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Electrochemical deposition of gold nanoparticles on graphite rod for glucose biosensing

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ABSTRACT

A biosensor based on glucose oxidase (GOx) immobilized on gold nanoparticles (Au–NPs) electrochemically predeposited on the surface of graphite rod (GR) electrode was developed (GOx/Au-NPs/GR). Main analytical characteristics of this biosensor were determined and compared with those determined using a biosensor setup without Au-NP modification (GOx/GR). The highest analytical signal of GOx/Au-NPs/GR electrode was observed after 13 nm Au-NP deposition on the electrode from 0.8 nmol L^{-1} solution lasting 20 min, when cyclic voltammetry was performed in the range from 0.0 to +1.0 V *vs* Ag/AgCl. The best analytical characteristics of the developed biosensor were obtained after 25 mg mL^{-1} GOx immobilization on the Au-NPs/GR electrode. Analytical signal registered using GOx/Au-NPs/GR electrode was 2.08 times higher in comparison to GOx/GR electrode. The registered currents of both electrodes were linearly dependent on glucose concentration in the range of 0.1–10 mmol L^{-1}. The developed GOx/Au-NPs/GR electrode was characterized by high sensitivity, which was equal to 101.02 μA mM^{-1} cm^{-2} in the linear glucose detection range. The limit of detection was 0.083 mmol L^{-1} with relative standard deviation of 6% for GOx/Au-NPs/GR electrode. This study demonstrates a successful practical exploitation of the developed biosensor in a human serum sample.

1. Introduction

Recently scientific and industrial impact of nanoscience and nanotechnology in analytical electrochemistry has been growing. Some challenging bioanalytical problems, such as sensitivity, specificity, reproducibility and reliability can be resolved by applying nanostructure-based electrochemical biosensors [1–4]. Nanotechnological methods enable to miniaturize biosensors and make them suitable for rapid and low cost analyte detection in low volume samples [5] or reliable for continuous analyte monitoring by implantable bioanalytical devices. Electrochemical biosensors have been applied in many areas, such as food industry, agriculture, military, veterinary, clinical applications, and environment [3,4,6].

Enzymes, which are mostly used in electrochemical biosensor design, are usually immobilized on various solid conducting supports by passive physical adsorption, covalent attachment, encapsulation or entrapment within an ultrathin polymeric film with an ultrahigh density [7,8]. Electrochemical biosensors with immobilized enzymes are characterized by high selectivity, sensitivity, reproducibility. In addition sometimes they are suitable for continuous and *in situ* monitoring of analyte in a complex matrixes [3,9,10]. Nowadays research is focused on the improvement of sensor properties by new sensing approaches. A noteworthy tendency in biosensor design is mainly based on advantages of nanotechnology, which allow reducing dimensions at the nanoscale, constructing arrays for high throughput analysis with the integration of microfluidics, and enhancing the performance of the biological components by using new nanomaterials [5]. Successful glucose biosensors are likely to be small, not expensive and portable, to reach the interest of millions of diabetic patients, which have daily need to perform glucose test in a simple way. In order to increase durability of electrochemical biosensors the biological components should display high storage and operational stability.

Different methods for electron transfer between active site of the redox enzyme and the surface of electrode were developed, including the application of diffusional redox mediators [11–13], incorporation of electron relaying redox centers to the proteins [14,15] and the immobilization of redox proteins within

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electroactive polymers [16,17]. The application of nanomaterials (nanoparticles, nanotubes, nanofibers, nanowires and nanocomposites) in the fabrication of biosensor allows the improvement of signal transduction. Usually nanoparticles, including metal and oxide nanoparticles, semiconductor and composite nanoparticles, exhibit unique chemical, physical and electronic properties that are different from these of bulk materials and such nanoparticles can be used for the construction of new electrochemical sensors and biosensors [1,6,18,19]. Nanoparticles can be used in electrochemical biosensor design as (i) substrates for biomolecule immobilization, (ii) catalysts of electrochemical reactions, and (iii) enhancers of electron transfer [6]. Gold nanoparticles of different diameters with relatively high monodispersity could be prepared by chemical reduction of gold salt in the presence of agents, which binds to nanoparticle surface to impart higher stability [1,20,21]. Colloidal solution of gold nanoparticles has a long shelf-life, it is non-toxic and has some amazing benefits for a wide variety of applications. The incorporation of nanoparticles into redox enzymes could provide a hybrid electrically active biomaterial. Au-NPs with the appropriate dimensions and functionalization adjacent to the enzyme redox center could act as an electron relay to a macroelectrode [22].

