



Single-step procedure for the modification of graphite electrode by composite layer based on polypyrrole, Prussian blue and glucose oxidase



Arunas Ramanavicius^{a,b,*}, Asta Inesė Rekertaitė^a, Raimondas Valiūnas^a, Aušra Valiūnienė^a

^a Department of Physical Chemistry, Faculty of Chemistry, Vilnius University, Naugarduko St. 24, LT-03225 Vilnius, Lithuania

^b Laboratory of NanoBioTechnology, Institute of Semiconductor Physics, State Research Institute Centre for Physical and Technological Sciences, A. Gostauto g. 11, LT-01108 Vilnius, Lithuania

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ABSTRACT

In this research single-step procedure for the modification of graphite electrode by polypyrrole (PPy), Prussian blue (PB) and glucose oxidase (GOx) based composite layer (PPy/PB/GOx) is reported. The PPy/PB/GOx-modified electrode was applied in the design of glucose biosensor. Optimal potential for the registration of amperometric response towards glucose was at +0.05 V vs Ag/AgCl_(KCl_{sat.}). Preliminary optimization of initial concentration of pyrrole in initial solution, which was used for the formation of PPy/PB/GOx layer, was performed. The sensitivity of several different PPy/PB/GOx-modified graphite electrodes, which were modified by PPy/PB/GOx layer at different pyrrole concentrations, was evaluated, and it was in a range of 1.0–1.9 μA cm⁻² mM⁻¹.

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1. Introduction

The redox activity of Prussian blue (PB), towards hydrogen peroxide, which is reduced by PB is of high importance in bioelectroanalytical chemistry [1]. Due to redox and catalytic activity PB and some its analogues have found a number of applications in electro-analytical devices and bioelectrochemical systems [2], e.g.: very stable hydrogen peroxide transducers were made by sequential deposition of the iron- and nickel-hexacyanoferrate layers; The layers based on both compounds demonstrated superior chemical and mechanical stability, therefore they are suitable for the development of glucose sensors [3]. PB is mostly used in the forms of electroactive layers, which could be chemically or electrochemically deposited onto the electrode surface [2]. Electrochemical deposition of PB on conductive surfaces is more efficient since it could be easily controlled by switching on/off electrical current. This procedure is usually carried out in aqueous solutions containing a mixture of Fe³⁺ and ferricyanide ([Fe(CN)₆]³⁻) ions,

either spontaneously at open-circuit regime or by applying a reductive electrochemical driving force [2]. PB-based glucose oxidase (GOx) modified electrode were successfully applied in the design of flow-injection biosensors, which were applied for the analysis of residual glucose in wines [4]. Moreover, the electrochemical formation of PB and homologous compounds allows the modification of microelectrodes and in this way some most promising structures consisting of alternating pattern of three layers of PB and three layers of Ni-HCF were applied for the modification of ultra-micro electrodes, which were used as sensors in scanning electrochemical microscopy based imaging of local hydrogen peroxide evolution in oxidase-based systems [5].

Next challenging problem in the development of bioelectrochemical systems is proper immobilization of biocatalysts. However, this procedure is not always successful due to very different natures of electrode material and protein-based biocatalysts, which are selected for the immobilization. Conducting polymers could be applied in the design of biosensors as a matrixes for the immobilization of enzymes and by this method electrocatalytic properties of the enzymes immobilized within layer of conducting polymers could be tuned and adjusted according to the requirements of electroanalytical system [6]. Moreover conducting polymers, such as polypyrrole (PPy), allows co-entrapment of

* Corresponding author at: Department of Physical Chemistry, Faculty of Chemistry, Vilnius University, Naugarduko St. 24, LT-03225 Vilnius, Lithuania.
E-mail address: arunas.ramanavicius@chf.vu.lt (A. Ramanavicius).

additional redox compounds and/or various nano-structures (e.g. gold nanoparticles, carbon nanotubes, etc.) that are facilitating the development of electroanalytical signal [7,8]. Recent attempts to create PPy/PB composites demonstrated that such composites are very suitable for the reduction of hydrogen peroxide [9–12]. PPy/PB composite could be very promising for oxidase-based biosensor design, because such biosensors are based on generation/detection of hydrogen peroxide. Among hundreds of recently used oxidases the glucose oxidase (GOx) is mostly used as a model enzyme, which could be applied in bioanalytical systems and biosensors [13].

During this research electrocatalytic properties of PB [1–5] and good compatibility of polypyrrole with biomaterials including GOx [14] were taken into account, and the main aim of this study was to develop simple, one-step based procedure, which will be suitable for the modification of graphite rod electrodes by PPy, GOx and PB based composite (PPy/PB/GOx).

2. Experimental

2.1. Materials

All solutions were prepared using ultrafiltrated water. All inorganic salts (highest purity), glucose oxidase (360 unit mg^{-1}) and glucose were purchased from ROTH (Germany). The solution of 0.1 M NaCl and 0.01 M NaH_2PO_4 , pH 7.3, was used as a buffer. Pyrrole was supplied by Alfa Aesar (Germany) and purified as described previously [6,7].

