COMPARISON OF GOLD NANOSTRUCTURED ELECTRODE AND FLAT GOLD ELECTRODE ON DOXORUBICIN MEASUREMENTS

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Abstract

Doxorubicin is a planar aromatic molecule and it is commonly used in cancer chemotherapy. In this study, we compared nanostructured electrodes with gold nanocolumns and flat gold electrodes by measurements with doxorubicin. Nanostructured electrodes were fabricated by electrochemical anodic oxidation when the aluminium layer was transformed to the porous alumina layer. Then gold material was deposited into the pores. After removal of aluminium template the nanostructured surface made of ordered gold nanocolumns on metal underlay was obtained. Flat gold electrodes were fabricated by physical evaporation of gold layer to the silicon substrate. After that, all electrodes were characterized by electrochemical impedance spectroscopy and then the electrochemical detection of doxorubicin was studied by differential pulse voltammetry. According to the impedance spectroscopy measurements a bigger electroactive area for nanostructured electrodes was proved. The gold nanocolumns have been found as a significant factor in increasing of electrodes active area. This fact is very important for sensors sensitivity. Fabricated electrodes were successfully used for determination of doxorubicin. Doxorubicin oxidation peak was observed at a potential - 0.6 V.

Keywords: Gold nanocolumns, Differential pulse voltammetry, Electrochemical impedance spectroscopy, Doxorubicin, Gold electrode

1. INTRODUCTION

Doxorubicin is anthracycline antibiotic that is commonly used in cancer chemotherapy. Doxorubicin is a planar aromatic molecule belonging to anthracycline. The drug can interact with double stranded DNA by intercalation and electrostatically with single stranded DNA [1, 2]. Many publications on this topic have been written. Interactions of DNA and doxorubicin have been investigated on hanging mercury drop electrodes, carbon paste electrodes, glassy carbon electrode, etc. [3-5].

Nanostructures are often used in sensor devices due to enlarging the active surface of geometrically small working electrodes. Indented surface means significant increase in sensor sensitivity and signal response. Template-based electrodeposition method is very simple and low-cost way for a production of enlarged surface area by ordered nanostructures. Nanotechnologies in conjunction with electrochemical methods are very useful tool for detection of various low–concentrated substances. Differential pulse voltammetry is high sensitive method for study of redox properties in trace amounts of chemicals in the sample. Therefore, this method is suitable for our purpose. Further electrochemical impedance spectroscopy is useful method for characterization of surface treated electrodes. According to the value of real part impedance and concentration of solution, the electroactive area of the electrode can be compared [6].

2. MATERIALS AND METHODS

2.1 Chemicals

Titanium (99.99%, Goodfellow, UK), aluminium (99.999%, Goodfellow, UK), gold (99.999%, Safina, CZ), tungsten (99.999%, Goodfellow, UK), sulphuric acid (H₂SO₄, 99.5%, p., Penta, CZ), oxalic acid ((COOH)₂),
Penta, CZ), potassium dicyanoaurate (K[Au(CN)]_2), 68%, Safina, CZ), boric acid (H₃BO₃, p., Penta, CZ), chromium trioxide (CrO₃, Penta, CZ), phosphoric acid (H₃PO₄, 98%, p.a., Penta, CZ), potassium sulphate (K₂SO₄, p., Lachema Neratovice, CZ), dihydrate sodium dihydrogen phosphate (NaH₂PO₄·2H₂O, 99%, Penta, CZ), dihydrate sodium hydrogen phosphate (Na₂HPO₄·2H₂O, 98%, Fluka, CZ) and doxorubicin hydrochloride (C₂₇H₂₅NO₁₃·HCl, Sigma Aldrich) were used as purchased without any purification. Deionised water (18.2 MΩ) was obtained from Millipore RG system MiliQ (Millipore Corp., USA).

2.2 Electrodes

Nanostructured electrodes were fabricated using electrochemical anodic oxidation when the aluminium layer was transformed to the porous alumina layer. At first, 120 nm thick titanium layer and 160 nm thick aluminium layer were physically evaporated to the silicon substrate. In the second step, aluminium layer has been transformed by anodization to hexagonally ordered porous alumina template (Al₂O₃) [7]. The thin porous anodic alumina template has been obtained by anodization process under constant voltage (5 V) in 3M sulphuric acid at 10 C. In the alumina template, the titanium layer is transformed by oxidation to titanium dioxide [8, 9]. Then gold material was deposited into the pores by electrochemical reduction of gold ions from potassium dicyanoaurate solution. The time of deposition was 4 seconds at a constant current of 20 mA. Finally, aluminium template was dissolved in 100 ml of mixture solution contain 3 g of chromium trioxide and 5 ml of phosphorous acid. Surface modified by gold nanocolumns on the titanium dioxide nanodots has been obtained and then characterised on the SEM (Tescan Mira II, Tescan, CZ). Fabricated nanocolumns were approximately 70 nm high and 10 nm wide.

The second type of nanostructured electrodes was obtained similarly. The tungsten material was used instead of the titanium. On the silicon substrate, 200 nm thick tungsten layer was sputtered and then the 150 nm thick aluminium layer was physically evaporated. Ordered template was fabricated by anodic oxidation and tungsten layer under aluminium layer was oxidized to tungsten trioxide nanodots. The oxide was removed using selective etching. The gold material was deposited into the porous template and obtained dimples. Anodization process was carried out in 0.3 M oxalic acid at an anodization potential of 40 V. Gold material was deposited into the pores by pulse deposition method. 23 pulses were applied. Current of pulses was 1 mA and length of pulses 400 ms. Obtained nanocolumns are approximately 100 nm high and 50 nm wide.

