



Amperometric detection of glucose in fruit juices with polypyrrole-based biosensor with an integrated permselective layer for exclusion of interferences



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ABSTRACT

A novel polypyrrole (PPy)-based bilayer amperometric glucose biosensor integrated with a permselective layer has been developed for detection of glucose in the presence of interferences. It comprises of a PPy-GOx film grown, in the absence of electrolyte, as an inner layer, and a permselective PPy-Cl film as an outer layer. The PPy-GOx/PPy-Cl bilayer biosensor was effective in rejecting 98% of ascorbic acid and 100% of glycine, glutamic acid and uric acid. With an outer layer thickness of 6.6 nm, the bilayer biosensor gave nearly identical glucose response to that of a single layer PPy-GOx biosensor. The biosensor also exhibited good reproducibility (1.9% *rsd*, *n* = 10), high stability (more than 2 months), wide linear range (0.5–24 mM), low *K_m* (8.4 mM), high *I_{max}* (77.2 $\mu\text{A cm}^{-2}$), low detection limit (26.9 μM) and good sensitivity (3.5 $\mu\text{A cm}^{-2} \text{ mM}^{-1}$). The bilayer biosensor was successfully employed for glucose determination in various fruit juices.

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1. Introduction

Monitoring of glucose in blood and its sources in diets is crucial for preventing or delaying the occurrence of diabetes and its related complications, such as heart disease, vascular (blood vessel) disease and poor circulation, blindness, kidney failure, poor healing, stroke, and other neurological (nerve) diseases (Torpy, Lynm, & Glass, 2009). Generally, fasting glucose levels equal or higher than 7 mM are considered to be diabetic (NCD (Noncommunicable Disease) Risk Factor Collaboration (NCD-RisC), 2016).

To enable regular and/or continuous monitoring of glucose, the development of amperometric enzyme-based biosensor with immobilized glucose oxidase (GOx) on electrode transducers have attracted considerable interest (Kong et al., 2009; Liang et al., 2015; Ramanavicius, Rekertaitė, Valiūnas, & Valiūnienė, 2017; Zhao, Gao, Sun, & Gao, 2015) due to the high enzyme selectivity, together with the simplicity and low cost it offers (Kong et al., 2009). These unique advantages have led to the attractiveness of glucose amperometric biosensors as valuable tools in the food and drink industries (Hobbs, Patel, Kim, Rugutt, & Wanekaya, 2013; Gokoglan et al., 2017).

However, the use of GOx for development of glucose amperometric biosensors have generally required the application of high overpotentials ($700 \pm 200 \text{ mV}$), (Witt, Wohlfahrt, Schomburg, Hecht, & Kalisz, 2000; Singh, Kathuroju, & Jampana, 2009), which can also oxidise a number of endogenous electroactive substances, such as ascorbic and uric acids that are often present together with glucose in blood and food samples. Several strategies have been employed to overcome the interferences caused by these substances, including the use of multi-enzyme systems (Gao, Guo, Zhang, Qi, & Zhang, 2011; Wu et al., 2007) and electrode surface modification with conducting organic salts to lower applied potentials (Centonze, Losito, Malitesta, Palmisano, & Zambonin, 1997), use of artificial electron acceptors, such as gold nanoparticles and carbon nanotubes (Kong et al., 2009), carbon nanotube (CNT) nanoelectrode ensembles (Lin, Lu, Tu, & Ren, 2004), and the use of various mediators, such as prussian blue (Ramanavicius et al., 2017), tetrathiafulvalene (German et al., 2015) and ferrocene (Liang et al., 2015). Ramanavicius et al. (2017) recently utilized prussian blue mediator (PB) to overcome the problem of overpotential in amperometric biosensors. The development of the PPy/PB/GOx-modified graphite electrode is simple and involves a single step procedure. The biosensor achieved a sensitivity of $1.0\text{--}1.9 \mu\text{A cm}^{-2} \text{ mM}^{-1}$ for 0.1–20 mM glucose. The values of Michaelis constant (*K_M*) determined in this study were 10.3, 14.4, 4.63 and 21.1 mM for 10, 20, 30 and

