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A functional immobilization matrix based on a conducting polymer and functionalized gold nanoparticles: Synthesis and its application as an amperometric glucose biosensor

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ABSTRACT

Combination of nanoparticles and biomolecules attracted considerable attention in biosensing applications. In this study, effective surface design was investigated by modifying the electrode surface with pristine and functionalized gold nanoparticles. For this purpose, spherical gold nanoparticles were synthesized and characterized with UV–vis spectroscopy and transmission electron microscopy (TEM) analyses. Then, gold nanoparticles were modified with mercaptopropionic acid (MPA) yielding Au–S bonds (Au NPs/MPA). Moreover, a novel functional monomer, 6-(4,7-bis(2,3-dihydrothieno [3,4-*b*][1,4] dioxin-5-yl)-2*H*-benzo[*d*][1,2,3]triazol-2-yl)hexan-1-amine (BEDOA-6), was synthesized and used as an immobilization matrix for glucose biosensor. After successful electrochemical deposition of the polymer; poly(BEDOA-6) on graphite electrodes, immobilization of glucose oxidase (GOx) was carried out covalently with the help of crosslinking agent. During immobilization, Au NPs and Au NPs/MPA were used in biosensor fabrication in order to achieve the most effective surface design for target biosensor. In addition, SEM and fluorescence analyses were utilized to characterize the surface properties. The biosensor shows a wide linear range between 0.025 mM and 1.25 mM glucose concentration with a low detection limit of 0.025 mM. Also, kinetic parameters, operational and storage stabilities were determined. Finally, the biosensor was tested on beverages for glucose detection.

1. Introduction

Nanomaterials exhibit remarkable properties and due to the constantly growing demand for high and long-term efficiency in every kind of research, nanomaterials are always preferred due to their distinct properties [1]. Particularly, gold nanoparticles with contributions of their localized surface plasmon resonance, improved light absorption, electron transport, excellent conducting properties as well as self-assemble structures [2] become convenient candidates for wide range of applications such as solar cells, electronic and biomedical applications [3].

As a class of organic conductive materials, conducting polymers with a conjugated π-electron system are a booming and increscent field to be investigated. They can be functionalized upon interest and used for various kinds of purposes. The polymer structure can be tuned according to the properties of resultant materials and widely applied in the construction of electrical, optical and biomedical devices. The introduction of nanomaterials into conducting polymers attracted attention in material science since both production of nanoparticles and conducting polymer is simple and easy to fabricate [4]. The synergetic effect due to the combination of these two valuable materials brings many advantages such as enhanced conductivity [5]. Moreover, in electronically important systems, introduction of nanoparticles can also provide substantial electronic interaction with the polymer which improves the charge

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transfer. Additionally, the charge can be travel along the conducting polymer chain and transferred to the desired positions via nanoparticles which bring on an improved electronic activity of the composite material [6].

In biological applications, it is aimed to interface biomolecules with polymers in a convenient way while maintaining the concrete structure of biomolecules in a sensitive and stabilized manner. Introduction of nanomaterials especially gold nanoparticles (Au NPs) conclude excellent results in bioanalytical applications [7]. Taking the advantage of simple and reproducible formation of organic polymer film via electropolymerization and covalent and stable immobilization of both gold nanoparticles and biomolecules, sensitive and reproducible structures can be constructed [8]. The use of Au NPs in addition to the prominent properties of conducting polymers leads the stacking of NPs. These combined structures pioneer raise in stability and functionality in the applications [2c].

Biosensors have attracted great interest throughout the world in last decades. They are considerably used in different areas like clinical diagnosis, food technology, biotechnology, environmental monitoring due to their fast response and ease of fabrication [9]. Conducting polymers (CPs) as immobilization platforms provide improvements in biosensor design. They offer extensive stability of enzymes on the electrode surface [10]. In an enzymatic biosensor, it is crucial to immobilize enzyme molecules onto the substrate stable and for long-term without activity lost. For this reason, conducting polymers are excellent materials with their structure, electronic character and compatibility. Furthermore, they can be functionalized and used in covalent immobilization technique to achieve a stronger biosensor construction.

The combination of nanomaterials and biomolecules in biorecognition process attracted considerable attention. Nanoparticles of noble metals especially gold nanoparticles (Au NPs) can play an important role in the construction of biosensors due to their large specific area, excellent biocompatibility, good conductivity capability, desirable catalytic properties and small size [11].