Flat gold electrodes were fabricated by subsequently physical evaporation of 180 nm thick titanium and 150 nm thick gold layers to the silicon substrate. All electrodes were fabricated to have the same geometric area (3 mm in diameter).

2.3 Electrochemical methods

All measurements were carried out using µAUTOLAB III/FRA2 in connection with NOVA 1.7 software (Metrohm Autolab, NL). Three-electrode cell with Pt auxiliary electrode and Ag/AgCl/3M KCl reference electrode (both Metrohm AG, CH) were used for all experiments. The impedance spectroscopy was measured at zero potential in a frequency range from 500 kHz to 0.1 Hz and amplitude of 20 mV. Electrochemical characterization of electrodes was done in potassium sulphate (K₂SO₄) of various concentrations from 0.01 µM to 10 mM. Calibration curves for doxorubicin were measured by differential pulse voltammetry in 0.1 M phosphate buffer solution (pH 7), potential step 5 mV, amplitude of modulation 25 mV, scan rate 10 mV·s⁻¹ and potential range from -0.75 V to -0.4 V.

3. RESULTS AND DISCUSSION

The titanium and tungsten electrodes modified by gold nanocolumns and flat gold electrodes were successfully fabricated and characterized optically and electrochemically. In the Fig. 1, there are SEM pictures of fabricated nanostructures of the titanium layer which were created by electrochemical growth of
gold through highly ordered nanoporous template. It could be observed that nanostructures lose their stability and create clusters after removing the alumina template. Despite this, the overall homogeneity of distribution is excellent. In the Fig. 2, there are SEM pictures of gold nanocolumns on tungsten layer which look very stable.

**Fig. 1** SEM microimages of fabricated gold nanocolumns on titanium layer: magnification 150 kx (1a) resp. 10 kx (1b)

**Fig. 2** SEM microimages of fabricated gold nanocolumns on tungsten layer: magnification 110 kx (2a) resp. 150 kx (2b)

The electrochemical impedance spectroscopy (EIS) characterization in a wide range of electrolyte concentrations was carried out. In that aspect, EIS is a powerful tool for nanostructured electrode surface characterization and for understanding of the electrochemical processes acting on it. In Fig. 3a, there are shown impedance spectra for both fabricated types of electrodes measured using the same solution concentrations. In Fig. 3b, there are two dependencies of impedance minima of spectra on the concentrations of potassium sulphate.

From EIS results, the different behaviour of nanostructured and flat gold electrode can be seen. From impedance spectra (Fig. 3a) is clear that the nanostructured electrodes show lower values of the real part of
impedance in the low concentrated electrolyte. According to the EIS results (Fig. 3) a bigger electroactive area for nanostructured electrodes was achieved. This fact is important for the sensitivity of the electrodes.

![Fig. 3 Impedance spectra for titanium nanostructured electrode and flat gold electrode (a), dependencies of spectra impedance minima on the potassium sulphate concentration (b)](image)

Fabricated electrodes were also used for determination of doxorubicin. Differential pulse voltammetry described in detail in subsection 2.3 was used for a detection of various concentrations of doxorubicin in range from 390 nM·ml⁻¹ to 100 µl·ml⁻¹. In Fig. 4, there is shown obtained voltammogram (Fig. 4a) and corresponding calibration curves for increasing concentration of doxorubicin (Fig. 4b) measured on flat gold electrode and tungsten nanostructured gold electrode after 10 minutes of drug accumulation [10]. An increasing anodic peak of doxorubicin can be seen at potential -0.6 V. Detection limit for tungsten nanostructured gold electrodes and flat gold electrodes was 790 nM·ml⁻¹. Dependency of anodic peak height and doxorubicin concentration is linear for nanostructured electrode and logarithmic for flat gold electrode. It could be caused by different electroactive area of electrodes. It was expected that in case of flat gold electrodes the whole electroactive area is covered by molecules at high concentrations. Therefore, the peak of doxorubicin did not increase. The titanium electrodes provided any doxorubicin peak. We assume that interface between gold nanocolumns and titanium dioxide blocked all signals.
Fig. 4: Differential pulse voltammogram for various concentrations of doxorubicin measured on tungsten electrode with gold nanocolumns (a), and calibration curves for increasing concentration of doxorubicin measured on flat gold electrode and nanostructured tungsten electrode after 10 minutes of drug accumulation (b).

4. CONCLUSION

Flat gold electrode and two types of electrodes modified by gold nanocolumns were fabricated and compared by electrochemical impedance spectroscopy and differential pulse voltammetry. The gold nanocolumns have been found as a significant factor in increasing of electrode active area. This fact is very important for sensors sensitivity. Finally fabricated tungsten and flat gold electrodes were successfully used for determination of doxorubicin. The detection limit of 790 nM·ml⁻¹ was achieved on flat gold and tungsten nanostructured electrodes.

5. ACKNOWLEDGEMENT

The work has been supported by project SIX (CZ.1.05/2.1.00/03.0072) and NaNoBioTECell (GA18109001 z.2200)

6. REFERENCES


