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Determination of Cystein in Biology Solids by Electrochemical Methods with Gold Colloidal Particles

[1]Eduard V. Gorchakov, [2]Dariya O. Perevezentseva, [1]Natalya V. Fedota, [1]Valeriy A. Belyaev and [1]Bagama M. Bagamaev

[1]Stavropol State Agrarian University, Zootechnical Lane, 12, 355017, Stavropol, Russian Federation
[2]NR Tomsk Polytechnic University, Lenina str 30, 634050, Tomsk, Russian Federation

Abstract: The electrochemical behavior of cysteine at a graphite electrodes modified with gold nanoparticles was studied by cyclic voltammetry. We compared the results of determination of cysteine in the particulate, graphite and glasgraphite electrodes modified with gold nanoparticles and single crystals of gold. Specific feature of the electrooxidation of cystein at a graphite electrodes modified with gold nanoparticles and at a graphite electrodes modified from solution $HAuCl_4$. It was found that graphite electrodes modified with gold nanoparticles have more electrocatalytic activity toward the oxidation of cysteine than single crystals of gold due to the increase in the number of atoms on the surface of the graphite electrode in comparison with single crystals of gold, which catalyze the oxidation process of cystein. The use of graphite electrode modified with gold nanoparticles reduces the detection limit for cystein under to $1 \cdot 10^{-12}$ M. The method for determination of cysteine in biological samples.

Key words: Cystein · Graphite electrode · Particulate electrode · Glassgraphite electrode · Gold nanoparticles · Gold single crystal · Voltammetry · Biological fluid · Fraction of blood

INTRODUCTION

Cysteine is a sulfur-containing interchangeable amino acid, involved in the metabolic processes in living organisms. Violation of cysteine concentration grade in the biochemical cycles of living organisms gives rise to various pathological processes, as well as nervous, psychological and other diseases. In this regard the development of highly sensitive methods for detecting cysteine in various bioobjects is an important objective of biological chemistry along with the methods of analytical chemistry [1-4].

The aim of this work was to study the possibility of using different types of graphite electrodes, modified with gold nanoparticles for detection of cysteine, as well as development of delicate electrochemical method (in particular, the voltammetric technique) for detection of cysteine in the aqueous solutions and the capability of cysteine detection in biologic fluids (blood). Carbon-black electrode (CBE-Au-$_{nano}$), graphite electrode, (GE-Au-$_{nano}$) and glassy-carbon electrode (GCE-Au-$_{nano}$), each modified with gold nanoparticles, were used in the current research. Lately, electrochemical analysis methods are increasingly used for the detection of cysteine due to their simplicity, quick-responsibility, sensitivity and low production cost of the required equipment. Electrochemical detection of cysteine on electrodes, modified with various types of dopes, is given increasingly greater preference [5-9]. This is due to the fact that nanoscale metal particles exhibit specific properties as a modifier to different types of substances and to cysteine in particular. Modification of electrode surfaces allows one to enhance the sensitivity of the method [10]. Therefore, modification of carbon-contained electrode surface by metal nanoparticles is not only a relevant objective, but a recent trend in electrochemistry.

Materials and research technique. All reagents used were of analytical grade and solutions were prepared based on double-distilled water. A cysteine solution was prepared immediately prior to measurements.

Corresponding Author: Gorchakov, Stavropol State Agrarian University, Zootechnical Lane, 12, 355017, Stavropol, Russian Federation.

Electrochemical signals were recorded with a voltammetric analyzer TA-4 (JSC"TomAnalyt", Tomsk). Graphite electrodes, modified with gold nanoparticles, CBE-Au-$_{nano}$, GE-Au-$_{nano}$ and GCE-Au-$_{nano}$ were used as working electrodes in a double-electrode cell, as well as the graphite electrode modified with gold particles from the $HAuCl_4$ solution with concentration of 10 mg/l (GE-Au). Voltammetric measurements were carried out against the background solution of 0.1 M NaOH.

Modification of graphite electrode surface was performed based on technique described in [11].

Solutions of biological fluids were prepared by fractionation of blood plasma, dissociation and separation of the protein fraction with its subsequent dilution to the concentration required for detection of cysteine.

RESULTS AND DISCUSSION

Gold colloidal solutions or sols are complex systems consisting of spherical particles, composed of oxidized or reduced forms of the metal. The size of the gold nanoparticles, used to modify the electrode surface, is 2.5 nm [12].

Voltammetric curves of cysteine were earlier obtained for GE-Au and GE-Au-$_{nano}$ in the background electrolyte solution of 0.1 M NaOH [11].

