



Short Communication

Scanning electrochemical impedance microscopy for investigation of glucose oxidase catalyzed reaction

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ABSTRACT

In this research biointerface based on immobilized glucose oxidase (GOx) was evaluated by scanning electrochemical impedance microscopy (SEIM), which consisted of merged scanning electrochemical microscopy (SECM) and electrochemical impedance spectroscopy (EIS). The gluconolactone, which is quickly hydrolyzed to gluconic acid, is produced during the enzyme-catalyzed glucose oxidation reaction. Gluconic acid formed above an enzyme-modified not-conducting plastic surface, was evaluated by EIS technique. A two electrode cell consisting of a scanning probe, which was based on 10 μm diameter ultramicroelectrode and stationary platinum counter/reference electrode was applied for the measurement. Locally measured solution impedance depends on the gluconic acid concentration close to the ultramicroelectrode surface and on the ion diffusion, which is hindered when the electrode is approaching close to the GOx-modified surface. EIS results were evaluated by applying an equivalent circuit consisting of elements representing solution resistance, double-layer capacitance, charge-transfer resistance and Warburg impedance. Solution resistance was calculated and showed to be dependent on the position of ultramicroelectrode. Also it was observed that the thickness of the conducting layer and gluconic acid concentration both are changing in time. The results indicate that here proposed SEIM technique could become a valuable tool for the investigation and characterization of enzyme-modified surfaces of biosensors and biofuel cells.

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1. Introduction

Electrochemical impedance spectroscopy (EIS) is a powerful, nondestructive and informative technique, which has been successfully applied for the characterization of glucose oxidase (GOx) based biosensor surfaces [1–5]. Moreover the EIS could be applied for glucose concentration measurements in electrochemical systems based on GOx-modified electrodes. However, conventional EIS based techniques represents only averaged response of the entire electrochemical system. In order to get more advanced mapping of electrochemical system scanning electrochemical microscopy (SECM) merged with EIS (SEIM) eventually could be applied. In SEIM based technique localized impedance measurements could be performed in the range of frequencies when the surface of interest is scanned by ultramicroelectrode (UME). The result of SEIM could be visualized by mapping one of calculated parameters, e.g. charge transfer resistance or double layer

capacitance as a function of 3D coordinates [6,7]. Conventional EIS produces averaged data, while the combination of SECM and EIS is providing information about local corrosion phenomena, which could help to identify the location of defects induced by corrosion [7]. In addition comparison of conventional EIS with localized EIS was done during the investigation of degradation of polyester coil-coated galvanized steel [8] and in the evaluation of the degradation of some coatings, which were based on deposited organic compounds or polymeric layers [9,10]. Another alternative technique, which was used for the determination of localized EIS data, is an impedance imaging combined with atomic force scanning electrochemical microscopy (AFM-SECM) [11]. In this technique the AFM-based topography and alternating current SECM (AC-SECM) based signals are recorded simultaneously with ring-shaped microelectrode, which is integrated on the AFM probe. Structured glass/gold substrate was evaluated by such advanced AFM probe and significant increase of current was registered when the probe was placed over spots consisting of gold [11].

The evaluation of enzymatic reaction intensity could be estimated by conventional SECM techniques [12,13]. However in conventional SECM the most commonly used electrochemical

