Imaging Glucose Oxidase by Scanning Electrochemical Microscopy

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***Abstract* — Scanning Electrochemical Microscopy is a tool for imaging and investigate surfaces by meaning of chemical reactivity, electric conductivity, and enzymatic activity. This technique is typical electrochemical cell plus three axis positioner. Positioner allows to measure typical electrochemical measurements, like Cyclic Voltammetry, Chronoamperometry, Impedance Spectroscopy, etc. in the vicinity of substrate and in the chosen position of working electrode. Moreover, this technique can be used as biosensor, e.g. measuring glucose concentration or other parameters, such as lactate and oxygen detection in living and unhealthy cells. In this paper, we present imaging of glucose oxidase by Scanning Electrochemical Microscopy in feedback mode. For experiments, glucose oxidase on the plastic surface was used as well-known enzyme. Imaging of glucose oxidase was performed in buffer with different glucose concentrations and with different redox mediator concentrations. Measurements showed that for higher image resolution glucose concentration has to be about 15-20 mmol·L^{-1} and redox mediator concentration needs to be lower than 0.2 mmol·L^{-1}. For image processing, Matlab programme was used. Another part of measurements was approaching the ultramicroelectrode to the surface and the current dependence on distance is shown in different concentrations of redox mediator and glucose.**

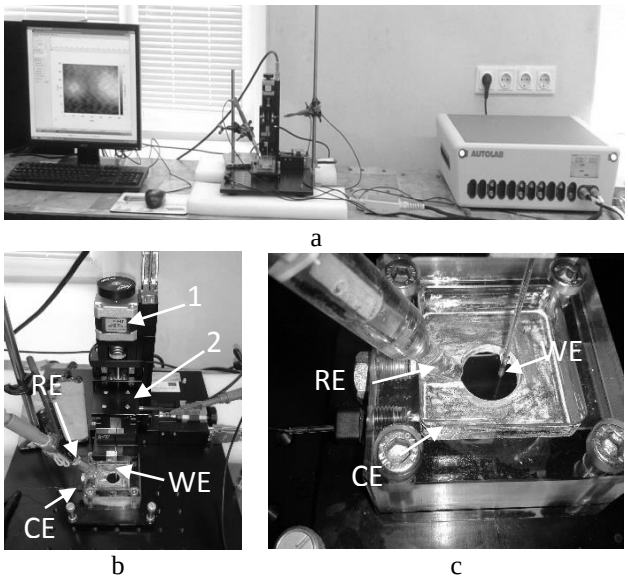
***Index Terms* — Glucose Oxidase, Imaging, Scanning Electrochemical Microscopy, Three Electrode Electrochemical Cell, Ultramicroelectrode**

I. INTRODUCTION

Scanning Electrochemical Microscopy (SECM) is a tool to electrochemical characterization of various surfaces, like as glass, metal, polymer, biological material [1] and liquids. The technique is used to study heterogeneous and homogeneous reactions, for high-resolution imaging of the chemical reactivity, electrical conductivity, enzymatic activity [2] and topography of various interfaces, and for microfabrication [3, 4]. SECM can be used as biosensor [5] for glucose, lactate, and oxygen detection in single cells [6].

Scanning electrochemical microscope is standard electrochemical cell (Fig. 1) with working (WE), reference (RE) and counter (CE) electrodes. Working electrode is ultramicroelectrode (UME) with a radius of the order of a few nm to 25 μm [3]. The UME is used for surface scanning by moving it by positioners in three directions – x, y, z.

The simplest mode of operation is the feedback mode, when only the current through the UME is measured. Current flow is caused by reduction reaction, occurred at the UME tip. The feedback can be positive or negative, depending on the kind of surface – conductive or insulating, respectively. In negative feedback, the diffusion of solution species to the tip is blocked, in positive feedback solution species is regenerated at the substrate [3]. Most SECM imaging experiments are carried out in constant height mode [7], where a probe is moved only laterally in the x and y directions. The constant height mode is appropriate for smooth surface (roughness is smaller than the UME diameter), because the tip current depends also on the tip-substrate distance, not only on the surface reactivity. So, image of surface reactivity can be recalculated to tip-surface distance. Use of a smaller probe in constant height mode results in a shorter working distance and even tip crash [1].



a b c

Fig. 1. Scanning Electrochemical Microscopy: a – general equipment, b – SECM three electrode electrochemical cell with view of stepper motor (1) and piezo (2) positioners, c – SECM electrochemical cell.

