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# Development of glucose biosensor based on reconstitution of glucose oxidase onto polymeric redox mediator coated pencil graphite electrodes

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ABSTRACT

In this study, a novel glucose biosensor was fabricated by reconstitutional immobilization of glucose oxidase (GOx) onto a poly(glycidyl methacrylate-co-vinylferrocene) (poly(GMA-co-VFc)) film coated pencil graphite electrode (PGE). The amperometric current response of poly(GMA-co-VFc)-GOx to glucose is linear in the concentration range between 1 and 16 mM (correlation coefficient of 0.9998) with a detection limit of 2.7 μM ($S/N = 3$). Experimental parameters were studied in detail and optimized, including the pH and temperature governing the analytical performance of the biosensor. The stability and reusability of the biosensor as well as its kinetic parameters have also been studied.

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## 1. Introduction

One of the worldwide leading causes of endocrine disorder of carbohydrate metabolism is diabetes mellitus, which further results in numerous complications from blindness, kidney failure to various heart diseases [1,2] and finally because of insulin deficiency and hyperglycemia [3] to death. In recent years research on glucose biosensors has drastically increased and more specifically World Health Organization last year reported that the number of patients with diabetes mellitus is most likely to reach more than 300 million by year 2025 [3], which thus requires some practical solutions for developing biosensor with excellent properties and abilities (sensitivity, selectivity, linear range, detection limit, inexpensive, etc.) [4].

Biosensor may be defined as a device composed of two intimately associated elements: a bioreceptor (e.g. enzyme, nucleic acid, and antibody) and a transducer (used to convert the biochemical signal into an electronic one) [5] for the purpose of identification and quantification of specific molecular entity [6]. In amperometric glucose biosensors, thick protein layer surrounding flavin adenine dinucleotide (FAD) redox center limits direct electron transfer between the GOx active site and the electrode surface, which, however, can be overcome by using organic conducting materials based on charge-transfer complex [7,8]. Working principle is based on glucose oxidase cofactors as catalyst and eventually as the initial electron acceptor [1,9], that the immobilized glucose oxidase catalyzes the oxidation of β-D glucose by molecular oxygen producing gluconic acid and hydrogen peroxide using two flavin adenine dinucleotide (FAD), glucose oxidase cofactors, as catalyst and eventually as the initial electron acceptor [1,9].

$$\beta\text{-d-Glucose} + \mathrm{GOx(FAD)} \rightarrow \mathrm{GOx(FADH_2)} + \text{d-glucono-}\delta\text{-lactone}$$

$$\mathrm{GOx(FADH_2)} + \mathrm{O_2} \rightarrow \mathrm{GOx(FAD)} + \mathrm{H_2O_2}$$

Glucose oxidase used in biosensors conveys levels of glucose by keeping track of the number of electrons passed through the enzyme [10]. It has high selectivity for glucose, easy to obtain, inexpensive, can withstand great extremes of pH and temperature, thus allowing relatively relaxed storage norms for use by lay biosensor users [11,12].

Ferrocene derivatives are widely used in the construction of mediated amperometric biosensors and they are excellent electron transfer mediators. The development of polymeric mediators is essential in biosensors, since polymers allow the incorporation of reagents to achieve reagent-less devices, as well direct attachment of ferrocene-based mediators onto polymeric films prevents leaching of the mediators [13]. Polyvinylferrocene is an excellent electron transfer mediator and because of its advantages such as a

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reversible electron process, simple coating of thin films and its high stability [14,15] it is widely used in the electrochemical system. Not only did Meral et al. reported many incredible properties of this polymer in glucose biosensor constructing amperometric glucose biosensor based on gold-deposited polyvinylferrocene film on Pt electrode and getting very high sensitive biosensor [16], but also Salig et al. reported its wide use together with different enzymes by amperometric xanthine biosensors based on electrodeposition of platinum on polyvinylferrocenium coated Pt electrode [17] and many other applications of ferrocene derivatives in biosensing [14,18,19].

However, having polymer with good properties is not enough, simply because of the crucial gap between enzyme and electrode which should transfer electrons. Willner et al. proposed stripping the flavin adenine dinucleotide redox center of glucose oxidase (GOx) and then reconstituting apo-enzymes with the modified cofactors [20]. This suggested method is of great importance in direct electron transfer, which is the most of the immobilization methods leading to random orientations of the redox center in relation to the electrode surface. So, direct electron transfer efficiency mainly depends on the average activity of the randomly oriented bioelectrocatalysts rather that the optimum charges transfers [21,22]. The native cofactor associated with the enzyme is eliminated from the protein to yield the respective apo-enzyme. The reconstitution of the apo-enzyme on the relay-cofactor monolayer yields an electrically wired configuration with some superior electrical communication features: (i) all enzyme units are linked to the electrode in an identical configuration that forces the redox center into a minimal spatial separation from the electrode. (ii) The relay unit is positioned between the cofactor and the electrode, and hence, mediated ET is the most efficient one. This concept was further extended to design integrated enzyme electrodes consisting of diffusional cofactors and enzymes that reveal ET communication with the electrodes [23,24].