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40 mM glucose, respectively. In another study, a glucose biosensor was prepared by immobilization of GOx on the surface of modified graphite electrode in mixed photocurable polymeric membranes of polyacrylamide (PAA)/polyvinylpyrrolidone (PVP) with electrodeposited layer of indium (III) and ruthenium (III) hexacyanoferrate films and used for glucose determination in commercial alcoholic beverages (Grassino, Milardovic, Grabaric, & Grabaric, 2012). The biosensor was stable for 2 months, achieved a linear concentration range up to 4.0 mM and a sensitivity of 0.55 $\mu\text{A}/\text{mM}$. Another strategy using vertically aligned carbon nanotubes (VACNT) and a conjugated polymer (CP) as immobilization matrix to entrap GOx on a modified indium tin oxide (ITO) coated polyethylene terephthalate (PET) electrode surface was proposed by Gokoglan et al. (2017). The biosensor response at an applied potential of -0.7 V versus Ag wire exhibited a linear range between 0.02 and 0.5 mM glucose and kinetic parameters (K_M^{app} , I_{max} , limit of detection (LOD) and sensitivity) were estimated as 0.193 mM, 8.170 μA , $7.035 \times 10^{-3}\text{ mM}$ and 65.816 $\mu\text{A}/\text{mM cm}^2$, respectively. German et al. (2015) reported on the use of gold nanoparticles covered with polypyrrole for investigation of the performance of different glucose oxidases for the development of an amperometric reagentless glucose biosensor. The biosensor based on the use of GOx_{p.funiculosum} gave higher analytical signal to glucose in comparison to two other biosensors that employed GOx_{A.niger} and GOx_{p.adametzii}. The registered current to glucose using GOx_{p.funiculosum}/PD/AuNP_{3.5}/GR electrode was linear from 0.1 to 10.0 mmol L⁻¹ and the limit of detection was 0.024 mmol L⁻¹. Additional PPy layer on the electrode surface reduced the influence of interfering species on the amperometric signal. Hobbs et al. (2013) used glassy carbon electrodes modified with carbon nanotubes and GOx based on layer-by-layer electrostatic self-assembly procedure for glucose determination in some beverages. The biosensor was stable over 3 months when stored at 4 °C, achieved a linear concentration range up to 15 mM, and enabled glucose determination in soft drink and beverages. Evidently, the use of mediators and nanoparticles have been found to improve the performance of glucose biosensor by overcoming the effect of oxygen variation from sample to sample, but this approach adulterates electrodes, which limits in vivo application and may also not be competitive with environmental oxygen for oxidation of the reduced enzyme (Lin et al., 2004).

To address the above limitations, Liang et al. (2015) investigated the direct electron property and GOx activity on graphene surface with and without mediators. Unfortunately, no glucose response was achieved with the biosensor without mediator. This suggested that simultaneous direct electron transfer (DET) and enzyme catalytic activity is not possible on the same GOx without mediators. On the other hand, Wang, Liu, Wu, and Cai (2009) achieved direct electron transfer with GOx without additional mediators or co-factors by electrochemically entrapping GOx onto the inner wall of a polyaniline nanotubes matrix (nanoPANi) with a template of an anodic aluminum oxide (AAO) membrane. The sensor demonstrated good anti-interference performance against common interfering species in blood, such as ascorbic acid, uric acid, and 4-acetamidophenol, due to the low detection potential (-0.3 V vs. SCE). Using a different strategy, Zhao et al. (2015) replaced graphene used by Liang et al. (2015) with carbon nanodots (CNDs) as immobilization supports and electron carriers to promote direct electron transfer (DET) reactions of glucose oxidase (GOx). The biosensor achieved a high rate constant (k_s) of $6.28 \pm 0.05\text{ s}^{-1}$ for fast DET and Michaelis–Menten constant (K_M^{app}) as low as $0.85 \pm 0.03\text{ mM}$ for affinity to glucose were found. A remarkable advantage of this biosensor include a high sensitivity of $6.1\text{ }\mu\text{A mM}^{-1}$ and limit of detection (LOD) of $1.07 \pm 0.03\text{ }\mu\text{M}$ (S/N=3). However, this biosensor has low dynamic range of 0–0.64 mM, which is unsuitable for the glucose detection range

