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ORIGINAL PAPER

Voltammetric determination of cysteine at a graphite electrode modified with gold nanoparticles

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Abstract The electrochemical behaviour of cysteine (Cys) at a graphite electrode modified with gold nanoparticles (G-Au_{nano} electrode) was studied by cyclic voltammetry. It was found that the graphite electrode-Au nanoparticles show an electrocatalytic activity towards the oxidation of Cys in 0.1 M NaOH. At 0.05 V, there is an "inverse" maximum in the cathodic voltammogram of Cys. Using a G-Au_{nano} electrode, the dependence of the peak current of the "inverse" maximum on Cys concentration was linear in the range from 1 to 14 pM, and the detection limit was 0.6 pM. The proposed analytical method is simple, rapid and sensitive.

Keywords Cyclic voltammetry · Electrocatalysis · Cysteine · Gold nanoparticles

Introduction

Cysteine (Cys) is a sulphur-containing amino acid which plays an important role in cellular functions, such as protein synthesis, detoxification and metabolism [1]. Changing the content of cysteine in the biochemical cycles of living organisms leads to various pathological processes, nervous, mental, and other diseases. Therefore, it is necessary to control the Cys content in pharmaceuticals, urine, serum, and blood plasma. The development of highly sensitive methods for the determination of cysteine in different biological matrices is an important task of analytical chemistry. Traditionally, the determination of cysteine in clinical investigations is carried out by chromatography [2], optical methods [3–6], capillary electrophoresis [7] and enzyme-linked immunosorbent assays [8]. Disadvantages of these methods are the long time of sample preparation and analysis and high costs of the equipments. At the same time, electrochemical methods are essentially simpler and cheaper than most of the other methods used in biochemistry, molecular biology, and medical diagnostics [9]. The main limitation of the use of electroanalytical methods in biochemical analysis is a non-selectivity with respect to the substance to be determined. The components are generally found in low concentration in the presence of a wide range of substances that may obscure the results of analysis or the presence of masking amino acids. The use of mercury electrodes allows the determination of Cys with a detection limit of 1 μM [10]. However, the toxicity of mercury compounds does not allow the use of this electrode in laboratory practice. Solid electrodes are used to expand the opportunities of voltammetric analysis of amino acids. However, the use of solid electrodes also does not allow the decrease of the detection limits of Cys due to the adsorption of the products of electrochemical oxidation of amino acids on the surface of the electrode. The detection limit of the voltammetric determination of Cys at a platinum electrode is 9.3 μM [11]. The detection limit of Cys at a diamond electrode [12] and a gold electrode [13] is 1 μM. Modifying the surface of carbon-containing electrodes by noble metals and their oxides allows the increase of the sensitivity of the voltammetric determination of Cys. The detection limit of Cys at a carbon paste electrode modified with ruthenium oxide is 1 μM [14].

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The detection limit of Cys at a glassy carbon electrode modified with the binary system gold–iridium is 0.05 μM [15]. Due to their specific properties, the use of nano-sized metal particles as modifiers can decrease the limit of detection of organic substances [16]. The purposes of this work are to investigate the electrochemical behaviour of Cys in aqueous solution at a G-Au_{nano} electrode by cyclic voltammetry and to propose a sensitive method of voltammetric determination of Cys in aqueous solutions.

Experimental part

Reagents and chemicals

All chemicals used were of analytical grade; solutions were prepared in double-distilled water. Cys (A_1) was prepared immediately before the measurements.

Preparation of gold nanoparticles

Gold nanoparticles were prepared by reduction of $HAuCl_4$ with $NaBH_4$ and sodium citrate according to the following technique: 0.2 ml of 0.005 M $HAuCl_4$ and 3 ml of 0.05 M of citrate sodium were placed in a beaker and then filled up to 100 ml with water. Further, a solution of $NaBH_4$ was prepared by dissolving 0.03 g in 10 ml of ice water. The obtained solution of borohydride was added dropwise to the gold solution until the appearance of a pink–red colour. This method allows obtaining gold nanoparticles with a particle diameter of 3–12 nm.

Instruments

Cyclic voltammetric measurements were carried out with the voltammetric analyzer TA-4 (Tomsk) with a two-electrode cell having the working electrode and a Ag/AgCl/KCl reference electrode at room temperature. The working electrodes were either an unmodified graphite electrode (GE), a gold electrode or the G-Au_{nano} electrode. pH values were measured with a PH-150 instrument. The morphology of the gold nanoparticles was characterized by a scanning electron microscope (JSM-5500).

