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Electrochemical Impedance Spectroscopy Based Evaluation of 1,10-Phenanthroline-5,6-dione and Glucose Oxidase Modified Graphite Electrode

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ABSTRACT

1,10-Phenanthroline-5,6-dione and glucose oxidase modified and unmodified graphite electrodes were analysed in buffer/glucose media by the electrochemical impedance spectroscopy (EIS) method. The EIS analysis was carried out under potentiostatic conditions. The gathered impedimetric data was evaluated applying estimated equivalent circuits. It was determined that equivalent circuit $R_\Omega(R_{F2}C_2)(C[R_FW])$ most optimally describes this electrochemical system. The study revealed redox mediating properties of 1,10-phenanthroline-5,6-dione deposited on graphite electrodes.

1. Introduction

Glucose oxidase (GOx) based electrodes are used in design of glucose biosensors [1] or biofuel cells [2]. GOx-based electrodes are suitable for the generation of electrical current, which is proportional to glucose concentration in the sample [1]. The reaction rate of GOx depends on electrolyte concentration, pH, temperature and presence and nature of redox mediators or other electron acceptors [3,4]. If GOx is used in biosensor design an efficient redox mediator, which is capable to transfer electrons efficiently from the active site of GOx to the electrode is required [5]. For example one natural electron acceptor of GOx is oxygen, but some other redox mediators can be used for the same purpose. Moreover there are several redox mediators that can compete with oxygen in electron transfer efficiency [6]. Various methods including optical microscopy [7], atomic force microscopy [8], and electrochemical methods, [9,10] can be applied to investigate properties of layers that are used for bioanalytical purposes. Electrochemical impedance spectroscopy (EIS) is one of the most informative out of many recently available electrochemical methods [10]. The EIS is based on electrochemical response of a system towards perturbations applied at different frequencies [10,11]. Using other electrochemical methods, such as amperometry or potentiometry there is always a problem of the measured system being non-linear because of changing electrochemical conditions, due to relatively high variation of electric current or voltage, and in this way influencing measured results. In potentiostatic EIS, which is based on constant electrode polarization voltage, a small sinusoidal perturbation of potential is usually applied. The amplitude of such perturbation usually is in the range of 5-20 mV. Such perturbation does not disturb the system's linearity and in this way it enables to acquire results, which are also linear in time and suitable for further analysis [12,13]. The EIS is mostly used in order to determine the double electric layer capacity and resistance of various modifiers, which carry the charge from electrolyte to electrode and also for the estimation of ion diffusion in solution towards the studied electrode [12]. Because of these abilities the EIS provides detailed information on characteristics of the electrochemical system. There are also some studies on the application of EIS for the characterization of enzyme-modified surfaces [13,14].

It was demonstrated that a phenanthroline derivative and glucose oxidase can be used together for the modification of electrodes,

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after which they can serve as glucose sensors or fuel cells [15]. The 1,10-phenanthroline-5,6-dione (PD), which was chosen for the research presented here, is known as an efficient redox mediator [16] and as a versatile bidentatic ligand in complexes with metal ions [17]. Formation of an enzyme-based layer on top of the electrode modified by this redox mediator provides unique opportunity to study redox properties of PD layer, when it accepts electrons from the enzyme and shuttles them to the electrode [18]. Because of this property, the 1,10-phenanthroline-5,6-dione based layer is expected to act as a conductor rather than an insulator [19]. On the other hand it is known that the transfer rate of charges, which are moving through the enzyme (e.g. GOx), is 'slowed down' due to relatively slow enzymatic electron exchange processes. It indicates that the enzyme is a poor conductor but a fairly good charge holder [6,20]. During preliminary evaluation of 1,10-phenanthroline-5,6-dione and glucose oxidase modified electrode basic $R_{\Omega}(R_A C_A)$ circuit was applied [20], however the circuit well fitted with experimental results only in very narrow frequency range from 1390 Hz to 14.37 mHz.

Therefore the aim of this research was advanced EIS-based evaluation of 1,10-phenanthroline-5,6-dione and glucose oxidase modified electrodes, in order to adjust most suitable equivalent circuit, which is describing this electrochemical system.

2. Experimental

2.1. Chemicals and equipment

Glucose oxidase (GOx) from a fungus *Aspergillus niger* (E.C.1.1.3.4.) 295 Umg^{-1} and 25% glutaraldehyde were purchased from AppliChem GmbH (Darmstadt, Germany). D-(+)-Glucose was obtained from Carl Roth GmbH&Co (Karlsruhe, Germany). The 0.04 M solution of glucose was prepared in a phosphate – acetate buffer, pH 6.0, (A-PBS) at least 24 hours before use, in order to reach equilibrium of α- and β-forms glucose was allowed to mutarotate. The 10.0 $mgmL^{-1}$ glucose oxidase solution was prepared by mixing the enzyme in A-PBS, pH 6.0, containing 50.0 mM sodium acetate and 50.0 mM sodium phosphate with 100.0 mM KCl. The 10.0 $mgmL^{-1}$ solution of 1,10-phenanthroline-5,6-dione in acetonitrile was prepared. All other chemicals were commercially available and were purchased from global suppliers of 'analytical grade' purity.