suggested by NCD (Noncommunicable Disease) Risk Factor Collaboration (NCD-RisC) (2016) for diabetes management. Consequently, there is still a need for an alternative approach that would reduce or eliminate interferences while still being able to achieve the NCD glucose detection range.

The benefits of using permselective membranes to reduce or eliminate interferences has been demonstrated in a number of studies by using different polymers, layer-by-layer deposition and mixed layers with varying transport properties based on size, charge, or polarity (Centonze et al., 1997; German et al., 2015). However, the multilayers used in some of these approaches are usually electrically non-conductive materials, such as polyphenylenediamine (PPD), over-oxidised polyaniline (oxPAN) and over-oxidised polypyrrole films (oxPPy). These materials often limit electron transfer in thin films. On the other hand, the use of composite film of cellulose acetate and Nafion as an inner layer has been shown to eliminate the interference of neutral acetaminophen and negatively charged ascorbic and uric acids, respectively (Wilson & Hu, 2000). Also reported is the use of composite film of sol-gel layer and Nafion coating for entrapping glucose oxidase and to reduce interference effects of urea, glycine, ascorbic acid, paracetamol and uric acid (Yang, Lu, Atanossov, Wilkins, & Long, 1998). However, this approach is often not reproducible and site-selective immobilization of GOx is difficult to achieve when the enzyme is deposited in the inner layer by dip coating or dispensing (Yang et al., 2002).

The use of various conducting polymers with doping ions to form bilayer for exclusion of interferences has proven to be an attractive alternative to this problem. Polyaniline (Stoček, Kalcher, Švancara, & Vytřas, 2011) and polypyrrole (Chen, Jiang, & Kan, 2006; German et al., 2015) have been successfully applied for selective detection of glucose. In most cases, the performance of the biosensors is significantly affected by the thickness of the mixed layers. There is therefore still a need for the development of an effective, simple, low cost and interference-free biosensor that can detect glucose at very low concentrations in real samples. Centonze et al. (1997) has previously reported a one-step procedure for potentiostatic entrapment of GOx in PPy film for amperometric detection of glucose in serum samples. They successfully suppressed anionic interferents, such as ascorbate and urate by converting a conducting PPy film into a non-conducting, permselective, antifouling membrane by a simple electrochemical overoxidation process. Despite the success of that study, the sensitivity of the sensor was compromised by the inclusion of over-oxidised polypyrrole (OxPPy) film as the outer layer, due to the reduction in film conductivity and the insulating nature of the non-conductive film.

However, we have previously demonstrated in our laboratory that the PPy film, in its conducting form, is effective for suppressing or eliminating interferences (Adeboju & Moline, 2001). This relatively simple approach involves the use of electrochemically grown conducting PPy films in supporting electrolyte-free monomer solution for entrapping GOx and as permselective membranes. The method allows easy control of film thickness by varying the quantity of electricity passed. The additional thin PPy-Cl layer was able to suppress the influence of ascorbic acid without much effect on the performance of the biosensor. However, until now, this method has not been exploited for amperometric detection of glucose, as a means of minimizing or eliminating interferences and for achieving improved sensitivity and to determine the glucose contents of some commonly consumed beverages.

In this study, we investigate the beneficial use of an ultrathin PPy-GOx film coupled with the addition of an outer layer for eliminating or minimizing interferences for achieving a more rapid and sensitive amperometric detection of glucose. The influence of key

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