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A bio-sensing platform utilizing a conjugated polymer, carbon nanotubes and PAMAM combination

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ABSTRACT

In this study, amperometric biosensing performance of a novel platform utilizing a conducting polymer (CP), multi-walled carbon nanotubes (MWCNTs) and poly(amidoamine) (PAMAM) dendrimer was constructed. In order to fabricate the proposed biosensor, a graphite electrode was modified with poly[9,9-di-(2-ethylhexyl)-fluorenyl-2,7-diyl] end capped with *N*,*N*-bis(4-methylphenyl)-4-aniline (PFLA), MWCNTs and PAMAM, respectively. Then, GOx was immobilized onto the modified surface with the help of glutaraldehyde (GA) as the crosslinking reagent. Then, analytical and kinetic parameters of the constructed biosensor were investigated and the biosensor showed a linear response for glucose between 0.05 and 0.75 mM with a detection limit of 0.014 mM. K_M^{app} and sensitivity values were calculated as 0.66 mM and 55.41 μA mM^{-1} cm^{-2}, respectively. To investigate the surface modifications, scanning electron microscopy (SEM) and cyclic voltammetry (CV) techniques were used. Finally, fabricated biosensor was tested on beverages for glucose detection successfully.

1. Introduction

Since health-care shifts away from the clinical setting, diagnostic tools become increasingly important. Biosensors based on electrochemical signal transduction represent a new trend emerging in the diagnostic technology [1]. A major concern in the development of a biosensor is the proper and reproducible immobilization of biomolecules on the electrode surface without any loss of enzyme activity [2]. Therefore, creation of an immobilization matrix which provides a proper orientation of the biomolecules has a crucial role. For this purpose, different types of materials can be used in biosensor construction. Dendrimers which are perfect monodisperse macromolecules having highly branched and regular structures are considered as suitable host molecules for accommodation of guest molecules due to their three-dimensional structure with an internal void space [3–5]. Due to their promising characteristics including high chemical and mechanical strength, controllable size, globular geometry, hydrophilicity, high surface functionality, homogeneity and biocompatibility, dendrimers are ideal matrices for biomolecule immobilization [6]. These properties enhance stability, sensitivity, target-capturing ability and specificity of biosensors. In particular, highly-branched dendritic macromolecules, poly (amidoamine) (PAMAM), can be used in biosensor applications since PAMAM contains a number of terminal amino groups which enhances the attachment of biomolecules [7]. However, dendrimers have low conductivity and they are oily liquids which limit their application in biosensors. In order to overcome these problems, dendrimers can be fixed on a solid-state carrier, such as multi-walled carbon nanotubes (MWCNTs) and nanoparticles to work effectively [8,9].

MWCNTs have also extensively used in biosensor applications since they have dimensional and chemical compatibility with biomolecules in addition to their mechanical strength, stability, high electrical and thermal conductivity properties. They enlarge the electroactive surface area of the electrodes, which results in an increased charge transfer and conductivity. As a result, they can catalyze biochemical reactions and promote charge-transfer between biomolecules and electrode surfaces [10–12]. In literature, several studies have been examined using MWCNTs for biosensing fabrication. Chang et al. fabricated a three-dimensional composite from polybenzimidazole and carboxylated MWCNTs and used this composite for the modification of gold electrode for hydrogen peroxide detection. They stated that conductivity and surface area properties were enhanced [13]. In another study, Pong and co-workers synthesized graphene oxide nanoribbons from the facile unzipping of MWCNTs with the help of microwave energy and fabricated MWCNT/GONR-modified glassy carbon electrode to detect