2.2. Preparation and characterization of PPy/PB/GOx-modified electrodes

6 mm diameter graphite rods (0.28 cm^2) were used as working electrodes that were modified by PPy/PB/GOx layer. Before deposition the surface of the working graphite electrodes was mechanically polished until a mirror finish and then electrodes were sonicated in an ultrasonic bath for 3 min. Then the electro-deposition of the PPy/PB/GOx layer was performed using 20 potential cycles in the range from 0.4 to 0.8 V at a sweep rate of 0.04 V s^{-1} . The solution, which was used for the electro-deposition of graphite electrode, contained 1 mM of FeCl_2 , 1 mM of $\text{K}_4[\text{Fe}(\text{CN})_6]$, 10–20 mM of pyrrole and 1 mg mL^{-1} of glucose oxidase. The electro-deposition of PPy/PB/GOx and investigations were performed by potentiostat/galvanostat $\mu\text{AUTOLAB}$ from ECO-Chemie (Utrecht, the Netherlands) in three-electrode mode. A platinum wire was used as an auxiliary electrode. The reference electrode was an $\text{Ag}/\text{AgCl}_{(\text{KCl}_{\text{sat}})}$ electrode, placed as close as possible to the working electrode by the mean of Luggin capillary.

2.3. Amperometric evaluation of biosensor

Amperometric responses of biosensor were evaluated in 0.1 M phosphate buffer, pH 7.3, at 0.05 V vs $\text{Ag}/\text{AgCl}_{(\text{KCl}_{\text{sat}})}$. When the background current reached a stable value, then corresponding amounts of glucose solution were added into the cell under stirring. Then the resulting current was recorded and the difference from background current was evaluated as analytical signal. All measurements were performed at room temperature (25°C).

3. Results and discussion

PPy/PB/GOx composite layer was deposited by one-step based electrochemical procedure (Fig. 1). By this the PB was formed directly within growing layer conducting polymer – Ppy – and contemporaneously the enzyme was immobilized within formed pyrrole. The deposition of PB on various conductive surfaces is usu-

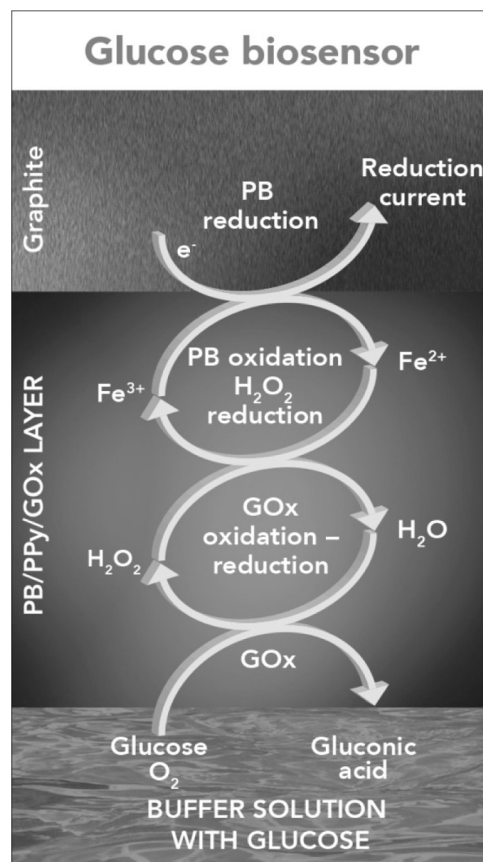


Fig. 1. Schematic representation of one-step based modification of graphite electrode by PPy/PB/GOx layer.

ally carried out from aqueous solutions containing a mixture of iron(III) (Fe^{3+}) and ferricyanide ($[\text{FeIII}(\text{CN})_6]^{3-}$) ions [2]. However, in order to keep higher activity of immobilized GOx the solution of FeCl_3 could not be used because it is stable only at relatively low pH, which is harmful for GOx. Therefore during here proposed deposition in initial solution we have replaced both in synthesis of PB traditionally used FeCl_3 and $\text{K}_3[\text{Fe}(\text{CN})_6]$ by FeCl_2 and $\text{K}_4[\text{Fe}(\text{CN})_6]$, respectively. Therefore in here proposed PB synthesis protocol the Fe^{3+} ions, which are required for the synthesis of PB, were formed in close proximity to the electrode by oxidation of FeCl_2 when sufficiently high electrode potential was reached during the potential cycling. PB was produced according to this reaction:



Simultaneously to the formation of PB pyrrole monomers also started to form the PPy layer at higher oxidative potentials and under chemical polymerization induced by formed Fe^{3+} ions. Moreover the GOx become entrapped within formed PPy layer in close proximity to the PB formed on the electrode surface. For the PB it is very beneficiary to be in close range to immobilized GOx, because then higher concentrations of hydrogen peroxide, which is formed in catalytic action of GOx, are reaching the surface of PB particles and significantly higher surface area of PB is capable to be involved into catalytic action.

In order to investigate the influence of ratio between formed PPy and entrapped GOx in the PPy/PB/GOx composite, several different PPy/PB/GOx layers were formed from solutions with different initial pyrrole concentrations.

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