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method is based on Faradaic response when fixed potential is applied to the UME in order to register the concentration of electrochemically active enzymatic reaction products [14,15]. Moreover, it should be noted, that the potential applied to the electrode drives the electrochemical system far from the equilibrium, and then the response to this perturbation is observed as disturbing signal. In order to avoid this problem the applicability of technique based on SECM in which the UME is modulated by selected frequency alternating current (AC-SECM) can be applied. The AC-SECM based technique also allows to study local corrosion processes in the entire solution volume without any redox mediator [16]. Using AC-SECM method the approach curves were recorded at several different frequencies and they demonstrated negative feedback behavior while the UME was approaching an insulating surface [17]. This phenomena is similar to that observed in Faradaic SECM based methods, however, different approach curves were observed when different frequencies were applied [18]. In order to advance this technique, the entire electrochemical impedance spectra can be registered at every measurement point and then the electrochemical system can be evaluated using the most suitable and informative equivalent circuits. This technique is called scanning electrochemical impedance microscopy (SEIM). In the case of SEIM conventional approach curves, which represent Ohmic resistance and/or other equivalent circuit parameters vs distance are plotted. The concept of SEIM was described recently in the evaluation of localized corrosion processes by Schuhmann's group [7,19]. When SEIM was applied for localized evaluation of corrosion processes the sample was minimally influenced by UME, which was applied as a scanning probe, and it was demonstrated that domains, which have different electrochemical properties, could be easily distinguished by SEIM even if they have similar topography and/or morphology [7]. In another research, impedance dependence on UME distance from insulating sample was revealed at feedback mode SEIM [20]. The spherical diffusion, which has most significant influence far from insulating surface, was evaluated by Cole–Cole impedance evaluation method, and the radial diffusion, which has most significant influence when the UME is approaching insulating surface, was evaluated by Cole–Davidson impedance evaluation method. It was determined that the low-frequency part of the EIS in a thin-layer between UME and surface of interest is controlled by both above mentioned types of diffusion. Moreover it was also demonstrated that the SEIM in feedback mode is suitable for constant-height based imaging. When SEIM was acting in feedback mode the local information on both topography and surface reactivity was obtained from the simultaneous analysis of the current and electrolyte-resistance variations [21]. Fundamental aspects of SEIM were investigated comparing UME responses while it was approaching to different surfaces e.g.: (i) insulator surface, (ii) conducting surface not-connected to electric circuit, and (iii) conducting surface, which was connected to electric circuit and was held at constant potential [22]. By this research it has been shown that the admittance of the UME located at relatively small distance from the surface of interest mostly depends on the distance between UME and the surface and on interfacial properties of the surface. Therefore the SEIM based imaging is informative even without any redox mediators. When AC-SECM and SECM methods are compared, it should be noted that the AC-SECM is performed at single frequency, while the SEIM is performed in broad range of frequencies what enables to select the most suitable equivalent circuit and to calculate number of EIS parameters. Therefore parameters, which are derived from SEIM evaluations, better describe an electrochemical system than the parameters that are derived from AC-SECM data. We believe that the SEIM could be suitable for the investigation of biosensor and biofuel cell surfaces in order to evaluate localized activity of immobilized enzymes and/or to perform

advanced evaluation of the diffusion of enzymatic reaction products.

The idea of this research is coming out from results of some investigations, which were described in earlier researches: (i) many researches were based on the EIS application for the evaluation of enzymatic reaction when the enzymes were immobilized on surfaces of electrodes [23]; (ii) conventional SECM is very often used for the investigation of enzyme-modified insulating surfaces while applying constant potential to UME [12–15]. Hence by assumption of previous achievements we have expected that it is possible to merge the EIS technique with SECM in order to get efficient SEIM system, which will be suitable for the evaluation of enzyme-modified surfaces. However, according to our best knowledge up to now no research, in the area of application of SEIM for the investigation of reaction product diffusion from enzyme-modified insulating surfaces, was reported.

The main aim of this research was to merge EIS with SECM into efficient SEIM system and, using developed SEIM for the investigation of solution conductivity at different positions, to demonstrate the suitability of SEIM for the evaluation of reaction product diffusion from enzyme-modified surface in real time.

2. Materials and methods

2.1. Materials

Glucose oxidase (EC 1.1.3.4, type VII, from *Aspergillus niger*, 215.3 units mg^{-1} , molar mass 160 kDa, polymer length 583 amino acids [24]) and 25% glutaraldehyde solution were purchased from Fluka Chemie GmbH (Buchs, Switzerland). D-(+)-Glucose was obtained from Carl Roth GmbH&Co (Karlsruhe, Germany). Before investigations glucose solutions were allowed to mutarotate overnight. All solutions were prepared in deionized water purified with Millipore S.A. (Molsheim, France).

2.2. Immobilization of glucose oxidase

A cylindrical poly(methyl methacrylate) (plastic) cell (diameter 55 mm, height 13 mm) was kept in a closed vessel above a 25% glutaraldehyde solution for 10 min to adsorb/graft glutaraldehyde onto the plastic surface. Afterwards 6 μL of 40 mg/ml GOx solution were dropped on the surface and dried in room temperature. Then the sample was again kept over 25% glutaraldehyde solution for 10 min to cross-link the enzyme and afterwards the GOx-modified surface was thoroughly washed with deionized water. Roughness of layer was measured by AFM: the roughness of unmodified plastic was 4 nm, while after enzyme immobilization it was 84 nm. Therefore supposed thickness of enzyme layer was 80 nm.

2.3. Impedance measurements

Scanning electrochemical microscope from Sensolytics (Bochum, Germany) was used for all experiments. The working electrode (WE) was situated as a SECM probe, which was approaching the substrate that was immersed in 0.1 mol L^{-1} glucose solution in deionized water. UME was purchased from Sensolytics GmbH (Bochum, Germany) and it was used as a working electrode. The UME was based on 5 μm radius platinum disk and RG (the ratio between the radius of entire electrode, which is including insulating part, and the radius of conducting part only) value was higher than 10 μm . Platinum wire was used as a counter/reference electrode (CE/RE). The distance between UME and substrate surface was determined by registration of current in feedback mode SECM while UME was approached to the surface of interest. EIS measurements were performed in the range of frequencies from 100 kHz to 20 mHz with sinusoidal

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