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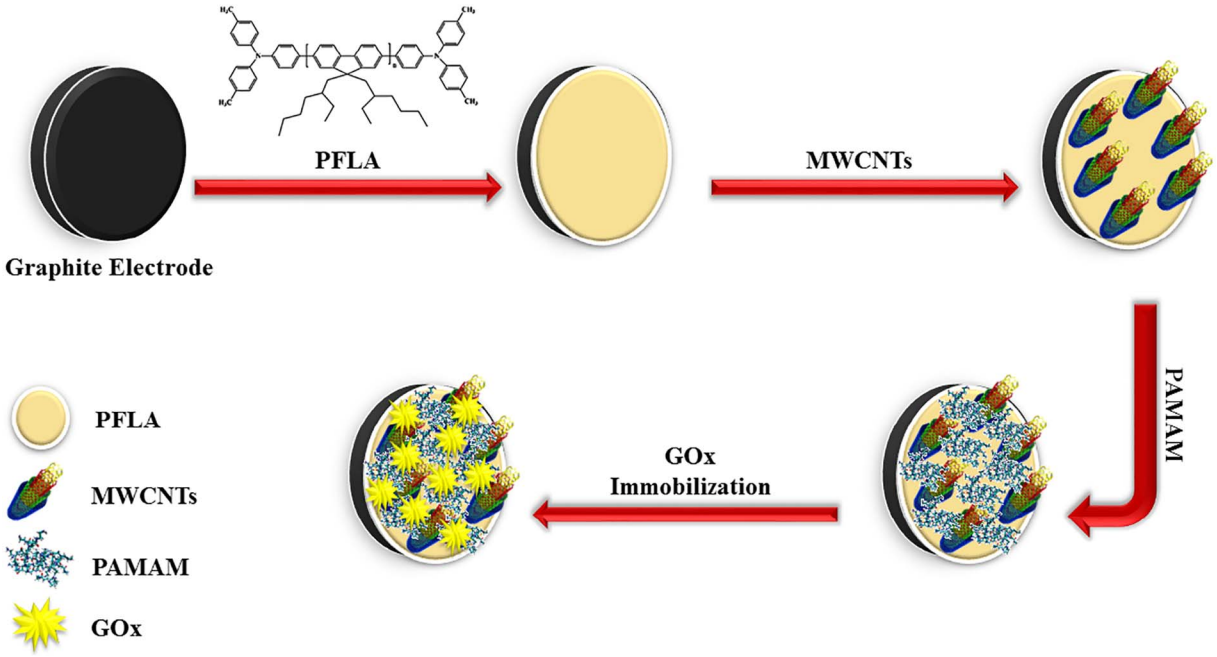
ascorbic acid, dopamine and uric acid. They employed MWCNTs due to their unique electronic structures and conductivities [14]. Moreover, several studies have been reported in which PAMAM and MWCNTs used together. Yao et al. proposed an electrochemical microRNA biosensor consisting of MWCNT-PAMAM dendrimer modified glassy carbon electrode and methylene blue redox indicator. They used MWCNTs due to their excellent electrochemical property for methylene blue electrochemistry and PAMAM dendrimers since they are suitable for immobilization of the capture probe due to their highly branched polymeric structure [15]. Youssoufi and co-workers produced the MWCNTs-polypyrrole nanocomposite by wrapping the polypyrrole film on MWCNTs during electrochemical polymerization of pyrrole on a gold electrode. They modified this nanocomposite layer with PAMAM dendrimers in order to provide association with a large number of bioreceptors due to the high number of amine groups on the dendrimer surface which resulted in increased sensitivity and lower detection limit [16]. Furthermore, conducting polymers (CPs), which have extended π-conjugated system, have become an important class of materials for biosensors due to their charge transport properties and biocompatibility [10]. In addition, CPs provide adjustable morphology, high surface area, processability and high mechanical strength resulting in extensive stability of the biomolecules incorporated [17].

Herein, with these motivations, a glucose biosensor utilizing poly [9,9-di-(2-ethylhexyl)-fluorenyl-2,7-diyl] end capped with *N*,*N*-bis(4-methylphenyl)-4-aniline (PFLA) as the conducting polymer, MWCNTs and PAMAM was constructed. Glucose oxidase (GOx), from *Aspergillus niger*, was used as the model enzyme. It is the most commonly used enzyme for glucose detection with a high glucose selectivity since it is easy to obtain, cheap and it can work under extreme pH, ionic strength, and temperature conditions [18]. GOx catalyzes the oxidation of β-glucose by molecular oxygen resulting in the production of δ-gluconolactone which is subsequently hydrolyzed into gluconic acid. Measurements are made by monitoring the level of the oxygen consumption [19]. Since the enzyme structure has both hydrophilic and hydrophobic parts, proper interaction with the alkyl chains on the polymer backbone resulted in the enhanced stability. In addition there is a non-covalent interaction as π-π stacking due to the presence of organic groups with π bonds in the polymer matrix. Possible π-stacking between aromatic residues of enzyme and polymer provides adhesion of the enzyme on top of the polymer coated surface [20]. To improve the immobilization and enhance the interaction between the polymer and enzyme molecules, MWCNTs were casted on CP modified electrode surface. After that, the biosensor was functionalized with PAMAM to get more reactive regions to attach GOx for more sensitive glucose detection. For this purpose, two different biosensors were prepared via surface modification of PAMAM G2 and PAMAM G4. When the performances of these two biosensors were compared, the electrode with PAMAM G2 gave higher and more stable responses. This is because as PAMAM dendrimer size increases, resistivity in flexibility increases and the detection sensitivity is limited [21]. Demirci et al. found out that K_M^{app} value is lower in the PAMAM G2 biosensor when it was compared with the PAMAM G4 biosensor [7]. This also shows that the enzyme activity is higher in the more flexible PAMAM G2 environment. Moreover, Miura et al. [22] compared the amount of protein adsorbed by different PAMAM generations. They showed that as dendrimer size increases, adsorption of protein molecules decreases indicating that dendrimer complex surface with a high generation results in higher bio-inertness. After that GOx was immobilized on the modified electrode surface using glutaraldehyde (GA) as the crosslinking reagent. The proposed biosensor was tested by amperometric detection technique by monitoring the decrease in oxygen level as a result of enzymatic reaction at − 0.7 V versus Ag/AgCl. Scheme 1 illustrates the construction procedure of the proposed amperometric glucose biosensor. The characterization studies of the biosensor were performed and the biosensor was tested on real samples successfully.

2. Experimental

2.1. Materials and methods

Poly[9,9-di-(2-ethylhexyl)-fluorenyl-2,7-diyl] end capped with *N*,*N*-bis (4-methylphenyl)-4-aniline (PFLA) was obtained from American Dye Source, Inc. (Quebec, Canada; www.adsdyes.com). Poly (amidoamine) (PAMAM)-25% C_{12} dendrimer, generation 2.0, 20 wt% solution in methanol, PAMAM-25% C_{12} dendrimer, generation 4.0, 10 wt% solution in methanol, multi walled carbon nanotubes (MWCNTs), glutaraldehyde (GA) and chloroform were purchased from Sigma–Aldrich Co., LCC. (St. Louis, USA). Glucose oxidase (GOx, β-D-glucose: oxygen 1-oxidoreductase,



Scheme 1. Schematic representation of PFLA/MWCNT/PAMAM/GOx biosensor.