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Application of planar materials obtained by photolithography containing equidistant ultramicroelectrodes for analyzing the properties of electroactive oligopeptides

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Abstract

The presented work is devoted to the study of the possibility of using planar materials consisting of ultramicroelectrode arrays for voltammetric analysis of compounds with close redox potential, but with different diffusion coefficients, which has great prospects in the analysis of various oligomers, including oligopeptides. A feature of the electrochemical behavior of materials containing arrays of ultramicroelectrodes is the realization of hemispherical diffusion, which can lead to the steady state or unsteady state regime of the electrode depending on the intersection or non-intersection of the hemispheres. There is also a transient mode of operation of electrodes at partial intersection: voltammetry diagrams obtained in this mode may contain analytical information on the concentrations of substances with one redox potential, which favorably distinguishes the approach from the classical cyclic voltammetry using macroelectrodes. The prospects of the proposed approach are confirmed by the example of analysis using ultramicroelectrode arrays of ferrocenemethanol and triglycene ferrocenemethanol ester. The results obtained prove the possibility of applying the approach to the analysis of electroactive oligopeptide derivatives.

Keywords: planar materials; lithography; ultramicroelectrode; voltammetry; ultramicroelectrode array; diffusion mode; peptide derivatization

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1. Introduction

During polarization of electrodes with a characteristic size of 1-10 μm in electrolytes, a special type of diffusion occurs: the substance diffuses not linearly onto the electrode plane as usual, but to a point from a hemispherical diffusion layer. This diffusion mode contributes to a higher mass transfer rate than in the case of flat solid electrodes. This leads to the fact that the rates of depletion of the near-electrode layer due to the electrochemical reaction and bringing the product from the depth of the solution are equalized. Thus, a situation arises in which the growth of the spherical layer, in which the concentration gradient is localized, stops, due to which the stationary mode of mass transfer is achieved Aikins (1983). The voltammetry diagrams of steady-state electrodes are sigmoidal Davies et al. (2005).

In the case of planar ensembles of ultramicroelectrodes, a steady-state diffusion mode is also realized; however, when the interelectrode distance decreases, leading to the intersection of diffusion spheres of neighboring ultramicroelectrodes, a transient mode occurs Lu et al. (2017). Since the intersection of the spheres depends on their mutual distance and the diffusion coefficient, the resulting change in the form of voltammetry curves can serve as an analytical signal for the study of substances with equal redox potential.

To achieve this goal, it is necessary to solve two main problems: the development of a method for creating an electrode material and the selection of electroactive compounds suitable for setting up an experiment.

The creation of ensembles of microelectrodes is associated with a complex materials science problem of obtaining materials with ordered arrays of electrically conductive sections in a dielectric matrix, which are connected to a common current collector. The special difficulty in creating such materials lies in the enormous influence of geometric parameters on the electrochemical behavior, which imposes a number of restrictions on the methods used.

One of the common methods leading to such objects is the creation of composites "dielectric polymer matrix/electrically conductive filler" by compounding and extrusion, infusion and other classical methods for the manufacture of polymer composites. Zakharova et al. (2012); Zakharova et al. (2014); Li et al. (2021); Qing et al. (2007) It is possible to create ensembles of microelectrodes by coating perforated films on electri-cally conductive substrates Fernando et al. (2015); Arbenin et al. (2020); Arbenin et al. (2021). A common disadvantage for these methods is the creation of disordered microelectrodes ensembles. As a result, despite the simplicity of the technologies used, their applicability for solving the problem is very limited: at different interelectrode distances, hemispherical diffusion layers can either be separated or crossed, which radically changes the electrochemical behavior of individual microelectrodes.

The most effective from this point of view are the methods of creating ensembles of microelectrodes by forced organization of the material structure by photolithography Arbenin et al. (2021) and printing Metters et al. (2011). These methods allow to create microelectrode ensembles with a very narrow microelectrode spacings distribution, so their behavior is more predictable.

Based on the above-mentioned, photolithography and printing are the most suitable techniques to create microelectrode ensembles suitable for the analysis of compounds with different diffusion coefficients, in which the degree of overlapping of the diffusion regions of neighboring microelectrodes is of decisive importance.