Preparation of the G-Au_{nano} electrode

Cleaning the surface of the carbon electrode was carried out mechanically by a dense filter paper, followed by electrochemical cleaning. Modification of the surface of the graphite electrode was carried out as follows: The electrode was placed in an electrochemical cell filled with 10 ml of 0.1-M HCl solution with the addition of 0.1 ml of gold sol prepared as described above. The gold nanoparticles were electrochemically deposited on the surface of the graphite electrode at the accumulation potential E_{acc}=−1.0 V during the accumulation time t_{acc}=300 s. Then, the electrode was removed from the solution, rinsed with double-distilled water and placed in the background electrolyte 0.1 M NaOH for the voltammetric determination of Cys.

Analytical procedures

A volume of 10 ml of the supporting electrolyte (0.1 M NaOH) was placed in the electrode cell. The voltammograms of the supporting electrolyte NaOH were recorded by CV under the following conditions: scan rate, 0.100 V s^{-1}; potential range E=−0.4 to 0.3 V. Then, 20 μl of a Cys solution of unknown concentration was added to the solution. The obtained solution was stirred for 1 min, and then, the cyclic voltammogram of Cys was recorded. Further, 20 μl of a Cys standard solution of known concentration was added to the solution. The obtained solution was stirred with the help of a magnetic stirrer for 1 min, and another cyclic voltammogram was recorded. Every voltammogram was recorded two times. After the measurements, the evaluation was performed as usual for the standard addition method.

Results and discussion

It is known that Cys exhibits an electrochemical signal at the GE at a concentration of 5 mM [15]. On a pure gold electrode, Cys shows an electrochemical signal when the concentration is at least 10 μM [13]. This paper is devoted to the investigation of the behaviour of Cys at the G-Au_{nano} electrode. Figure 1 depicts the gold nanoparticles which possess a spherical shape. The average size of gold

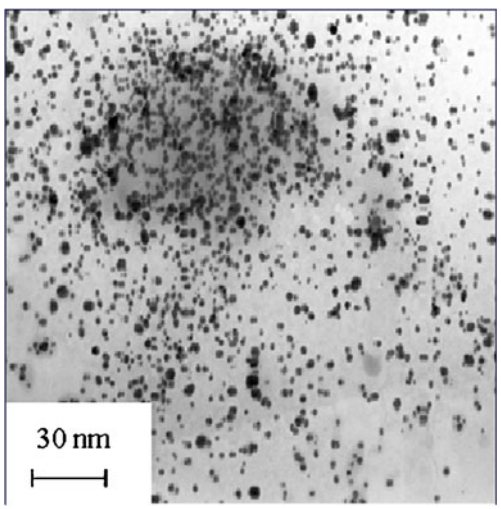

Fig. 1 SEM image of gold nanoparticles

nanoparticles is 5–15 nm. The size of the gold particles depends on the nature of the reducing agent and the conditions of formation and storage [17]. This is similar to the case of silver nanoparticles [18]. For the purpose of comparison, the electrochemical behaviour of Cys was studied on graphite and gold electrodes (Fig. 2).

At a GE, Cys does not show an electrochemical signal at a concentration of 0.01 μM in 0.1 M NaOH. (curve b′b″, Fig. 2). However, Cys shows at that concentration a signal at the gold electrode. With further addition of Cys, the signal does not grow anymore due to the adsorption of Cys at the gold electrode. This observation is consistent with literature data [19].

When scanning in the range from −0.3 to 0.4 V, there is a current maximum at E_a=0.15 V on the anodic branch at the gold electrode (curve c′, Fig. 2). The appearance of this maximum is associated with the formation of Au_2O_3 on the electrode surface [20, 21]. On the anodic branch of the cyclic voltammogram at the G-Au_{nano} electrode (curve a′, Fig. 3), the potential of that maximum is shifted to 0.15 V, i.e. to more negative potentials compared with the gold electrode where it is situated at E_a=0.0 V.

There is a maximum at E_c=−0.15 V on the cathodic branch of the cyclic voltammogram when scanning in the range from 0.4 to −0.3 V; there is a maximum at the gold electrode in supporting electrolyte 0.1 M NaOH (Fig. 2, curve d″). The appearance of the cathodic maximum may be due to the transition of a freshly hydrated oxide of gold $Au_2O_3{\cdot}nH_2O$ to an adsorbed monolayer of $Au_2O{\cdot}nH_2O$ in an alkaline solution [22, 23]. On the cathodic branch, the cyclic voltammogram at the G-Au_{nano} electrode, the potential maximum, is shifted to more positive potentials (curve a″, Fig. 3) at 0.15 V.

