Three-component working electrode micron-sized Ag particles/tiO$_2$ layer/Ti: template electrochemical synthesis and potential use as electrochemical sensor for glutathione detection

A Yu Arbenin, E G Zemtsova, S S Ermakov, A M Gaskov, P I Baburova, D N Sokolova, S V Yaroshenko and V M Smirnov

1 Saint Petersburg State University, Universitetskii pr. 26, 198504 St. Petersburg, Russia
2 Lomonosov Moscow State University, Department of Chemistry, Leninskie gory 1/3, 119992, Moscow, Russia
3 Author to whom any correspondence should be addressed.

E-mail: vms11@yandex.ru

Keywords: chemical sensor, glutathione, biological liquid, composite electrode, titanium-silver-TiO$_2$ film, clinical diagnostics

Abstract

In the present work the possibility is considered of a chemical sensor synthesis for quantitative glutathione (GSH) determination. Sensor is based on a composite working electrode containing an array of micron-sized Ag particles immobilized on a conductive substrate (Ti) coated by dielectric TiO$_2$ film. To determine GSH in biological fluids, particularly, in saliva, electrochemical silver-based sensors can be used, since such sensors contain –SH group. With the use of cyclic voltammetry (CV) with a composite working electrode containing an Ag microparticles array, the threshold of quantitative GSH determination is reduced to nM level. Since other modern analogues are inferior at least one order of magnitude in the limit of quantitative GSH detection, we assume that the proposed sensor may be of great interest for clinical diagnosis.

1. Introduction

Currently, a very important task of biomaterial chemistry is the development of new sensory materials for the analysis of biological markers that indicate the presence of certain metabolic [1] or pathological [2] processes. Glutathione (GSH) determination is of the great importance in the diagnosis [3–5]. This is due to the fact that GSH is responsible for antioxidant activity in the body, and its content in saliva can serve as a criterion for the diagnosis of a number of diseases [3, 6, 7]. To determine GSH in biological fluids, particularly in saliva, silver-based electrochemical sensors can be used. Currently, many works are devoted to the synthesis of Ag microparticles arrays: devices based on them are suitable as sensors for peroxides [8], hydrogen sulfide [9] and many other compounds. In this work, we present the template electrochemical synthesis of micron Ag particles array on a solid substrate. This approach includes formation of the template by the sol-gel method using a structure-forming polymer dopant. Thus, this is two-stage templating. The final material is polished titanium with an array of surface-deposited Ag microparticles separated by a dielectric TiO$_2$ xerogel. Such kind of material is promising for the GSH determination due to small required volume of biological samples—usually, tens of microliters; moreover, this technology allows to produce micron-sized sensors.

2. Experimental

2.1. Fabrication of TiO$_2$ thin films

At the first stage, Ti substrates were coated by textured TiO$_2$ to obtain a template by sol-gel method modified by us [10]. Commercial grade VT1-0 Ti was used as a substrate. The samples with size of 10x38 mm with rounded edges have been sanded on the abrasives with grid size of P300, P600 and P1200. Subsequently, they were polished with nano silica (Mastermet Buehler). This treatment provides roughness $R_z < 0.01$ micron. Synthetic
solution consisted of absolute isopropanol (iPA, Vekton, Russia), titanium tetraisopropoxide (TTIP, Sigma Aldrich), diethanolamine (DEA, Sigma Aldrich) and polyethylene glycol Mw = 20000 D (PEG, Merck) was used to synthesize the film. The components ratio in this solution was as follows: iPA/TTIP/DEA/PEG = 773/227/105/50. Before coating, the solution was preheated up to 45 °C. The film was sol-gel fabricated by dip-coating using KSV Nima Dip Coater, Singlevessel. The extraction speed was 100 mm min⁻¹. The deposited film was heat treated on the hot plate at 400 °C, which results, as previously shown [11], in partial cracking. After that, film was extracted with boiling deionized water and air dried at 200 °C.

