

NON-LITHOGRAPHIC TEMPLATE SYNTHESIS OF ULTRAMICROELECTRODES ENSEMBLES MADE OF SILVER WITH ADJUSTABLE DISTANCE ON A PLANAR MATRIX FOR THE CREATION OF ELECTROCHEMICAL SENSORS FOR MEDICAL PURPOSES

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ABSTRACT

In our work, we are developing a material for an electrochemical sensor detecting cysteine and glutathione based on non-lithographic template synthesis consisting of an array of micron silver particles on a Ti substrate separated by a dielectric TiO₂ xerogel with adjustable mutual removal. The array of microelectrodes has a number of advantages over planar electrodes consisting of a single conductive metal phase. The smaller the electrode area and the larger the anolyte flow due to diffusion to its surface, the more capacitive interference is leveled, and it becomes possible to determine concentrations of electroactive substances at low concentrations.

The use of such materials for the amino acids determination is promising due to the fact that the volume of biological samples (saliva), as a rule, is at the level of tens of microliters, and the use of this technology allows the manufacture of micron-sized sensors and rapid analysis at low amino acids concentrations. Also, the detection of biological substances in low concentrations will make it possible to make a clinical diagnosis at earlier stages of the disease. During the study of cyclovoltammograms, the possibility of using these materials to determine electroactive compounds with similar diffusion coefficients was shown.

The work carried out studies with cysteine and glutathione, since currently the identification of these amino acids in low concentrations in biological media is of great interest. These amino acids play an important role in cellular homeostasis, as well as those responsible for antioxidant activity in the cytoplasm of the cell, and the level of their content in saliva can serve as a criterion for the diagnosis of a number of diseases.

1. INTRODUCTION

The formation of structures with a given topology is one of the most important areas of materials science in recent decades. Particular interest is shown in the development of new methods for obtaining an ensemble of microelectrodes from various metals on the surface of

planar electrically conductive substrates. The surface of such electrodes consists of ordered or randomly arranged arrays of metal conductors separated from each other by an insulator.

Arrays of microelectrodes are increasingly used in electrochemistry, as such structures have great advantages over planar electrodes made of a single conductive metal phase. Due to hemispherical diffusion, the rate of mass transfer to and from the electrode increases, which affects a decrease in the detection limits of various components in solution and an increase in current output [1]. Also, due to the reduction of the surface area, the capacity of the double layer decreases, the ohmic drop in potential, and the rapid establishment of a stationary signal is realized.

Such matrices of conductive particles can be used to produce various functional materials, for example, coatings for implants are implemented when hydroxyapatite is deposited on metals micromassifs [2], and blanks for lithium-ion batteries are deposited when amorphous silicon is deposited [3]. Also, arrays of microelectrodes made of metals can be widely used as electrochemical sensors with a high sensitivity threshold for the analysis of biological markers indicating the course of certain metabolic [4] or pathological [5] processes in the human body. This will make it possible to use sensors with arrays of microelectrodes to diagnose infectious diseases and functional disorders. Currently, the determination of amino acids such as cysteine and glutathione is of great interest [6-8]. It has been proven [9] that cysteine and glutathione play an important role in cellular homeostasis. Cysteine acts as a cancer indicator, an antioxidant and a free radical scavenger. Cysteine is also involved in the antibodies formation and removes toxins from the body, including Pb, Hg and Cd, and is a precursor to glutathione.

Abnormal glutathione concentrations are an important signal of changes in oxidative stress in cells, which can lead to stroke, cancer and many neurodegenerative diseases. Therefore, the rapid and selective detection of these two amino acids in biologically available samples, such as saliva, can make it possible to make an early diagnosis and prevent complications. Currently, glutathione and cysteine in biological samples can be determined in various ways: chromatographic, electrochemical, spectrophotometric methods, capillary electrophoresis, chemiluminescence. Among all the methods, electrochemical methods of analysis have significant advantages, since they are the most sensitive. Ag-based electrochemical sensors can also be used to determine amino acids in biological fluids, particularly in saliva. Ag-based sensors are used to recognize organic compounds - proteins, amino acids, DNA, enzymes, glucose, as well as various inorganic substances.

Thus, Ag-based sensors act as stable, selective and sensitive instruments for the electrochemical determination of substances. In the future, such materials can be widely used for qualitative and quantitative biochemical analysis [10].

