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A novel functional conducting polymer as an immobilization platform

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ABSTRACT

Here, we present the fabrication of conducting polymer based enzymatic and microbial biosensors. To obtain immobilization platforms for both pyranose oxidase (PyOx) and *Gluconobacter oxydans*, the graphite electrode surface was modified with the polymer of 4-amino-N-(2,5-di(thiophen-2-yl)-1H-pyrrol-1-yl)benzamide (HKCN) which has free amino groups on the surface for further bioconjugation reactions with the biomolecules. Initially, the electrode surface was covered with HKCN via electropolymerization. Then, either PyOx or *G. oxydans* cell was stabilized using glutaraldehyde as a cross-linker. After optimization of biosensors, analytical characterization and surface imaging studies were investigated. The change of current depends on glucose concentration between 0.05–1.0 mM and 0.25–2.5 mM with HKCN/PyOx and HKCN/*G. oxydans* biosensors in batch systems. Also, the calibration graphs were obtained for glucose in FIA mode, and in this case, linear ranges were found to be 0.01–1.0 mM and 0.1–7.5 mM for HKCN/PyOx and HKCN/*G. oxydans*, respectively.

1. Introduction

Biomolecule-based detection technology has become a dynamic area of research because of its remarkable potential for a variety of applications such as in food/beverage industries [1–4], diagnosis of disease [5–9], bioprocess monitoring [10–12] and screening of environmental pollutants [13–17]. To improve the stability of the biosensors for different requirements, modification of transducer surface for immobilization of bio-molecule is a key point. Different strategies such as self-assembly monolayers [18,19], natural polymers (gelatin, chitosan etc.) [20,21], sol–gel method [22], and conducting polymers [23–25] have been used to prepare immobilization platforms. The use of conducting polymers in the design of biosensors is very common because of homogeneous and manageable film character, ability of modification of physical and optical properties, stability and biocompatibility, availability of various types of monomers, reproducibility and easy production, and efficient electron transfer ability [26–28]. The synthesis of novel conducting polymers gives the creativity of the fabricated bio-detection systems. And also the addition of functional groups to the monomer backbone provides special and targeted immobilization of bio-components and also improves the stability of bio-components onto conducting polymers because of linkage via covalent attachment [29].

Here we described the use of a novel monomer with amino groups as an immobilization matrix. In the first part, 2,5-di(2-thienyl)pyrrole derivative namely 4-amino-N-(2,5-di(thiophen-2-yl)-1H-pyrrol-1-yl) benzamide (HKCN) has been synthesized via the reaction of 1,4-di(2-thienyl)-1,4-butanedione and p-aminobenzoyl hydrazide. Using hydrazide instead of amine has not only increased product yield, but also improved properties of the corresponding polymer. Recently, optical properties of HKCN were described [30]. Herein, electrochemically deposited HKCN onto the graphite surfaces was used for the immobilization of PyOx and whole cell of *Gluconobacter oxydans* to fabricate electrochemical enzyme and whole cell biosensors. After optimization, the characterization studies such as linearity were carried out using both batch and FIA modes.

2. Materials and method

2.1. Reagents

Pyranose oxidase (PyOx; pyranose:oxygen 2-oxidoreductase, E.C. 1.1.3.10, from *Coriolus* sp.), D-glucose, D(+)-xylose, ethanol, glycerol, and ascorbic acid were purchased from Sigma Chem. Co. and tetrabutylammoniumhexafluorophosphate ($TBAPF_6$) was purchased from Aldrich. Dichloromethane (DCM) was obtained from Merck. Glutaraldehyde solution (25%, v/v) was purchased from Sigma-Aldrich. Commercial enzyme assay kit (Glucose MR, Cat. No. 1129010) was obtained from Cromatest. Aluminum chloride ($AlCl_3$) (Aldrich), succinyl chloride (Aldrich), hydrochloric acid (Merck), $NaHCO_3$ (Aldrich), $MgSO_4$ (Aldrich), propionic acid (Aldrich), and toluene (Aldrich) were used for the synthesis of the monomer. All other chemicals were of analytical grade.

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