2.2. Electrochemical silver deposition.
For silver deposition, the following electrolyte composition is used: AgNO₃ (Belar)—3.15 g, sulfosalicylic acid (Vekton, Russia)—11 g, aqueous ammonia 25% (Vekton, Russia) to reach pH 9. The resulting solution was adjusted to 100 ml by deionized water. For the deposition we used potentiostat-galvanostat Ellins R45H. The deposition was performed in a polypropylene cell with magnetic stirring. As a working electrode, a sample of textured TiO₂-coated Ti was used. As a counter electrode, graphite was used. Pulsed mode was applied: 750 cycles—1 V—5 ms, 0.3 V—3 ms, 2 V—10 ms, 0 V—65 ms.

2.3. Cyclic voltammetry study
CV diagrams were registered in a three-electrode electrochemical cell on a potentiostat Ellins P-30I within the range −0.6 V to +0.6 V using AgCl reference electrode and auxiliary platinum wire electrode. Phosphate buffer solution with pH 7 served as supporting electrolyte. Sweep speed of 50 mV s⁻¹ was applied to study the concentration dependence. Additional experiments at 25 and 100 mV s⁻¹ were performed to demonstrate the reproducibility of the determination at various sweep speeds.

3. Results and discussion
At the first stage, TiO₂ xerogel thin films were obtained on the polished Ti surface. Xerogel consists of amorphous titania that is confirmed by XRD. These films have star-shaped perforations (holes). This is due to polyethylene glycol precipitation as a separate phase during cooling, with formation of a micron-sized droplets array in the bulk gel.

Further, the template was removed from the film by extraction and heat treatment, which eventually led to the structure depicted on figure 1. This is an insulator layer with an array of perforations that liberate the conductive metal substrate. Due to this structure, electrochemical deposition can occur in perforations, that assists template electrochemical synthesis. However, at a constant potential Ag particles cover the entire sample surface (figure 2).

Pulsed deposition mode allows to realize template electrochemical synthesis (figure 3).

EDS console (Oxford Instruments INCAx-act) to Zeiss Merlin scanning microscope was used to make microanalysis and to build elemental maps of Ti with Ag on the surface. The template pattern is replicated by electrochemically deposited Ag (figure 4) that indicates the effectiveness of the developed approach.
Figure 2. Electron microphotograph of the sample obtained at a constant potential.

Figure 3. Electron microphotograph of a sample obtained in pulsed mode.

Figure 4. Elemental maps (a–Ag, b–Ti) of the Ti sample with Ag on the surface obtained in pulsed mode and constructed using an energy dispersive spectrometer (EDS).
The height of the deposited Ag profile of 0.5–1 μm is confirmed by microphotographs of plate edge (figure 5).

To study the sensory properties of the material, CVs were recorded in phosphate buffer with addition of reduced GSH ($10^{-9}$ to $2\times10^{-7} \text{M}$) at room temperature and 50 mV s$^{-1}$ (figure 6).

Obtained data suggested that the silver dissolution current decreases with the GSH addition. This is probably due to the oxidation of the reduced GSH by Ag$^+$ ions. In turn, Ag$^+$ ions are reduced to the metal, which leads to ionization current decrease. We carried out two additional experiments at sweep speeds of 25 and 100 mV s$^{-1}$ (figure 7, in comparison with experiment at 50 mV s$^{-1}$).

The relationship between the GSH peaks location and height confirms the possibility of reproducible glutathione determination at various scan speeds.

The dependence of the current difference of the maximum Ag ionization with GSH additives on its concentration becomes linear in semi-logarithmic coordinates (figure 8). The determination coefficient calculated for the current density dependence on concentration quantitatively indicates the analysis reproducibility: $R^2 = 0.9864$. The logarithmic dependence of the current on the concentration indicates that GSH oxidation by silver is accompanied by its adsorption on the silver surface.
4. Conclusion

In this study the template electrochemical synthesis is discussed: metallic Ag was deposited on the perforated TiO₂ xerogel, repeating the pattern of perforations. This was achieved by pulsed electrochemical deposition. The synthesized Ag microparticles surface layer on Ti separated by a dielectric xerogel of TiO₂ has nanomolar sensitivity to GSH in model solutions. This means that the developed material is a promising sensor for the GSH determination, since other known materials for GSH analysis are inferior in quantitative determination by at least one order of magnitude as compared to the developed material [12].