Both lithographic and self-organization methods can be used to create electrochemical sensors. Non-lithographic methods have a significant advantage in terms of forming the sensor surface structure. The process of creating the material becomes simpler and cheaper, since it is not required to design and produce masks, and it also becomes possible to use both plane-parallel and curved surfaces to form Ag arrays.

In our work, we are developing a material for an electrochemical sensor detecting cysteine and glutathione based on non-lithographic template synthesis consisting of an array of micron Ag particles (ultramicroelectrodes) on a Si substrate separated by a dielectric TiO₂ xerogel with adjustable mutual removal. The use of such materials for the determination of amino acids is promising due to the fact that the volume of biological samples, as a rule, is at the level of tens of microliters, and the use of this technology allows the manufacture of micron-sized sensors.

An array of ultramicroelectrodes has a number of advantages over planar electrodes consisting of a single conductive metal phase. Such structures are characterized by a higher signal-to-noise ratio when used in electrochemical analysis as working electrodes, since the signal is proportional to the total diffusion layer of microelectrodes, and the noise is proportional only to the microelectrodes area. The smaller the electrode area and the larger the analyte flow due to diffusion to its surface, the more capacitive interference is leveled, and it becomes possible to determine concentrations of electroactive substances in the low concentration range.

2. METHODS

2.1. MULTISTAGE TEMPLATE SYNTHESIS OF Ag MICROPARTICLES ARRAYS (ULTRAMICROELECTRODES) ON THE Si SUBSTRATE SURFACE WITH ADJUSTABLE DISTANCE

For high selectivity and sensitivity of the amino acids determination with a similar structure of the electroactive center (thiol group), such as glutathione and cysteine, the distance between microelectrodes should be 5-10 microns. Such a system structure will allow to achieve hemispherical diffusion and stationary current measurement mode. The too close location of the microelectrodes does not provide a stationary current measurement mode. For this purpose, a technique has been developed for the multistage template synthesis of microelectrode arrays from Ag microparticles on a Si substrate. The methodology consisted of the following items:

1. The electrophoretic microspheres deposition on a Si substrate.

In order to variate the distance between microelectrodes, it is necessary to use a polymer template, the concentration of which can be adjusted on a flat substrate. Emulsion microspheres made of polystyrene were chosen as the polymer template. For electrophoretic polystyrene (PS)

microspheres deposition from the emulsion, plates of polished silicon with a size of 2*1 cm were prepared. A 200 nm thick layer of Cr was pre-coated on Si using a Quorum 150T Plus magnetron atomizer to improve the substrate conductivity. Electrophoretic anode deposition was carried out at room temperature using a DC power supply B5-50 at a voltage of 60 V for 30 s. The distance between the electrodes was fixed to 1 cm.

2. Production of perforated TiO₂ xerogel film.

The TiO₂ was coated on polished silicon capped with polystyrene microspheres using the dip-coating method on the KSV NIMA dip-coating single small device. The following synthetic conditions were applied to obtain the films: the rate of Si plate stretching is 100 mm/min; drying of the silicon plate at 250 °C. Next, the Si wafers were ultrasonically treated in acetone for 10 minutes to remove polystyrene spheres from the coating. After polymer extraction, microperforations with different mutual distances were formed in the TiO₂ film.

3. Electrochemical deposition of Ag in the perforation of a TiO₂ xerogel film and the formation of microelectrodes array of on the solid substrate surface.

Silver was deposited by electrochemical template synthesis on an Ellins P-45X potentiostat-galvanostat device using Ag anode. The deposition was carried out in a polypropylene cell with stirring using a magnetic stirrer. The working electrode was a Ti sample coated with textured TiO₂. To precipitate Ag from the solution, 1000 cycles were performed with the following sequence of pulses and pauses: -1V - 5ms, 0.3V - 3ms, 2V - 6ms, 0V - 65ms.

The block diagram of synthesis is shown in Fig. 1.