Cysteine is nonelectroactive with graphite electrodes in solution of 0.1 M NaOH [12]. The cysteine oxidation pick height on GE-Au is observed in solution with a concentration of $1\cdot10^{-8}$ M and increases 1.5-fold as compared with the oxidation pick height, corresponding to the modifier oxidation. The cysteine is electrochemically active on GE-Au-$_{nano}$ at concentration of $2\cdot10^{-12}$ M. This fact is associated with the increase of active sites on the surface of the GE-Au-$_{nano}$ as compared to single crystals of gold, catalyzing the cysteine oxidation process.

On cathodic branch of the cyclic curve, cathodic maximum is observed at the potential E_c = –0.14 V on the GE-Au. The appearance of the cathodic maximum can be caused by a freshly hydrated gold oxide $Au_2O_3\ nH_2O$ into the adsorbed monolayer of $Au_2O\ nH_2O$ [13] in an alkaline medium. On GE-Au-$_{nano}$ maximum is observed at a potential E_c = 0.05V that corresponds to the formation of metallic gold. Anodic maximum, obtained on the Ge-Au-$_{nano}$, is shifted to negative potentials at 0.2V. The cathodic maximum shifts to the positive potential region at 0.1 V. The offset potential in the area of positive potentials on the GE-Au-$_{nano}$ can be accosiated with oxidation of gold nanoparticles in an alkaline medium due to reducing the activation energy [14].

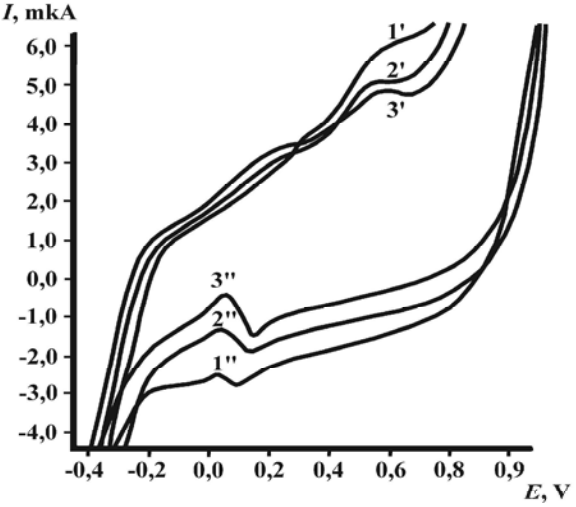

Fig. 1: Cyclic voltammetric curves for GE-Au-$_{nano}$: 1', 1''- 0.1 M NaOH background solution; 2', 2''– presense of $2\cdot10^{-12}$ M cysteine; 3', 3'' - presense of $4\cdot10^{-12}$ M cysteine.

The oxidation peak height of the cysteine on the GE-Au-$_{nano}$ is increased by 30% as compared to the peak height, corresponding to oxidation of modifier.

Process, occurring on the GE-Au-$_{nano}$, can be attributed to adsorption process, as evidenced by a positive slope of $I\sqrt{v} - \sqrt{v}$ functional connection, obtained for GE-Au-$_{nano}$ [12].

Cysteine electrooxidation process and its reactivation can be described by the following reaction scheme:

$$6RSH + Au_2O_3\,D \rightleftarrows 3RSSR + 2Au + 3H_2O$$

In the presence of cysteine at concentration of $2\cdot10^{-12}$ M on GE-Au-$_{nano}$, reverese maximum is observed at the potential E_c=0.0 V on the cathodic branches of cyclic curves (Fig. 1).

The cause of reverse maximum origin on GE-Au-$_{nano}$ may be associated with the oxidation of gold oxide from a lower oxidation state Au_2O to Au_2O_3 [10, 15]. Increment of the useful signal relative to modifier oxidation is 170%.

The activity of CBE-Au-$_{nano}$ and GCE-Au-$_{nano}$ with respect to cysteine was investigated under similar conditions and has shown a similar pattern, i.e. when using CBE-Au-$_{nano}$ and GCE-Au-$_{nano}$ in the presence of cysteine in amount of $2\cdot10^{-12}$ M, the reverse maximum is observed at a potential E_c=0.0 V on the cathodic branches of cyclic curves. Increment of the useful signal relative to the modifier oxidation is less than that for the GE-Au-$_{nano}$ and equals 65% and 110%, respectively. Thus, electrodes, which include in their structure carbon in different states

Table 1: Activity factors of carbon-containing electrodes, modified by gold nanoparticles, with respect to cysteine

Electrode	GE-Au-nano	CBE-Au-nano	GCE-Au-nano
The activity factor of the electrode	0,87	0,33	0,56

