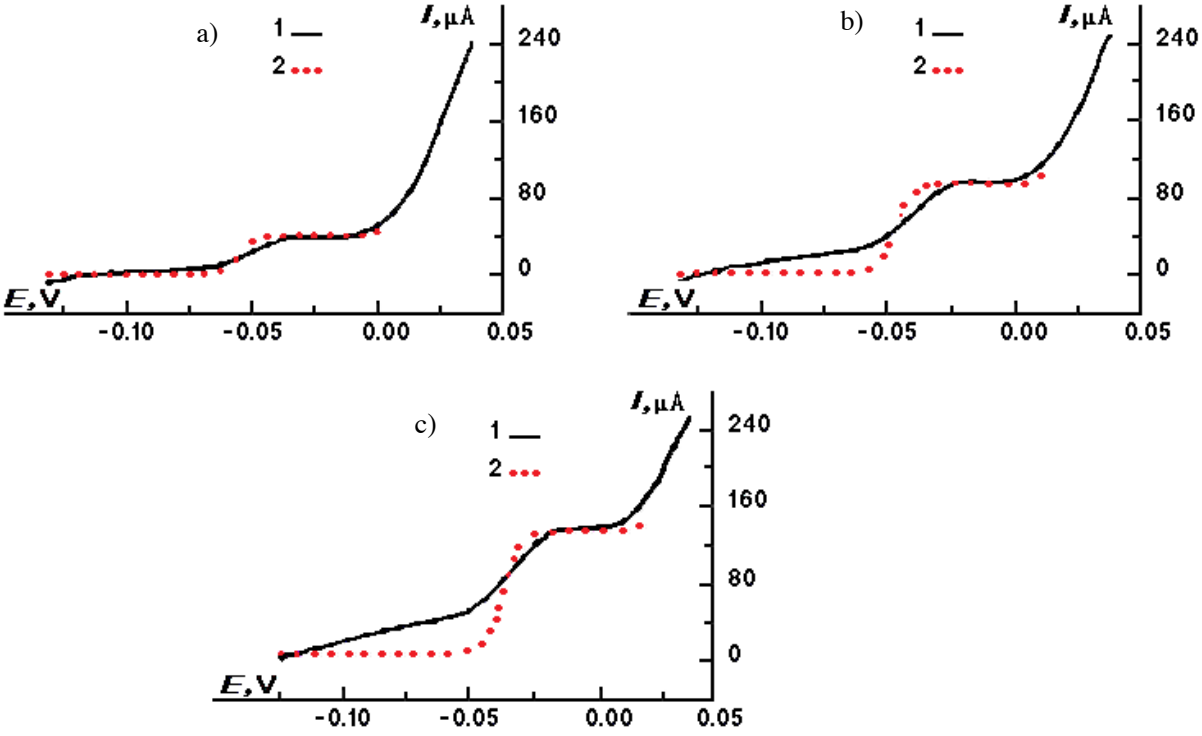


Fig. 2. The experimental (1) and theoretical (2) voltammograms of the oxidation process on MFE in borate buffer (0.01 mol/dm^3 $Na_2B_4O_7$, pH 9.18) with 0.28 mmol/dm^3 of glutathione at potential scan rate: (a) 30 mV/s; (b) 60 mV/s; (c) 100 mV/s

As can be seen from Figure 2, the discrepancy between the experimental and theoretical results is minor, especially in case of low scan rate. The data obtained indirectly confirm CE mechanism of the process.

The methods of experiment design were used to assess the adequacy of the mathematical models. Firstly, the coefficient of determination (D) was calculated by the difference between the points on the theoretical and experimental curves:

$$D = r^2 = 1 - \frac{\sum_{i=1}^{N}(y_{\exp} - y_{teor})^2}{\sum_{i=1}^{N}(y_{\exp} - y_{teor})^2 + \sum_{i=1}^{N}(\bar{y}_{\exp} - y_{teor})^2}$$

where y_{exp} is a response function in the point on the experimental curve, y_{teor} is a response function in the point on the theoretical curve, N is a number of points on the experimental and theoretical curves.