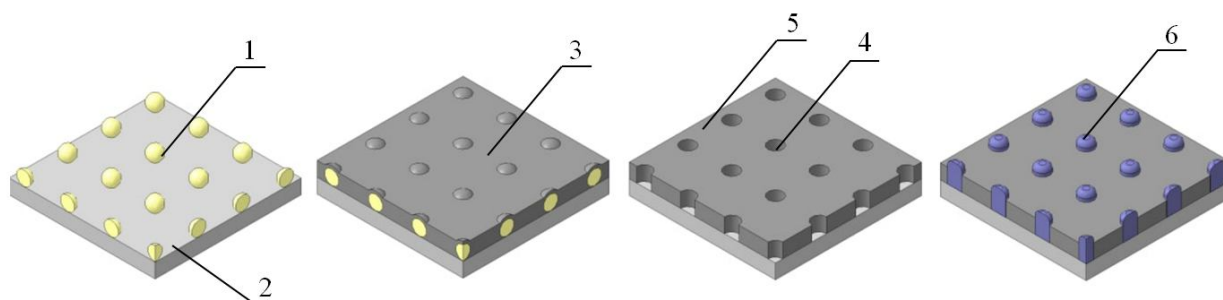


Figure 1. Block diagram of obtaining a sample with hemispherical Ag microparticles. 1 - polystyrol microspheres, 2 - Si substrate with a Cr layer, 3 - TiO₂ gel, 4 - perforations, 5 - TiO₂ xerogel, 6 - Ag microparticles

2.2. INVESTIGATION OF THE SENSORY PROPERTIES OF SAMPLES BY CYCLIC VOLTAMMETRY

Cyclic voltammetry (CV) is the most popular method of studying modified electrodes. CV provides information about charge transfer. In addition, this method allows to determine the stability of the modified electrode, as well as select the electrolytes suitable for the experiment

and the analysis conditions. When using traditional working electrodes, cyclic voltammograms with anodic and cathodic peaks are obtained. With ultramicroelectrodes, voltammograms of stationary processes are recorded, and the curves of oxidation and reduction almost coincide. There are also transition modes in which the curve is transitional between classical and sigmoid due to the intersection of diffusion hemispheres. Due to hemispherical diffusion to an array of conductive particles (ultramicroelectrodes), overvoltage is removed from the electrode, and the dependence of current on voltage takes the form of a polarographic curve. Due to the hemispherical diffusion to the ultramicroelectrode, the currents turn out to be close to the currents of an electrode with a radius equal to the diffusion sphere. However, the electrode area turns out to be significantly smaller, and this, due to the lower capacitance of the double electric layer, leads to a smaller capacitive contribution to the cyclovoltammogram curve, and, accordingly, to a greater sensor sensitivity.

Cyclovoltammograms were recorded in a three-electrode electrochemical cell on an Ellins P-30I potentiostat with a fixed working electrode area 0.196 cm^2 . An electrode with an Ag microparticles array was used as a working electrode, AgCl served as a reference electrode, and a Pt wire served as an auxiliary electrode. The potential sweep ranged from -1.5 V to $+1.5 \text{ V}$. The background electrolyte was a phosphate buffer with $\text{pH} = 7$. CV was processed using the "Origin" software.

2.3. SAMPLES CHARACTERIZATION

Morphology and elemental analysis of the samples was performed using a Zeiss Merlin scanning electron microscope with additional Oxford Instruments INCAx-act X-ray microanalysis attachments and the Oxford Instruments CHANNEL5 electron backscattered diffraction (EBSD) registration system. The elemental analysis was performed from dry particles deposited on a monocrystalline Si substrate.

2. RESULTS AND DISCUSSION

2.1. STRUCTURE AND ELEMENTAL COMPOSITION OF THE PERFORATED FILM

We investigated the dependence of the average distance between microspheres as a result of their electrophoretic deposition on a Si substrate with a Cr layer on the suspension concentration.

Fig. 2 shows the resulting stable suspension of polystyrene microspheres with a diameter of $1 \text{ }\mu\text{m}$.

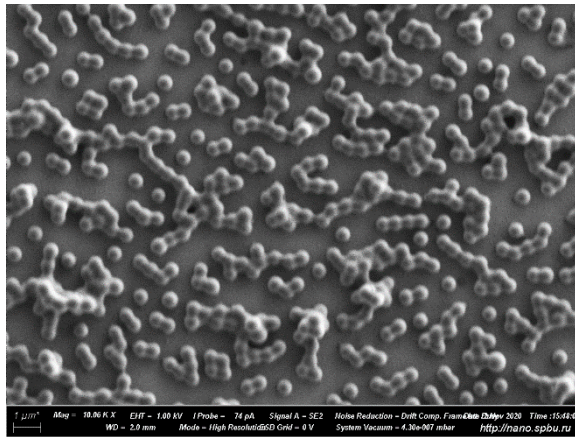


Figure 2. Micrograph of a polystyrene microspheres suspension

It is worth noting that polystyrene microspheres have electrophoretic mobility due to the presence of negatively charged DEL.