At the G-Au_{nano} electrode, the offset of potential in the region of positive potentials may be due to the oxidation of gold nanoparticles (Fig. 2) in the alkaline medium, which leads to a decrease in activation energy, which is consistent with published data [16]. As can be seen from Fig. 2, curve c ′, to Fig. 3, curve a′, the maxima on the anodic branches of the cyclic voltammograms obtained on the G-Au_{nano} electrode and the gold electrode can be described by the following schemes [20, 21]:

$$2Au + 6OH^- \leftrightarrows Au_2O_3 + 3H_2O + 6e \quad (1)$$

The maximum on the cathode branch of cyclic curve (Fig. 2a″) at the gold electrode is described by the scheme of the process [22, 23]:

$$Au_2O_3 + 6OH^- \leftrightarrows Au_2O + 2O_2 + 4e \quad (2)$$

There are two maxima on the cathode branch at the G-Au_{nano} electrode (Fig. 4, curve b″). The first maximum corresponds to the cathode scheme (2). The second

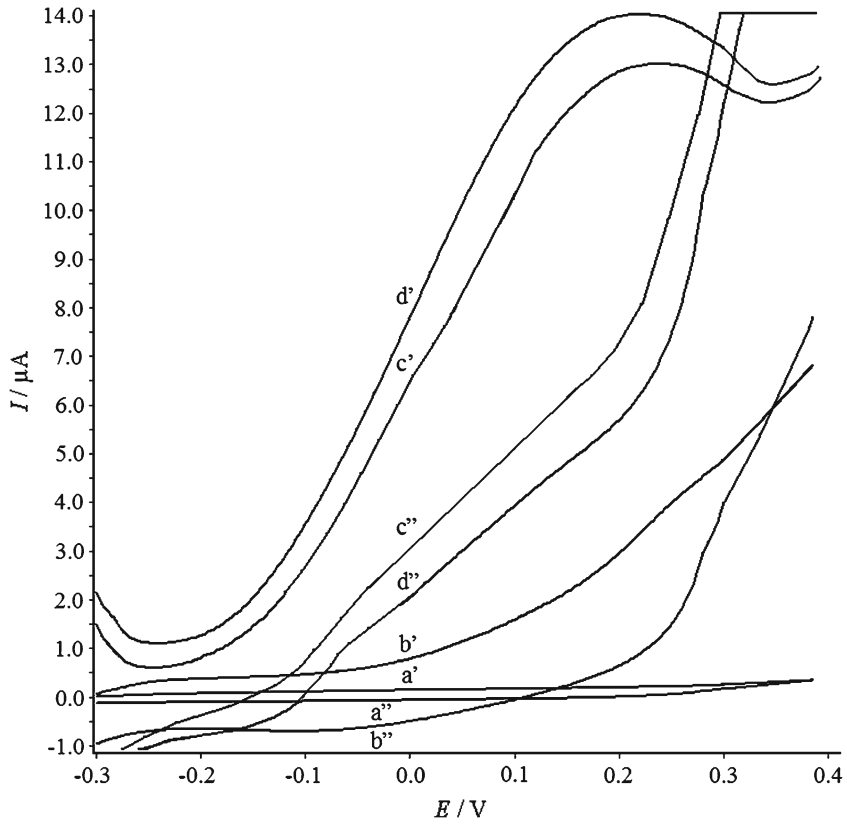

Fig. 2 Cyclic voltammograms of Cys in a solution of the background electrolyte 0.1 M NaOH, sweep rate 0.1 V s^{-1} at GE: *a′a″* 0 pM Cys, *b′b″* 2 pM Cys; at the gold electrode, *c′c″* 0 pM Cys, *d′d″* 2 pM Cys