2. Materials and Methods.

2.1. Reagents

Diglycine ⩾ 99.0% (Sigma-Aldrich), N,N-Dicyclohexylcarbodiimide, ⩾ 99% (Ferak), 4-(Dimethylamino)pyridine, ⩾ 99% (Sigma-Aldrich), Dimethylformamide, ⩾ 99% (JSC LenReactiv), Ferrocenemethanol, ⩾97% (Sigma-Aldrich), Trifluoroacetic acid, ⩾99% (Sigma-Aldrich), Methylpiperidine ⩾ 99.0% (Sigma-Aldrich), Sodium nitrate, ⩾99% (LLC Vekton), photoresist AZ MIR 701 (AZ Electronic Materials.), photoresist developer AZ MIF 726 (AZ Electronic Materials.)

2.2. Lithographic production of ultramicroelectrodes ensembles

At first stage a one-sided polished single-crystal silicon wafer were covered by chromium (5 nm) and gold (200 nm) layers via thermal vacuum deposition. At the second stage perforation array was fabricated using optical lithography. The substrate was spin-coated with photoresist layer. Then, a hexagonally ordered array of circles was deposited on this layer using a maskless photolithographic system Heidelberg DWL 66FS. Next, the photoresist layer was processed with a developer, which led to the opening of circular metal contacts. The remaining photoresist provided the dielectric layer for further electrical measurements.

2.3. Synthesis of glycine and triglycine esters of ferrocenemethanol.

Synthesis of electroactive derivative was carried out by carbodiimide esterification between ferrocenemethanol and FMOC-protected triglycine. FMOC-protected triglycine was synthesized via condensation of diglycine and FMOC-protected glycine in presence of dicyclohexylcarbodiimide and dimethylaminopyridine. Electroactive derivative of tryglycine was deprotected by methylpiperidine in DMF media with following quick evaporation of solvent and deprotector. That synthetic sequence results in ferrocenemethanol ester of triglycine. Water solution of that compound mas obtained by dissolving in water in presence of hydrochloric acid pH=4.

2.4. Recording cyclic voltammograms

Cyclic voltammograms were recorded using an Elins potentiostat-galvanostat. The studied ultramicroelectrode ensembles were used as working electrode, AgCl as reference electrode, and a glassy carbon as auxiliary electrode. We used solutions of ferrocenemethanol and electroactive derivate of triglycine in 0.01 M phosphate buffer with the addition of 0.2 M sodium nitrate as a supporting electrolyte. The concentration of the main substance is $1*10^{-4}$ M.

3. Results.

3.1. Microscopic study of microelectrode ensemble's structure

Micrographs of the prepared microelectrode arrays confirm that lithographic process was carried out successfully Fig. 1. Hexagonally ordered array of circular perforations was obtained.

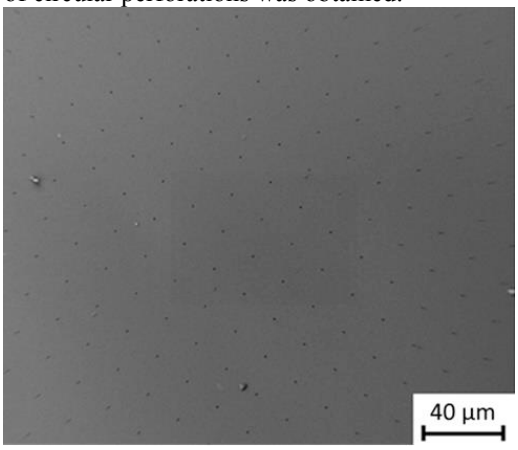

Fig. 1. Electron micrograph of the microelectrode ensemble.

The interelectrode distance, estimated from the image in Fig. 1, corresponds to 20 µm, perforation diameter – 2 µm.

3.2. Determination of the structure of ferrocenemethanol ester of triglycine

To study the esterification products of ferrocenemethanol and Fmoc-triglycine high-resolution mass spectra were recorded on a Shimadzu LCMS-9030 Q-TOF spectrometer. Molecular ion peak with m/z=609.1557 was observed, which corresponded to the formula $C_{32}H_{31}N_3O_6Fe$. Obtained chemical formula is equal to FMOC-protected ferrocenemethanol ester of triglycine

3.3. Experimental study of electrochemical behavior of ultramicroelectrode ensembles

The voltammetry diagrams of pure solutions of ferrocenmetanol and triglycine ether differ significantly in shape: for ferrocenmetanol, as a substance with a large diffusion coefficient, there is a greater shift to the steady-state mode than for ether Fig. 2.