The electrochemical impedance spectroscopy measurements were performed in a three-electrode electrochemical cell inside of a Faraday-cage with AUTOLAB PGSTAT30 at ambient temperature (25 °C). Potentiostat/Galvanostat was controlled by the frequency response analyser (FRA) software Eco Chemie (Utrecht, Netherlands). Ag/AgCl electrode in saturated KCl ($Ag/AgCl/KCl_{sat}$) from Metrohm was used as reference electrode. Handcrafted graphite rod (GR) auxiliary electrode was constructed from 3 mm diameter×40 mm length, 99.999% graphite rod, which was purchased from Sigma–Aldrich (Berlin, Germany). Bare or modified graphite rod electrodes were used as working electrodes, depending on requirements of the experiment. The cell's electrochemical impedance spectrum was recorded under potentiostatic conditions at 0.0 V *vs* $Ag/AgCl/KCl_{sat}$. Although the selected potential of 0.0 V is not the best for the charge transfer process [19], the 0.0 V potential was chosen as the most informative for EIS evaluation, because at this potential the initial spectrum of a graphite electrode modified with 1,10-phenanthroline-5,6-dione and glucose oxidase, in buffer solution possesses a more informative characteristic shape (Fig. 1), which is suitable for the evaluation of layered electrochemical system. Also the 0.0 V potential allows to avoid additional influence of electrode polarisation on formation of complicated ion layers on the electrode surface. The EIS spectra for further data analysis are presented as Nyquist plots.

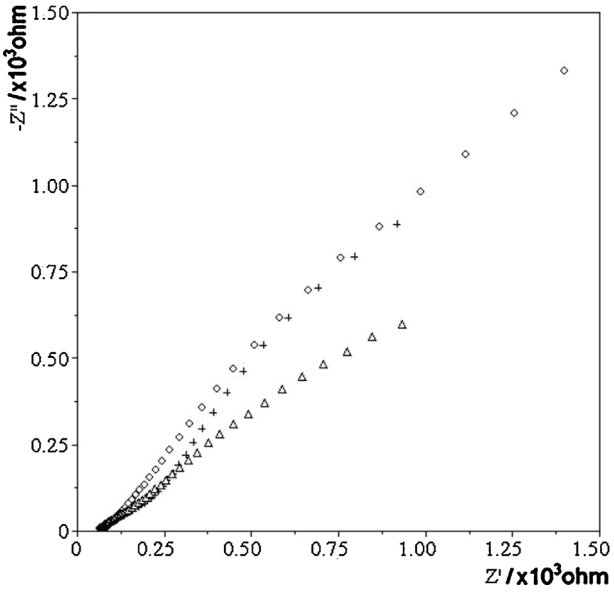

Fig. 1. Initial EIS spectrum for potential selection of graphite electrode modified with 1,10-phenanthroline-5,6-dione and in buffer solution. Plus (+) symbolized data series was gathered at the potential of 0.0 V, triangle (Δ) symbolized data series at 0.1 V and rhomb (◊) symbolized data series at 0.5 V vs Ag/AgCl/KClsat.. The impedance spectra were recorded in the logarithmic frequency range from 40 kHz to 8 mHz while applying 10 mV sinusoidal perturbation amplitude.

2.2. Electrode preparation, modification and characterization

In order to obtain a clean electrode surface, the surface of the graphite electrode was polished with fine emery paper. Then the electrodes for 10 minutes were boiled in a 4:1 mixture according to volume of 25% ammonia and 30% H_2O_2. After this they were washed with acetone, ethanol and distilled water prior to use, and dried at room temperature. To achieve the best results the edge of the graphite rod surface was limited to only a circular disk of 0.071 cm^2 of geometrical surface, which was polished to mirror smoothness in order to minimize the charge holding double electric layer disturbance and to have a similar roughness of all electrodes. Preparation of enzyme-modified electrodes was performed as previously described in other researches [21,22]. In order to obtain a 1,10-phenanthroline-5,6-dione and glucose oxidase–modified graphite (GR/PD/GOx) electrode surface: (i) firstly the GR electrode was covered by 1,10-phenanthroline-5,6-dione, for this 3.0 μL of 1,10-phenanthroline-5,6-dione 10.0 $mgmL^{-1}$ solution was distributed on the surface of GR electrode; (ii) then, the immobilization of glucose oxidase onto the 1,10-phenanthroline-5,6-dione modified graphite (GR/PD) electrode was performed by procedure described previously [22]. The electrodes were evaluated by EIS during different steps of preparation: (i) bare GR electrode, then (ii) developed GR/PD electrode, after this (iii) formed GR/PD/GOx in buffer and (iv) in buffer/glucose solution was investigated. Then the EIS spectra were evaluated by adjusting several equivalent circuits and their parameters were calculated in order to achieve the best fits between measured results and theoretical values [23].

3. Results and discussion

3.1. Electrochemical impedance spectroscopy characterizations of electrode at different modification steps

Glucose oxidase alone cannot transfer electrons to the electrode directly and for this reason a charge transferring agent is necessary. The 1,10-phenanthroline-5,6-dione is quite compatible with GOx [15,18]. Active site of glucose oxidase is oxidizing