Resolution studies of scanning electrochemical microscopy (SECM) shows quantitative correlation of the loss in resolution and the increase in distance between tip and sample is found [8]. To determine the distance for appropriate measurement resolution, the current-distance curve has to be obtained approaching the tip to the surface. The maximum anodic currents can be converted

to estimated distances [7]. Example of such approaching curve is showed in Fig. 2. Distance can now be calculated:

$$\frac{i_T}{i_{T,\infty}} = \frac{d}{a} \quad (1)$$

where i_T – current in the small distance, d – distance, $i_{T,\infty}$ - current far from a surface

Steady-state diffusion-controlled current when the tip is far from a surface (Fig. 2) is given by:

$$i_{T,\infty} = 4nFDca \quad (2)$$

where F is the Faraday constant, D and c are the diffusion coefficient and the initial concentration of the mediator, and a is the radius of the UME.

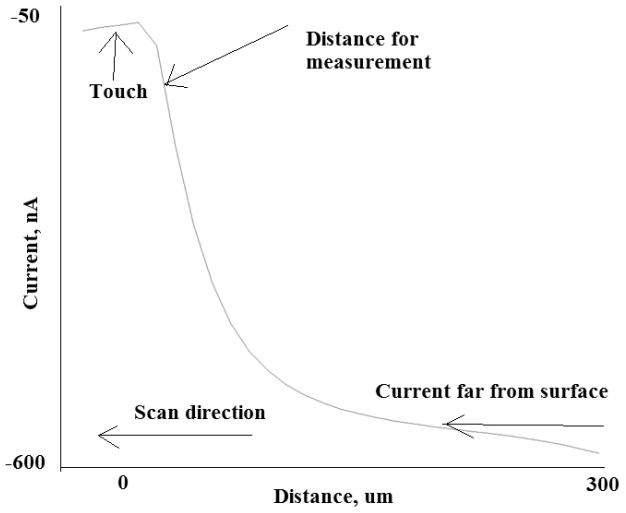


Fig. 2. Approaching the UME to the GOx surface. Line scan has an insulating behavior

Imaging of glucose oxidase

Imaging of DNA, keyhole limpet hemocyanin, mouse monoclonal IgG, and glucose oxidase on a mica substrate can be performed with resolution of the order of 1 nm [9]. Scanning electrochemical microscopy (SECM) can be used for imaging an enzyme chip with spatially-addressed spots for glucose oxidase [10] and to investigate biocatalytic reactions inside the enzyme layer of a biosensor during its operation [11]. SECM current–distance curves enabled the determination of kinetic information about GOx in GOx/PDDA multilayers as a function of layer number, film termination, inert covering layers, and enzyme substrate concentration after fitting to numerical models [12].

SECM in feedback mode analysis provides a characterization of the modified surface and the measurement of the enzymatic activity depending on the concentrations of glucose and mediator. Kinetics analysis indicates that GOx maintains a large enzymatic activity [13]. However, such big quantity of researches gives different results, because of different immobilization conditions of GOx.

II. EXPERIMENTS

Reagents and chemicals

Glucose oxidase (EC 1.1.3.4, type VII, from Aspergillus niger, 215.266 units mg−1 protein) was purchased from Flukay. d-(+)-Glucose was obtained from Carl Roth GmbH&Co (Karlsruhe, Germany). Before investigations glucose solutions were allowed to mutarotate overnight. All solutions were prepared using deionised water purified with water purification system Millipore S.A. (Molsheim, France). The solution buffer was prepared by mixing sodium acetate trihydrate, potassium chloride, monopotassium phosphate, Sodium phosphate dibasic which were obtained from Reanal (Budapest, Hungary) and Lachema (Neratovice, Czech Republic). 25% glutaraldehyde solution was purchased from Fluka Chemie GmbH (Buchs, Switzerland).

Plastic cell was evaporated by glutaraldehyde 25% for 10 min, Gox 1 mg/ml 0.5uL was sprayed on the surface to get little drops and dried in the room temperature. Then again Gox drop was evaporated 10 min by glutaraldehyde 25% and washed with buffer.

SECM experiments

Measurements were performed using Sensolytics SECM (Bochum, Germany). The UME was slowly approached towards the sample in order to measure steady state current [3]. The UME tip was a 10 µm diameter. The UMEs were characterized by cyclic voltammetry and polarized at -0.8V vs. Ag/AgCl. The applied potential for imaging at the UME is chosen +0.6V vs. Ag/AgCl. The electrochemical cell was used in a typical three-electrode configuration, with a platinum counter electrode and an Ag/AgCl, 3 M KCl reference electrode. In all experiments, the redox mediator was the $[Fe_3(CN)_6]^{3-}$, applied potential for approach curves -0.6V vs. Ag/AgCl at different concentrations in buffer with pH 6.8.

III. RESULTS

From approach curves, the averaged current in 40-50 µm distance was used to compare measurements in different concentrations. We get that redox mediator in concentration from about 0.2 $mmol \cdot L^{-1}$ has no more influence to image resolution and stay constant (Fig. 3). Important factor is that concentration of redox mediator has to be small as possible. Influence of glucose concentration is different (Fig. 4). We get that about 15 $mmol \cdot L^{-1}$ glucose concentration in buffer is the point for good imaging resolution. Adding more glucose shows the same current in all the scanned picture and image is featureless. Fig. 5 shows GOx surface with and without glucose, scanned on the same place. Approach curves for different concentrations doesn't look the same, therefore the scanning distance from surface is bigger in the part a.