The research was carried out using the equipment of the resource centers of the Science Park of Saint Petersburg State University ‘Center for diagnostics of functional materials for medicine, pharmacology and nanoelectronics’, ‘Innovative technologies of composite nanomaterials’, ‘Nanotechnologies’.

Acknowledgments

The work was financially supported by St. Petersburg state University (event 3, grant id: 26520408).

ORCID iDs

A Yu Arbenin  https://orcid.org/0000-0002-0168-7612
V M Smirnov  https://orcid.org/0000-0002-7358-1884
References

[1] Berdowska I 2004 Cysteine proteases as disease markers *Clin. Chim. Acta* 342 41–69
[2] Keicho N, Matsushita I, Tanaka T, Shimbo T, Le Hang NT, Sakurada S and Lien LT 2012 Circulating levels of adiponectin, leptin, fetuin-A and retinol-binding protein in patients with tuberculosis: markers of metabolism and inflammation *PLoS One* 7 e38703
[3] Ngamchuea K, Batchelor-McAuley C, Cowen PJ, Williams C, Gonçalves LM and Compton RG 2016 Can saliva testing replace blood measurements for health monitoring? Insights from a correlation study of salivary and whole blood glutathione in humans *Analyst* 141 4707–12
[4] Ngamchuea K, Batchelor-McAuley C and Compton RG 2016 The copper(II)-catalyzed oxidation of glutathione *Chem. Eur. J.* 22 15937–44
[5] Ngamchuea K, Batchelor-McAuley C and Compton RG 2017 Rapid method for the quantification of reduced and oxidized glutathione in human plasma and saliva *Anal. Chem.* 89 2901–8
[6] Özütürk I K, Furuncuoğlu H, Atala M H, Ülûkoylu O, Akyüz S and Yarat A 2008 Association between dental-oral health in young adults and salivary glutathione, lipid peroxidation and stalic acid levels and carbonic anhydrase activity *Braz. J. Med. Biol. Res.* 41 956–9
[7] Godlew ska BR, Sharpley AL, Cowen PJ and Compton RG 2018 Salivary glutathione in bipolar disorder: A pilot study *J. of Affective Disorders* 238 277–80
[8] Zhao W, Wang H, Qin X, Wang X, Zhao Z, Miao Z and Chen Q 2009 A novel nonenzymatic hydrogen peroxide sensor based on multi-wall carbon nanotube/silver nanoparticle nanohybrids modified gold electrode *Talanta* 80 1029–33
[9] Fam DWH, Tok AJY, Palaniappan A, Nopphawan P, Lohani A and Mhaisalkar SG 2009 Selective sensing of hydrogen sulphide using silver nanoparticle decorated carbon nanotubes *Sensors Actuators B* 138 189–92
[10] Zemtsova EG, Orekhov JV, Arbenin AY, Valiev RZ and Smirnov VM 2016 The creation of nanocoatings of various morphology on the basis of titanium dioxide on a titanium matrix for bone implant *Materials Physics and Mechanics* 29 138–44 http://www.ipme.ru/e-journals/MPM/no_22916/MPM229_05_zemtsova.pdf
[11] Zemtsova E, Arbenin A, Yudintceva N, Valiev R, Orekhov E and Smirnov V 2017 Bioactive coating with two-layer hierarchy of relief obtained by sol-gel method with shock drying and osteoblast response of its structure *Nanomaterials* 7 323
[12] Timur S, Odaci D, Dincer A, Zinhigolu F and Telefoncu A 2008 Biosensing approach for glutathione detection using glutathione reductase and sulphydryl oxidase bienzymatic system *Talanta* 74 1492–7