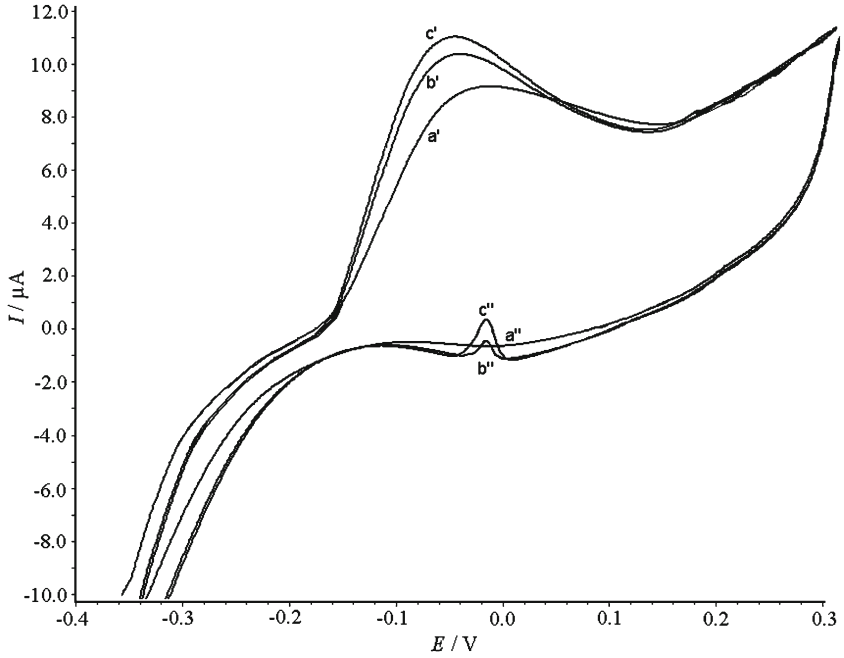

Fig. 3 Cyclic voltammograms of Cys at the G-Au_{nano} electrode: 0 pM (*a′a″*) and 2 pM (*b′ b″*), 4 pM (*c′c″*) Cys. Conditions: supporting electrolyte, 0.1 M NaOH; scan rate, 0.1 V s^{-1}

maximum corresponds to the oxidation of oxide gold (I) to oxide gold (III) in alkaline medium [22, 23] scheme (3):

$$Au_2O + 4OH^- \leftrightarrows Au_2O_3 + 2H_2O + 4e \quad (3)$$

The process (3) takes place in a cyclic mode when scanning in the potential range from 0.8 to −0.4 V at the G-Au_{nano} electrode in the solution of supporting electrolyte 0.1 M NaOH (Fig. 4).

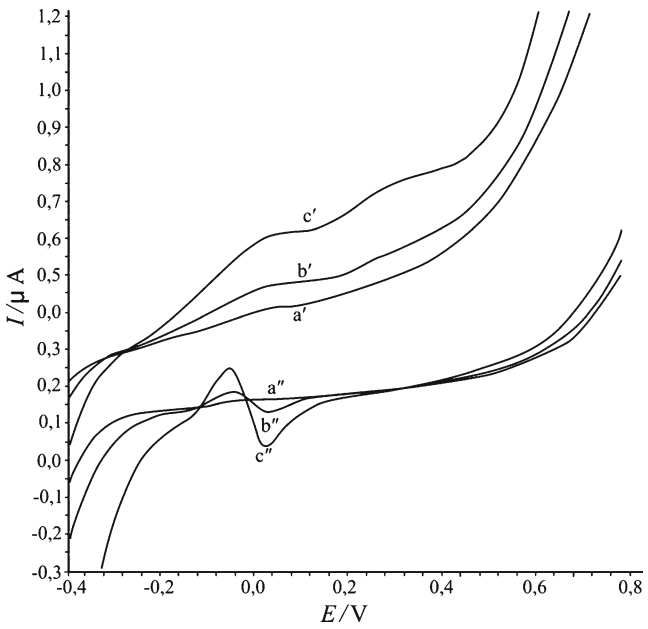

Fig. 4 Cyclic voltammograms of Cys at the G-Au_{nano} electrode: 0 pM (*a′a″*), 2 pM (*b′b″*) and 4 pM (*c′c″*). Conditions: supporting electrolyte, 0.1 M NaOH; scan rate, 0.1 V s^{-1}; in the range of potential from −0.4 to 0.8 V

In the presence of 2 pM Cys at the G-Au_{nano} electrode, a maximum is observed at E_a=−0.05 V on the anodic branch of curve b′ (Fig. 4). The height of the maximum of Cys oxidation at the G-Au_{nano} electrode is increased by 1.5–2 times compared to the height of maximum corresponding to the oxidation of the modifier.

In the presence of 2 pM Cys, there is an "inverse" maximum observed at a potential E_c=0.0 V on the cathodic branch of curve b″ at the G-Au_{nano} electrode (Fig. 4, b″). Cys does not exhibit electrochemical signals at the GE and gold electrode at a concentration of 5 mM in agreement with published data [13, 15]. The "inverse" maximum at the gold electrode was not obtained.

The cause of the appearance of an "inverse" peak at the G-Au_{nano} electrode can be associated with a gold oxide from lower to higher oxidation states (e.g. Au_2O to Au_2O_3), similar to the process of oxidation of Ag→AgO in alkaline solution [24] or process of electroreduction of oxygen, catalyzed by the gold deposition on the electrode surface [25]. A nonlinear increase of the anodic maximum of Cys is observed in the presence of 4 pM Cys at the G-Au_{nano} electrode (Fig. 3, curve c′). With further increase in the concentration of Cys, the analytical signal does not change, which is caused by the adsorption of Cys on the electrode surface.