When an emulsion polymerizate is deposited on a Si wafer from a 18 $\mu\text{g/ml}$ solution, a distance between microspheres of ca. 2-3 microns is observed. When diluting the emulsion polymerization solution to 12 $\mu\text{g/ml}$, polystyrene microspheres are located at 5-8 microns. With a further decrease of the concentration to 9 $\mu\text{g/ml}$, the distance between them on the substrate increases to 12 microns, and when the emulsion is diluted to 3 $\mu\text{g/ml}$, it is possible to spread the microspheres 15-20 microns apart (Fig. 3). The average distances between microspheres on a planar Si matrix are given.

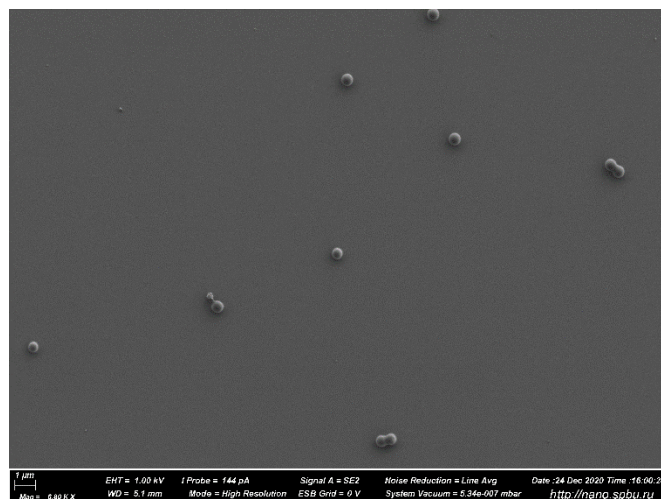


Figure 3. Micrograph of the polystyrene microspheres distribution on a Si substrate after EFO from a 12 $\mu\text{g/ml}$ solution

We revealed that during electrophoretic deposition of polymeric spheres obtained by emulsion polymerization on a conductive substrate, the distance between the particles depends on the suspension concentration.

Next, perforated TiO₂ coatings on a Si substrate were studied by SEM. When the substrate was ultrasonically extracted in acetone, the polystyrene spheres dissolved, and perforations remained in their place. The morphology of the resulting coating of perforations on a Si substrate after extraction in acetone from a 12 µg/ml solution is shown in Fig. 4. This concentration gives the optimal ratio between the locations of perforations in the film (located at 5-8 microns) to obtain an ultramicroelectrode.

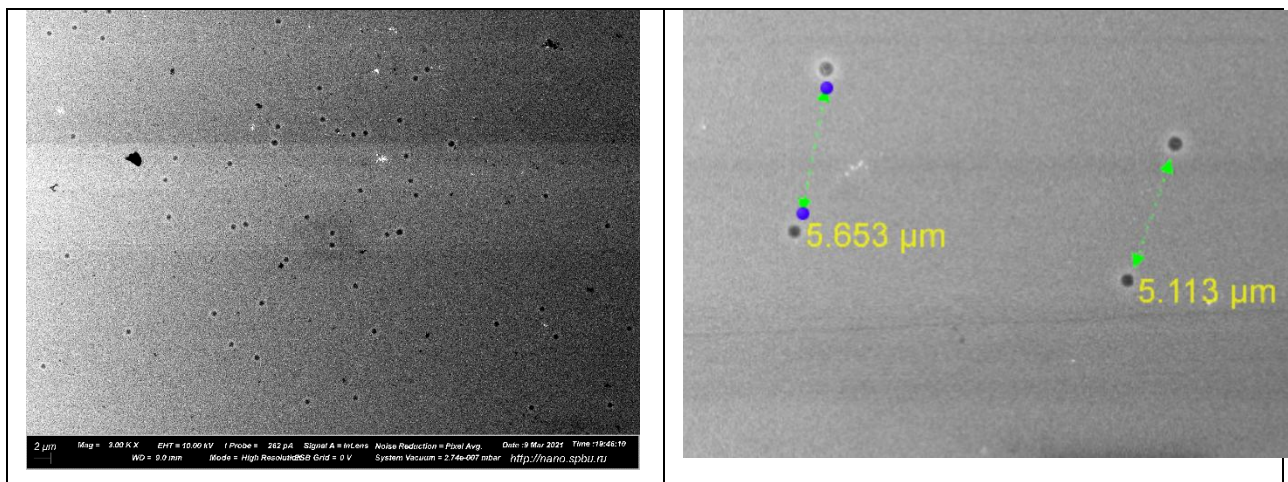
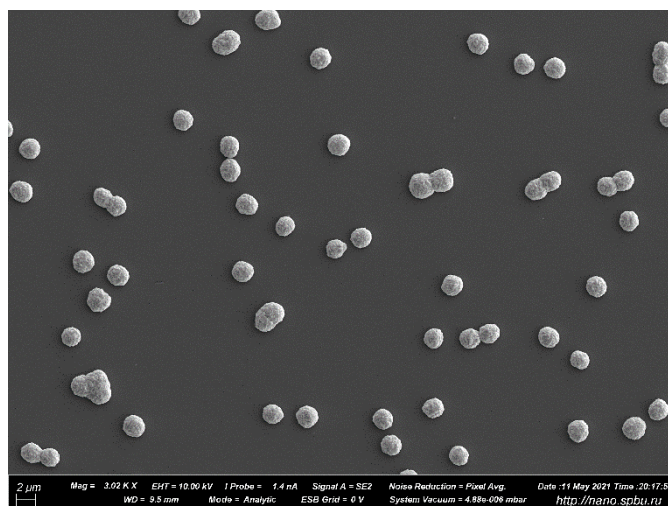