The current from various measurements can be compared in the same plot using (1). The approach curves with different solutions is plotted using normalized values

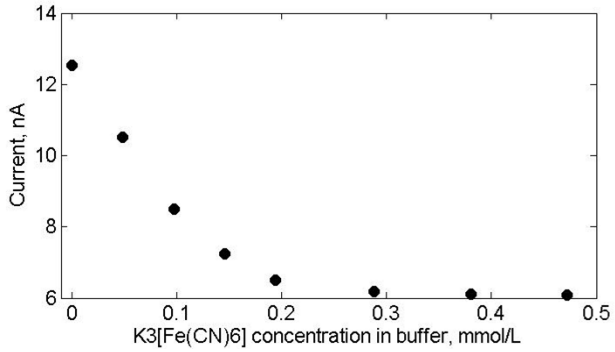


Fig. 3. Current dependence on mediator concentration in buffer with 10mmol·L^{-1} glucose, applied potential 0.6V vs. Ag/AgCl

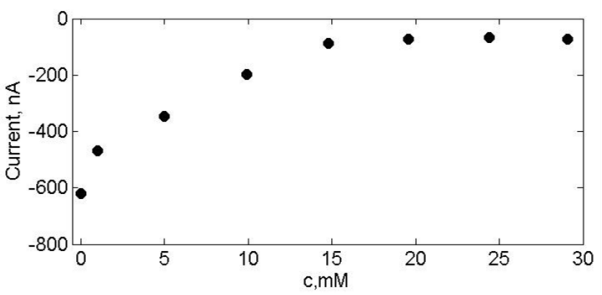
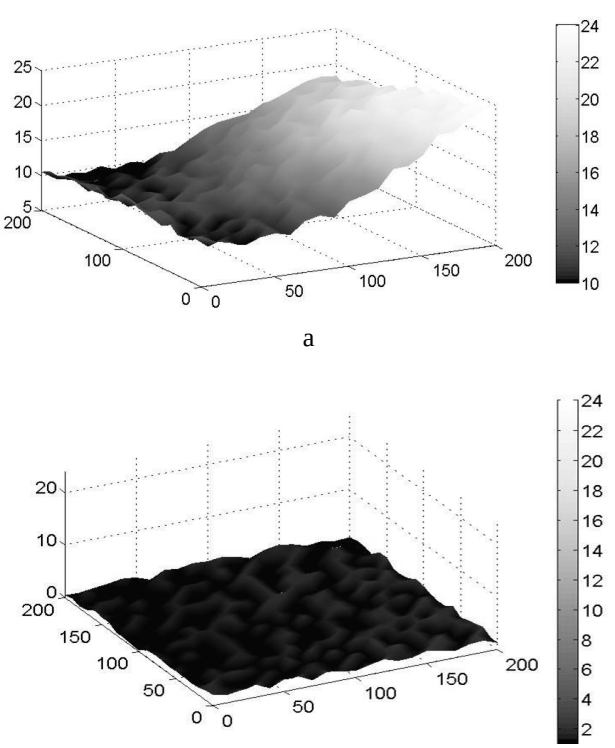


Fig. 4. Current dependence on glucose concentration in buffer without any mediator, applied potential -0.6 V vs. Ag/AgCl.



a

b

Fig. 5. Imaging the same place of GOx surface: a – 10 mmol·L^{-1} glucose in buffer; b – buffer. Applied potential +0.6V vs. Ag/AgCl

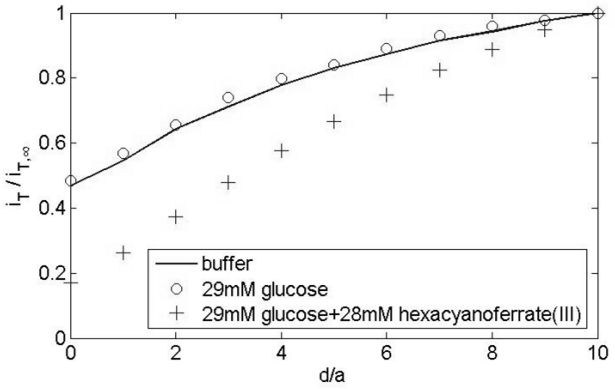


Fig. 6. Normalized current dependence on normalized distance in different solutions, approaching to the GOx surface. Applied potential -0.6V vs. Ag/AgCl

IV. CONCLUSION

Higher $K_3[Fe(CN)_6]$ concentration shows reduction of current, higher glucose concentration shows increase in current. Matlab program is successfully used for image processing, calculation and analysis of results.

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