The process taking place at the G-Au_{nano} electrode in the presence of Cys can be referred to electrocatalysis with a contribution of adsorption to the process of Cys electrooxidation and regeneration of the initial form of the catalyst:

$$6RSH + Au_2O_3 \leftrightarrows 3RSSR + 2Au + 3H_2O \quad (4)$$

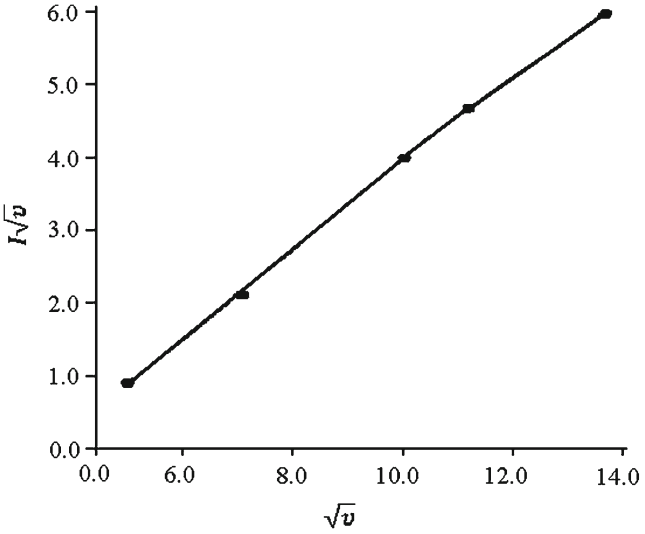

Fig. 5 Dependence of the maximum current of Cys against scan rate at GE the G-Au_{nano} electrode. C_{Cys}=4 pM

The positive slope of the dependence of $I\sqrt{v} - \sqrt{v}$ indicates a contribution of adsorption to the process of electrocatalytic oxidation of Cys at the G-Au_{nano} electrode (Fig. 5).

At concentrations of Cys of 2 and 4 pM at the G-Au_{nano} electrode, an "inverse" maximum is observed at E_c=0.0 V on the cathode branches of cyclic curves b″ and c″ (Fig. 3). With further increase in the concentration of Cys, the maximum of the "inverse" current is linearly dependent on the concentration of Cys. This dependence is linear in the range of concentration from 2 to 14 pM at the G-Au_{nano} electrode (Fig. 6). The linear regression equation of Cys can be expressed as I_p (in microamperes)=0.1946C (in micromolars)+1.0607. The detection limit of Cys is 0.6 pM.

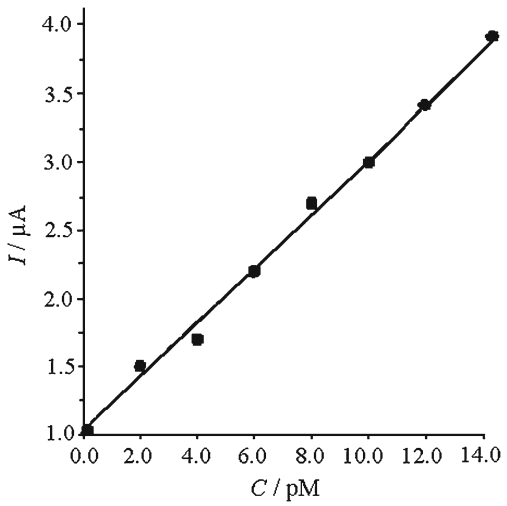

Fig. 6 Dependence of the maximum current against concentration of Cys at the G-Au_{nano} electrode in 0.1 M NaOH

Conclusion

In this work, the G-Au_{nano} electrode is shown to exhibit an electrocatalytic activity towards the oxidation of Cys in 0.1 M NaOH. For the first time, the voltammetric determination of Cys at a G-Au_{nano} electrode is proposed using 0.1 M NaOH as supporting electrolyte. There is an "inverse" maximum on the cathode branch of the cyclic voltammograms at the G-Au_{nano} electrode. The current of this maximum depends on the Cys concentration. A linear dependence of the current of the "inverse maximum" on the cathodic branch of the cyclic voltammograms on Cys concentration is observed in the range from 1 to 14 pM, and the detection limit is 0.6 pM. The proposed method is simple, sensitive and does not need toxic substances to be used.

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