Figure 4. Micrograph of the distribution of perforations on a Si substrate after extraction in acetone from a solution of 12 µg/ml

3.2. SURFACE STRUCTURE OF A TiO₂/Ag COMPOSITE FILM OBTAINED USING AN EMULSION POLYMERIZATE TEMPLATE

Using template electrochemical synthesis, a TiO₂/Ag composite was obtained on TiO₂ films template with micron perforations. The pulse mode provided Ag deposition directly into the xerogel pores (Fig.5).



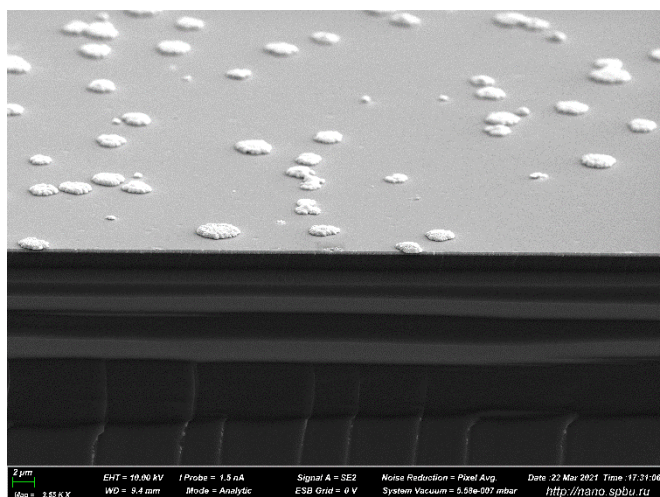


Figure 5. Micrographs of Ag electrochemically deposited at pulsed current on a xerogel TiO₂ film

TiO₂/Ag composite was also investigated by energy dispersive X-ray fluorescence spectroscopy. The element maps (Fig. 6) confirm the successful implementation of electrochemical template synthesis.