Calculated coefficients of determination for different values of the potential scan rate are shown in Table 1. The coefficient of determination tendency confirms that obtained mathematical models adequately describe the mechanism of the process, especially for small values of the potential scan rate.

Also, a Fisher's criterion was used for the adequacy hypothesis check of a mathematical model in points around theoretical and experimental potential of maximum oxidation current of glutathione.

$$F = \frac{S_{ad}^2}{S_y^2} \qquad S_y^2 = \frac{\sum_{i=1}^{N} S_i^2}{N} \qquad S^2{}_{ad} = \frac{\sum_{i=1}^{N} \sigma^2{}_x}{N-k} \qquad \sigma^2{}_x = (\overline{Y} - Y^*)^2$$

where $\overline{Y}$ is an experimental result, Y^* is a calculated result according to the obtained mathematical model, N is a number of the experiments, $S_y{}^2$ is dispersion of reproducibility, $S_i{}^2$ is interlinear dispersion.

The experimental voltammograms of glutathione oxidation in the same experimental conditions (parallel experiment) were recorded to assess the dispersion of reproducibility.

The variances of homogeneity were assessed by a Cochran test. Estimated values of the Fisher's criterion for different scan rate are shown in Table 1. From the obtained data, the discrepancy between theoretical and experimental values is the smallest at low speeds of potential sweep. Thus, the model is adequate for the scan rate of 30mV/s. This confirms the suggested scheme of the process with the presence of a previous chemical reaction with a formation of an intermediate complex.

Table 1. Coefficient of determination and Fisher's criterion for different scan rate

W (mV/s)	D	Fisher's criterion	
		F_{exp}	F_{theor}
30	0.964	2.32	2.6
60	0.921	4.71	2.6
100	0.917	5.03	2.6

The deviations of the mathematical model from the experimental values of the glutathione reduction current at high speeds potential sweep can be attributed to the large capacitive component of the current and the limitations of the diffusion process. The rate constant preceding chemical reaction of the intermediate complex (k, s^{-1}) and formal potential of the process (E_o, V) have been determined (Table 2), based on the obtained results and initial parameters of the modifier layer thickness (L), the diffusion coefficient of the depolarizer (D_{ox}), the equilibrium constant for the preceding chemical reaction (K). Also, the rate constant of the limiting stage of the electrode process (k_s, cm/s) has been determined. Its value indicates a quasi-reversible electrode process, which coincides with the experimental data.

The obtained results indicate that under these experimental conditions the chemical reaction proceeds at a higher rate than electrochemical one, which limits the overall process. The formal process potential E_o has a thermodynamic value.

Table 2. Theoretical kinetic parameters of the process

Initial parameters				
L (cm)	D_{ox} (cm^2/s)	C_0 (mmol/dm^3)	S (cm^2)	K
0.8	$1 \cdot 10^{-5}$	0.00028	0.196	10^{10}
N	E_0, V	k_s, cm/s	k, s^{-1}	D
W = 30 mV/s				
10	1.22	$1\ 10^{-3}$	3.26	0.96
W = 60 mV/s				
10	1.22	$1\ 10^{-3}$	6.8	0.79
W = 100 mV/s				
10	1.22	$1\ 10^{-3}$	65	0.65

4. Conclusions

It has been found that the process of glutathione electroreduction proceeds through the formation of a complex with mercury in a quasi-reversible process.

Mathematical modeling of the glutathione electrochemical reduction in the simulated solution allowed indirect confirming the existence of a preceding chemical reaction.

The kinetic and thermodynamic parameters of the process have been calculated. It has been shown that the mathematical model adequately describes the process at low scan rate. The electrochemical properties of glutathione can be used to develop a methodology for determining the thiol compounds in human serum using the voltammetry method, according to the obtained mathematical model of the thiol compounds electroreduction process in simulated solutions.

Acknowledgements

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