In this study, a novel glucose biosensor was constructed by immobilization of glucose oxidase enzyme onto polymeric mediator coated pencil graphite electrode via reconstitution. The novel side and impetus of the current study compared to the previous reconstitution studies comes from its aim to prepare a new polymeric mediator of boronic acid. The poly(GMA-co-VFc) films provided polymeric mediator coated electrode and were used in the fabrication of reconstituted enzyme based amperometric biosensors. Also, apo-glucose oxidase was efficiently reconstituted on FAD monolayer and optimized experimental conditions were assessed for pH efficiency, temperature and stability of the biosensor.

## 2. Materials and methods

Glucose oxidase (GOx) (EC 1.1.3.4), glucose and flavin adenine dinucleotide sodium salt (FAD) were obtained from Sigma Chemical Co. 4-aminophenyl boronic acid (APBA);2,2′-azobis(isobutyronitrile) (AIBN) from Sigma–Aldrich. All other chemicals were of analytical grade and were used without further purification.

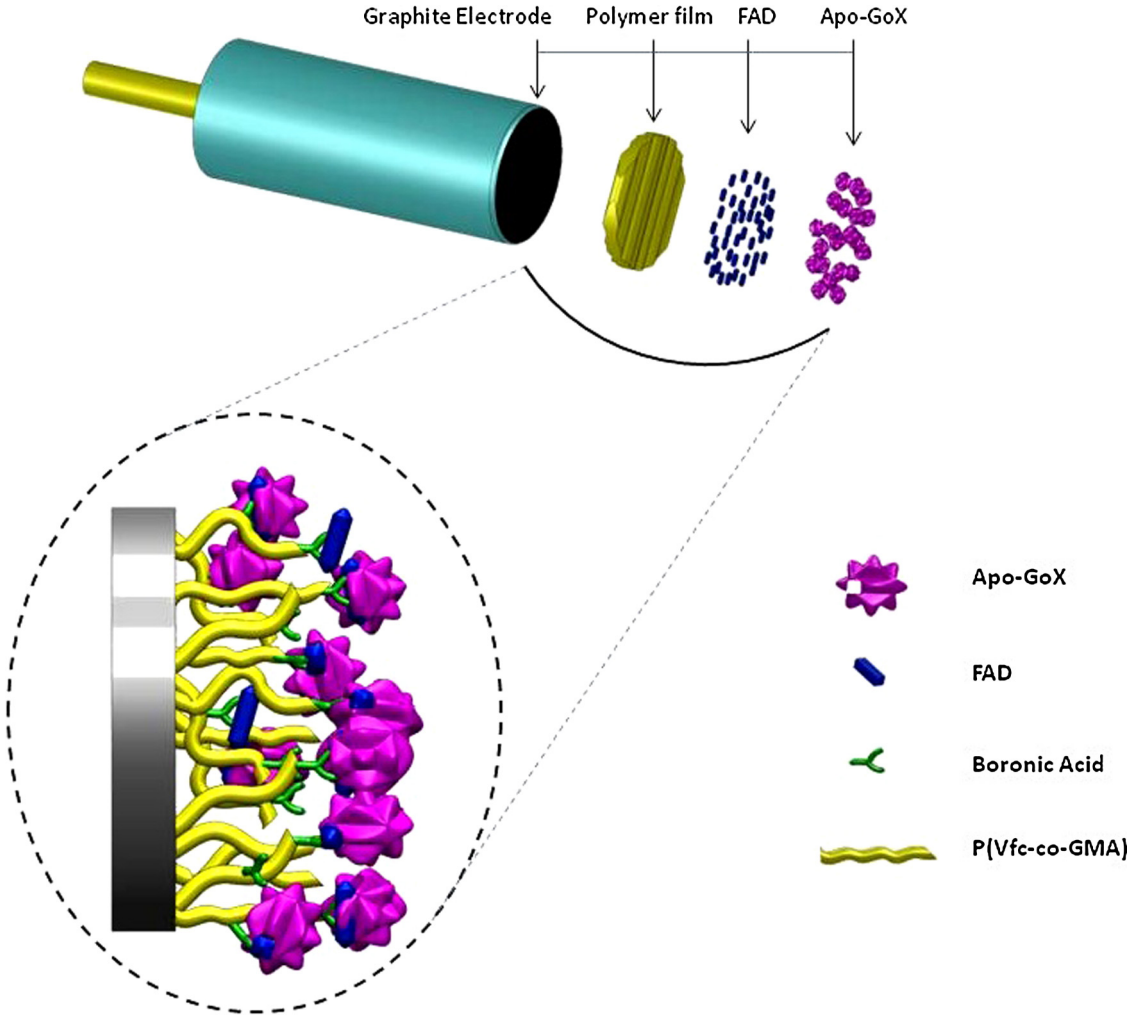


**Fig. 1.** Illustration of the prepared glucose biosensing electrode.

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