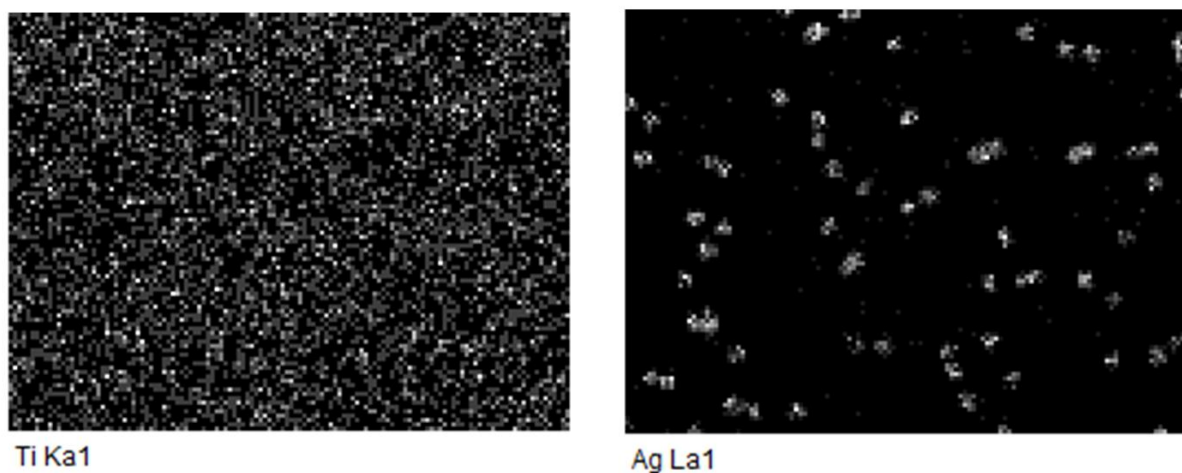


Figure 6. Element maps of TiO₂/Ag composite on micron perforations

Based on experimental data, a technique for non-lithographic template electrochemical synthesis of various Ag microparticles arrays was developed. It is shown that the use of a pulsed mode of Ag electrochemical deposition according to a template based on perforated TiO₂ films xerogel makes it possible to realize electrochemical template synthesis of Ag microparticles in the perforations.

3.3. VOLTAMMETRIC BEHAVIOR OF THE COMPOSITE IN AQUEOUS SOLUTION IN THE PRESENCE OF CYSTEINE

To study the sensory properties of the obtained hemispherical arrays of Ag microelectrodes, CV was recorded in a phosphate buffer solution with the addition of 10^{-6} M reduced cysteine (Fig. 7). The distance between the arrays was 5-7 microns.

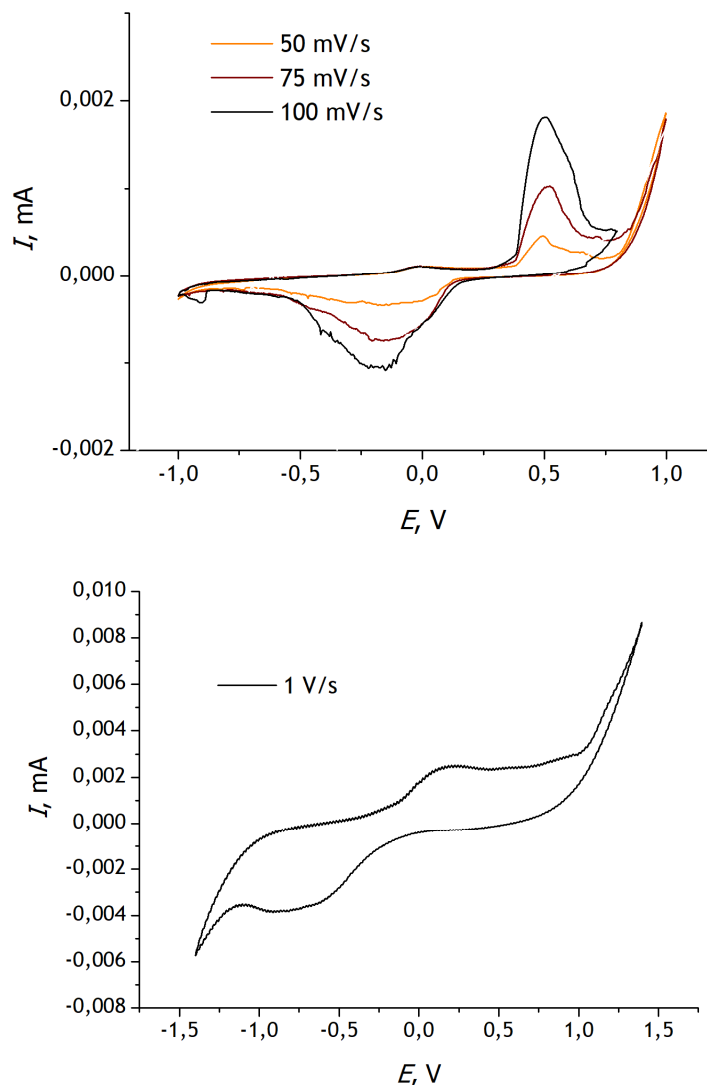


Figure 7. CV of cysteine solution at different scanning speeds

As the scanning speed increases, the voltammogram type changes, which indicates the stationary mode. This can be explained by the absence of intersection of the diffusion spheres of individual microelectrodes. The change in the curve shape may be associated not only with the scanning speed, but also with the diffusion coefficient of the substance under study, which in the future will allow the determination of compounds with similar redox potentials.