Among many methods, which are suitable for the deposition of Au-NPs on the electrode surface, electrochemical methods are very attractive. Au-NPs assembled on the electrode surface *via* electrostatic interaction or covalent bond formation enhance the electrode conductivity, facilitate the electron transfer and improve the analytical sensitivity, selectivity and stability of biosensors [22–24]. These systems based on directly electrochemicaly deposited biocomposite consisting of chitosan hydrogel, glucose oxidase, and gold nanoparticles exhibited a rapid response, low detection limit of glucose and high stability due to strong adsorption and prevention of leakage of enzyme [25]. A novel amperometric glucose biosensor based on Pt electrode coated by polyvinylferrocene film and Au-NPs was developed [26]. The electrocatalytic effect of Au-NPs on enzymatically generated H_2O_2 offers more sensitive and selective monitoring of glucose than that in biosensors without any Au-NPs. To achieve high sensitivity and the linear current dependence on glucose concentration (in the range of 1–10 mmol L^{-1}) electrochemical synthesis of Au-NPs on multiwall carbon nanotubes from $HAuCl_4$ solution by potential cycling from 1.0 to 0.0 V was performed [27]. The electrochemical deposition of Au-silicate-GOx biocomposite could be achieved from sol of Au-NPs containing GOx by potential cycling between −0.4 and 1.0 V *vs* Ag/AgCl [28]. This co-entrapment of glucose oxidase in a gold nanoparticle-silicate network imparts biocatalytic activity of the film and increases operational and long-term stability of designed biosensor. Another biosensor based on gold nanoparticles electrochemically deposited from $HAuCl_4$ solutions under the constant −0.2 V *vs* SCE on the surface of glassy carbon electrode showed good performance in electrochemical oxidation of tryptophan [21]. Smaller nanoparticles are more suitable for enzyme immobilization [6,29], because changes of protein structure and function upon adsorption are lower on higher curvature surfaces [30]. Nanoparticles could act as nano-scaled electrodes and therefore they are often used in the design of electrochemical biosensors [1,20].

The main aim of this study was the development of glucose biosensors based on glucose oxidase immobilized on a graphite rod electrode precoated by electrochemically deposited gold nanoparticles from a colloid solution. The optimal concentrations of Au-NPs, GOx, and a redox mediator were selected, electron transfer between GOx and electrode in the presence of a soluble redox mediator and immobilized gold nanoparticles was evaluated, and analytical characteristics of glucose biosensors were assessed. The newly developed electrodes were used for determination of glucose in human serum samples.

2. Materials and methods

2.1. Materials

Glucose oxidase (EC 1.1.3.4, type VII, from *Aspergillus niger*, 215.3 unit mg^{-1} protein) and *N*-methylphenazonium methyl sulphate (PMS) were purchased from Fluka and Sigma-Aldrich (Buchs, Switzerland), respectively. D(+)-glucose, D(+)-saccharose, D(+)-xylose, D(+)-galactose, D(+)-mannose, D(-)-fructose, tetrachloroauric acid ($HAuCl_4 \cdot 3H_2O$) and tannic acid were obtained from Carl Roth GmbH&Co (Karlsruhe, Germany), sodium citrate – from Penta (Praha, Czech Republic), hydrochloric acid 37% – from Acta Medica (Hradec Kralove, Czech Republic). Before investigations glucose solution was stored overnight to reach equilibrium between α and β optical isomers. All other chemicals used in the present study were either analytically pure or of highest quality. All solutions were prepared using deionized water purified with water purification system Millipore S.A. (Molsheim, France). The solution of sodium acetate (SA) buffer (0.05 mol L^{-1} $CH_3COONa \cdot 3H_2O$) with 0.1 mol L^{-1} KCl was prepared by mixing of sodium acetate trihydrate and potassium chloride, which were obtained from Reanal (Budapest, Hungary) and Lachema (Neratovice, Czech Republic). Graphite rods (3 mm diameter, 99.999%, low density) were purchased from Sigma-Aldrich (St. Louis, USA), alumina powder (grain diameter 0.3 μm, Type N) – from Electron Microscopy Sciences (Hatfield, USA), 25% glutaraldehyde solution, L-ascorbic acid and uric acid – from Fluka Chemie GmbH (Buchs, Switzerland) and AppliChem GmbH (Darmstadt, Germany).

2.2. Synthesis of gold nanoparticles

The 13 nm diameter Au-NPs were synthesized by the reduction of $HAuCl_4 \cdot 3H_2O$ by sodium citrate in the presence of tannic acid as previously reported [19]. The concentration of 13 nm Au-NPs was calculated according to the amount of starting material, density of gold, the approximate diameter of nanoparticles, and assuming that the reaction yield is 100%. It was determined that concentration of Au-NPs is 3.6 nmol L^{-1} [31]. The solution of 13 nm Au-NPs was stored in dark glass flask at +4 °C.

2.3. Pre-treatment of the working electrode

The working surface area of graphite rod electrodes was 0.071 cm^2. Graphite rod was cut and polished on fine emery paper and then polished by slurry of alumina powder containing 0.3 μm grains of Al_2O_3. After this the surface of electrodes was rinsed with distilled water, dried at 20 ± 2 °C and sealed into silicone tube in order to prevent contact of the electrode side surface with the solution.

2.4. Optimization of electrochemical Au-NP deposition using cyclic voltammetry (CV)

For the preparation of GOx/Au-NPs/GR electrodes the graphite rod was placed in the 0.1 nmol L^{-1} solution of 13 nm Au-NPs, where gold nanoparticles were electrochemically deposited on the surface of electrodes using a computerized potentiostat PGSTAT 30/Autolab (EcoChemie, The Netherlands) with GPES 4.9 software. CV mode with scan rate of 0.05 V s^{-1} was applied. For the evaluation of the optimal electrode modification time Au-NPs were deposited on GR electrodes for different periods of time ranging from 5 to 45 min and using cycling potentials from 0.0 to +1.0 V *vs* Ag/AgCl. The optimization of cycling potential was performed in the range from 0.0, −0.25, −0.5, −0.75, −1.0 to +1.0 V and from +0.25, +0.5, +0.75, +1.0 to −1.0 V *vs* Ag/AgCl. After enzyme immobilization the analytical signal was evaluated at 1.08, 6.14 and 17.3 mmol L^{-1} glucose in