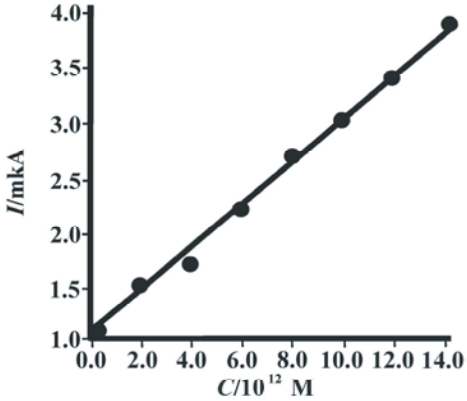

Fig. 2: Dependence of the maximum oxidation current on cysteine concentration on the GE-Au-$_{nano}$ in 0.1 M NaOH solution.

and having the surface, modified by colloidal gold particles, exhibit greater activity towards to cysteine, as compared to micro residuas. We have evaluated electrode activity factors for the electrodes, used in the studies when detecting the cysteine. The obtained data are presented in the Table 1.

It is obvious from the data presented that GE-Au-$_{nano}$ has the greatest activity factor that corresponds to the detactable concentration equal to $2•10^{-12}$ M, whereas for the other electrodes the detactable concentration will be greater due to the lower activity factor of the electrode. Thus, for CBE-Au-$_{nano}$ detectable concentration of cysteine is $4•10^{-12}$ M, while for GCE-Au-$_{nano}$ it is $2.6•10^{-12}$ M.

Thus, for Ge-Au-$_{nano}$, the cathodic reverse maximum, shown in Fig. 2, linearly dependends on the cysteine concentration within the concentration range from $2•10^{-12}$ M to $14•10^{-12}$.

For CBE-Au-$_{nano}$ and GCE-Au-$_{nano}$ linear dependence on the cysteine concentration is observed within the range from $4•10^{-12}$ M to $12•10^{-12}$ and from $2.6•10^{-12}$ M to $11•10^{-12}$ M, respectively.

During the studies conducted, we have selected GE-Au-$_{nano}$ as the working electrode to detact the cysteine in biological fluids (blood fraction, containing cysteine). This choice was made due to the high sensitivity of the selected electrode with respect to cysteine, better metrological characteristics compared to other working electrodes and greater range of detectable cysteine concentrations.

A solution of a biological fluid containing cysteine was prepared according to the technique, described in [16]. The principle of this technique is that a blood sample is subjected to fractional separation. The residue, resulted from centrifuge process, contains the protein fraction and amin acids, included in the blood. The residue was separated and subjected to rectification for formed elements. Separated fraction, conteining cysteine, was collected and diluted by a background electrolyte up to concentrations, falling within the linear range of the concentration dependence. Subsequent detection of cysteine was carried out as described in [11, 12].

It is known that the average content of cysteine in the blood is 0.004-0.019 g/dm^3 ($3.3•10^{-5} – 1.6•10^{-4}$ M); this allows one to dilute the blood by factor of 10^7 to reduce the impact of associated components during the fractionation of blood components.

On the basis of the results obtained, authors have developed a voltammetric method for detacting cysteine in biological fluids by the reverse cathodic maximum on GE-Au-$_{nano}$ electrode.

CONCLUSIONS

- Thus, GE-Au-$_{nano}$ electrode can be used for the voltammetric detection of cysteine in aqueous solutions and samples of biological fluids (blood). Employment of GE-Au-$_{nano}$ makes it possible to increase the sensitivity of cysteine detection by four orders of magnitude compared with GE-Au electrode. Enhanced detection sensitivity of cysteine on the GE-Au-$_{nano}$ is associated with increasing in number of atoms on the surface of the GE in contrast with single crystals of gold, catalyzing the cysteine oxidation process.
- For tested electrodes CBE-Au-$_{nano}$, GE-Au-$_{nano}$ and GCE-Au-$_{nano}$ the reverse maximum has been found on cathodic branch of the cyclic curve. This reverse maximum is not observed on single crystals of gold.
- A linear dependence between the reverse maximum current and cysteine concentration was revealed for electrodes CBE-Au-$_{nano}$, GE-Au-$_{nano}$ and GCE-Au-$_{nano}$. Noted linear dependence was observed within the intervals $(1-14)•10^{-12}$ M, $(4-12)•10^{-12}$ M and $(2.6-11)•10^{-12}$ M, respectively, in the solution of 0.1 M NaOH.

- Authors have developed a voltammetric method for the detection of cysteine in biological fluids, allowing one to exclude the effect of concomitant blood components in dilute solutions.

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