In biosensor fabrication, Au NPs are recently used and one of the most effective methods is binding gold nanoparticles via functionalization as a self-assembled monolayer (SAM). This modification takes advantages of increased surface area of three-dimensional electrode surfaces [12]. Sulfur containing compounds like alkanethiol have high affinity to metals. In recent studies, Au NPs are self-assembled with short-chain molecules such as cysteamine (Cyst) and 3-mercaptopropionic acid (MPA) [13]. Biosensors fabricated with SAM technique can possess high sensitivity and short response time.

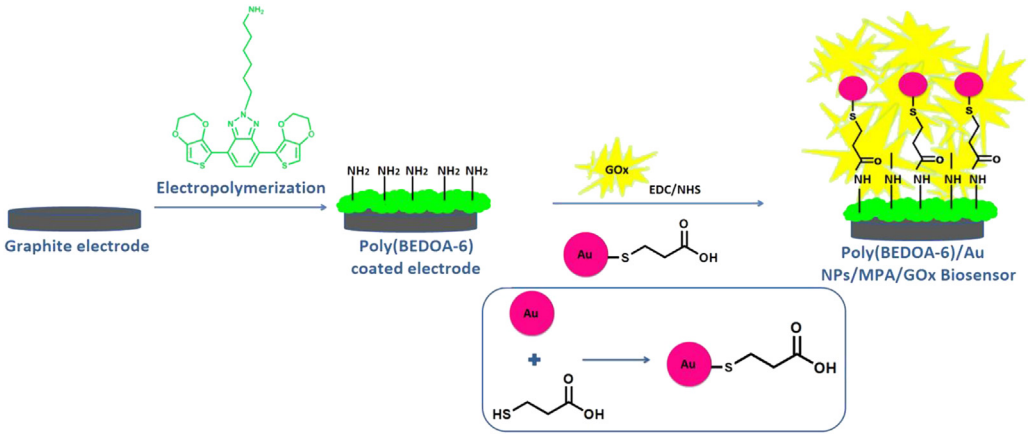
MPA is a bifunctional molecule containing both thiol and carboxylic acid functional groups. The thiol groups serve as binding sites for covalent attachment of MPA to Au NPs. Moreover, the carboxylic acid groups can further react covalently with amino groups of a functional material and also with the enzyme molecules in order to achieve effective immobilization and increase lifetime stability [10c].

In this study, conducting polymer of 6-(4,7-bis(2,3-dihydrothieno[3,4-*b*][1,4]dioxin-5-yl)-2*H*-benzo[*d*][1,2,3]triazol-2-yl)hexan-1-amine (BEDOA-6) was used as the immobilization matrix for enzymes to achieve an effective surface for biosensor. The synthesis of the monomer, BEDOA-6, and its electrochemical polymerization and characterization were previously described [14]. The polymer backbone bearing pendant amino groups serves as an excellent immobilization patterning forming covalent attachment of enzymes while facilitating electron transfer. Gold nanoparticles were functionalized with mercaptopropionic acid. MPA monolayers were first self assembled on gold nanoparticles via Au—S bond. In constructing the biosensor, after polymerization of the monomer on a graphite electrode, immobilization of glucose oxidase (GOx) and Au NPs/MPA (SAM) onto the polymer surface were achieved through covalent binding using two step carbodiimide coupling method, simultaneously [15]. This procedure results in a stable biosensing interface and outstanding stability and sensitivity. GOx was used as the model enzyme in this study. GOx from *Aspergillus niger* catalyzes the oxidation of β-glucose to δ-gluconolactone in the presence of molecular oxygen which is subsequently hydrolyzed into gluconic acid [16]. Scheme 1 displays the procedure for the construction of the proposed amperometric glucose biosensor. The biosensor was characterized and applied for the estimation of glucose contents in various beverages successfully.

2. Experimental

2.1. Materials

Glucose oxidase (GOx, β-D-glucose: oxygen 1-oxidoreductase, EC 1.1.3.4, 39,800 units/g) from *A. niger*, D-glucose were purchased from Sigma (St. Louis, USA; www.sigmaaldrich.com). Dichloromethane (DCM) was obtained from Merck (Darmstadt, Germany; www.merck.com). Tetrabutylammonium hexafluorophosphate ($TBAPF_6$) was supplied by Aldrich. All chemicals for the synthesis of the monomer were purchased from Aldrich except tetrahydrofuran (THF) which was obtained from Acros (Geel, Belgium, www.acros.com). N-Hydroxysuccinimide (NHS) and N-(3-dimethyl aminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) were purchased from Fluka (Buchs, Switzerland) and Sigma, respectively. All other chemicals were analytical grade. For nanoparticle



Scheme 1. Schematic representation of poly(BEDOA-6)/Au NPs/MPA/GOx biosensor.

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