3.4. INVESTIGATION OF THE POSSIBILITY OF QUANTITATIVE DETERMINATION OF GLUTATHIONE BY CYCLIC VOLTAMMETRY USING THE DEVELOPED ELECTRODE

To study the sensory properties (lower sensitivity range) of Ag micromassives, cyclic voltammograms were taken in a phosphate buffer solution with addition of reduced glutathione. Concentrations: $1 \cdot 10^{-9}$, $2 \cdot 10^{-9}$, $5 \cdot 10^{-9}$. The potential sweep ranged from -0.5 V to +0.8 V. One complete measurement included 8 cycles with a sweep speed of 50 mV/s. A phosphate buffer with pH =7 was used as the background electrolyte. The voltammograms are shown in Figure 8.

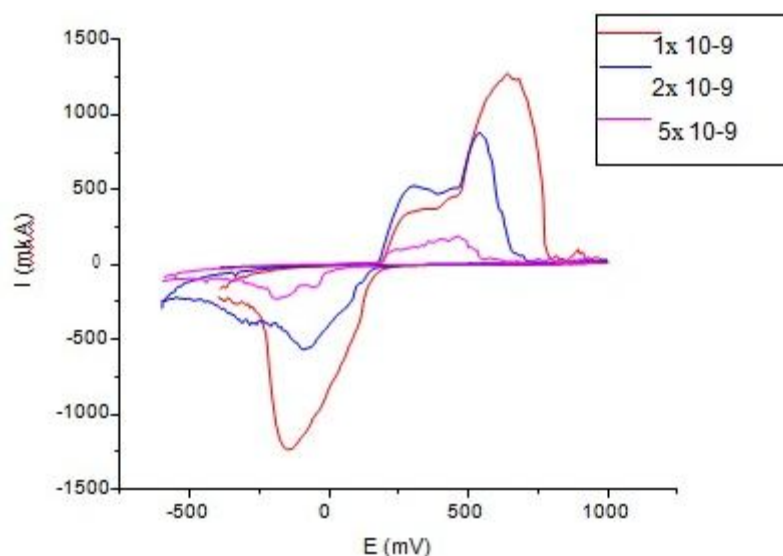


Figure 8. CVs with different glutathione concentrations in phosphate buffer solution

With an increase of glutathione concentration, the value of the Ag dissolution current decreases. This is due to the fact that silver ions convert the reduced glutathione form into the oxidized one. In this case, Ag^+ ions are reduced to metal, which leads to an ionization current decrease. The dependence of the current difference of the maximum Ag ionization in the background solution and with glutathione additives on its concentration becomes straight in semi-logarithmic coordinates.

The results obtained indicate the possibility of using this sensor for the quantitative determination of glutathione in concentrations above 2 nM.

CONCLUSION

In this work, a technique was developed for producing perforated TiO_2 xerogel films with a controlled average. It was found that during electrophoretic deposition of polymeric spheres obtained by emulsion polymerization onto a conductive substrate, the distance between the

particles depends on the suspension concentration. Thus, when diluting a polystyrene suspension from 18 µg/ml to 3 µg/ml, it is possible to realize electrophoretic deposition of microspheres with mutual removal from 2.5 microns to 25 microns, respectively.

The use of a pulsed mode of electrochemical deposition of silver according to a template based on perforated films of TiO₂ xerogel made it possible to realize electrochemical template synthesis of Ag microparticles in the perforation region. The developed method is non-lithographic. This simplifies the technology of obtaining the texture of the layer on the substrate surface, and allows to work on non-planar parallel substrates: due to the use of electrochemical deposition and dip coating methods, this limitation is absent for the developed approach. As a result of the work, a material was developed for the working electrode of an electrochemical sensor based on non-lithographic template synthesis for the determination of the amino acids glutathione and cysteine. We revealed that the obtained materials have nanomolar sensitivity to glutathione in model solutions. This indicates their prospects for the development of sensors for its determination. During the study of cyclic voltammograms taken using electrodes with an average mutual separation of Ag microparticles of 5-7 microns, the possibility of implementing a stationary diffusion regime was shown. This opens up the possibility of using these materials to determine electroactive compounds with similar diffusion coefficients.

COMPLIANCE WITH ETHICAL STANDARDS

The work is not related to research in which people or animals act as the object.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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