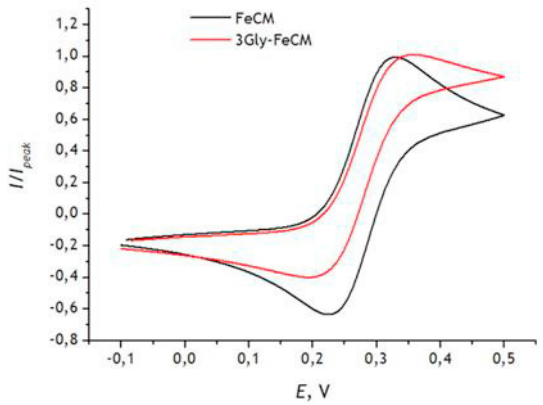

Fig. 2. Voltammetry patterns of ferrocenemethanol and triglycylferrocenemethanol at a sweep rate of 20 mV/s on an ensemble of ultramicroelectrodes with a distance of 20 μm.

To confirm the possibility of using the curve shape as an analytical signal, voltammetry diagrams of mixtures with different ratios of ferrocenmetanol and its ester were obtained Fig. 3.

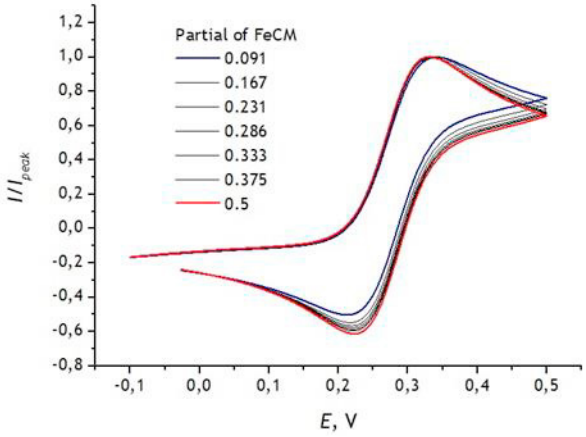

Fig. 3. Voltammetry patterns of ferrocenemethanol and triglycylferrocenemethanol mixtures at a sweep rate of 20 mV/s on an ensemble of ultramicroelectrodes with a distance of 20 μm.

A numerical parameter to quantify the concentration of the components can be the shift of the peak potential of the voltammetry peak, which, as can be seen, increases with concentration. To understand the course of the dependence, a graph was plotted with the concentration on the abscissa axis of the concentration and the potential shift on the ordinate axis Fig. 4.

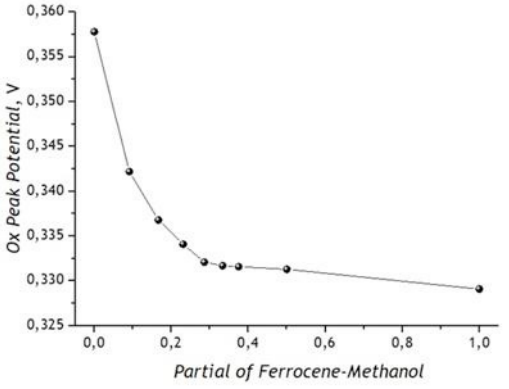

Fig. 4. Dependence of the oxidation peak potential on the ratio of ferrocenemethanol/ether in the mixture.

4. Conclusion.

As a result of this study, it was shown that planar materials containing ensembles of ultramicroelectrodes obtained by the photolithographic method are promising for the analysis of electroactive compounds with equal oxidation-reduction potential and different diffusion coefficients. The study was performed on the ferrocene methanol/ferrocene methanol triglycine ester system; the results obtained indicate the possibility of applying the developed approach for the analysis of electroactive derivatives of oligopeptides by studying the shift of peak potentials of cyclic voltammetry patterns.

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