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Original Research Paper

Superior performance of a carbon-paste electrode based glucose biosensor containing glucose oxidase enzyme in mesoporous silica powder

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ABSTRACT

A carbon paste electrode (CPE) based glucose biosensor was developed with an optimized composition. It had 60 wt.% graphitic carbon powder mixed with 15 wt.% silicone oil binder, to which was added 2.3 wt.% glucose oxidase (GOD) enzyme (the sensing molecule) immobilized in 12.7 wt.% mesoporous silica (SBA-15) host particles, and 10 wt.% ferrocene, as an electron mediator. Optimization was achieved by first fixing the SBA-15 content in the CPE based on detection of hydrogen peroxide, an intermediate formed during oxidation of glucose by GOD. Subsequent glucose sensing measurements necessitated addition of ferrocene to improve sensitivity. This sensor gave a very high linear sensing-range of glucose (2.0–18.2 mM) from the hypo- to hyperglycemic range, a high sensitivity (1.5 $\mu A\,cm^{-2}\,mM^{-1}$), a fast response time (<5 s), a good storage stability (of at least 8 days), without any loss in sensitivity. The sensor is also selective against interfering species such as urea and dopamine, but not against uric acid and ascorbic acid. A large mesopore diameter (11.4 nm) of the SBA-15 particles ensured GOD molecule was well impregnated, translating into a superior linear range and storage stability. Simultaneously, the high specific surface area of SBA-15 (406 m^2/g) resulted in a large contact area between carbon and SBA-15 particles, leading to increased inter-particle connectivity. The latter, together with ferrocene, resulted in increased sensitivity.

1. Introduction

The importance of accurate and rapid detection of glucose is well established in the diagnosis and management of diabetes mellitus. This has motivated research into the development of a better glucose biosensor [1]. Enzyme based glucose biosensors are popular because they are highly selective to glucose and are effective in increasing sensitivity; thus they are useful in combining both recognition and amplification steps. Many techniques such as fluorescence [2], optical [3], electrochemistry [4], flow injection [5], etc. have been developed along with suitable enzyme immobilization approaches, in order to achieve desirable sensor characteristics. It is required to have a broad linear range of detection, high sensitivity, fast response time, reusability and long term storage stability. Among electrochemical techniques, glucose detection by amperometry is the most popular method because of its better sensitivity and potential selectivity, small sample volume requirement, and relatively low detection cost [6,7]. Therefore, efforts have been made to develop a glucose biosensor, by immobilizing glucose oxidase (GOD) enzyme into a suitable host, which can measure glucose concentrations in both hypoglycemic (2–4 mM) and hyperglycemic (7–15 mM) conditions, in addition to the normal glucose range (4–7 mM) [1].

Among different hosts reported in literature, particles of mesoporous silica have been of specific interest because of its inherent desirable properties such as high specific surface area for immobilization, large pore volume for ease of enzyme diffusion, precise control over the pore diameter, and finally good chemical, thermal and mechanical stability of silica [8,9]. In literature, GOD immobilized mesoporous silica based glucose biosensors have been made with three approaches. The first being detection of enzymatically generated hydrogen peroxide [1]. The maximum glucose linearity range reported by this simple approach was 0.1–7.7 mM [10]. Thus

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the biosensor was not suitable for detecting glucose in the hyperglycemic (>7 mM, up to 33 mM) range. The second approach was based on direct electron transfer between active centre of GOD and the electrode surface, without the use of any electron mediators. For example, a direct electron transfer was reported when GOD was immobilized in mesoporous silica host particles. However, the sensor resulted in a smaller linearity range of only 0.5–6.0 mM glucose [11]. Moreover, use of only GOD on the electrode surface did not result in the direct electron transfer [4]. Therefore, this approach was found to be limited because of the thick insulating protein layer surrounding the active centre of GOD, which makes direct electron transfer difficult. The third approach was based on the use of electron mediators (such as ferrocene) from the active centre of GOD to the electrode surface. So far, this route has resulted in a linear glucose sensing range of 0.4–13 mM [12], and in another report 0.32–15.3 mM glucose [4]. Thus these biosensors were suitable for diabetic patients. However, there is a need to further increase the linearity range of the sensor in order to prevent glucose level reaching life threatening hyperosmolar glucose level [13]. In the present work, we demonstrate a highly sensitive and stable glucose biosensor with the highest glucose linear range, based on GOD and mesoporous silica containing carbon paste electrode (CPE).

2. Experimental

2.1. Materials

Tetraethyl orthosilicate (TEOS, >99%, Fluka), pluronic123 (P123, Sigma Aldrich), decane ($C_{10}H_{22}$, Sigma Aldrich), hydrochloric acid (HCl, Merck, 35%) and ammonium fluoride (NH_4F, Hi Media) were used for synthesis of mesoporous silica (SBA-15) particles. GOD from *Aspergillus niger* and horseradish peroxidase (HRP) were from Sigma Aldrich. Sodium dihydrogen phosphate (Merck) and disodium hydrogen phosphate (Merck) were used for phosphate buffer solution preparation. Acetic acid (Merck) and sodium acetate (Qualigens) were used for making acetate buffer solution. Sodium azide (S.D. Fine Chemicals) was used as a preservative. Phenol (Merck), 4-aminoantipyrine dye (4-AAP, make Spectrochem) and D-glucose (HiMedia) were used for GOD assay. Ferrocene (Sigma Aldrich, 98%) was used as an electron mediator. Graphite powder (S.D. Fine chemicals) and silicone oil (Merck) were used for making carbon paste. 2 ml plastic syringe (BD Discardit II) was used as casing for carbon paste. Deionized water was used in all experiments. All chemicals were used as received without further purification.

2.2. Synthesis of mesoporous silica (SBA-15) particles

SBA-15 particles were synthesized with following molar ratios of reactants [14]:

P123: HCl: NH_4F: $C_{10}H_{22}$: H_2O: TEOS = 1: 261: 1.8: 135: 11,278: 60.

Details of the synthesis protocol are given as follows: 1.5 g Pluronic 123 was dissolved in 55 ml of 1.3 M HCl solution at 30 °C. Then 0.027 g NH_4F was added, followed by 11.762 ml decane and stirred for 5 h. Finally, TEOS (3.464 ml) was added in drops for 2 min. The mixture was stirred at 30 °C for 20 h and then kept in a closed vessel under static conditions, for further reaction at 100 °C for 48 h. The molar ratio of reactants used was as mentioned above. Finally, the solid product was filtered, washed, dried at ambient condition and calcined at 540 °C for 6 h.

2.3. Preparation of carbon paste electrode (CPE) for glucose sensing

Graphite powder and silicone oil were mixed in a mortar with a pestle for 2 h to obtain the carbon paste, which was further modified by mixing SBA-15 particles for 30 min. Subsequently, the CPE was obtained by tightly packing the modified carbon paste in a 2 ml open barrel syringe, followed by insertion of a copper wire for electrical contacts. Four different electrodes were made by varying SBA-15 content in CPE, from 0 to 15 wt.% (Table 1), in order to optimize the sensitivity of the sensor for H_2O_2 detection. Based on these results (discussed later in Section 3.2), CPE with 15 wt.% SBA-15 samples (S15-CPE) was modified further for conducting the final glucose sensing measurements (Table 1).

The CPE for glucose sensing was prepared as follows: 9 mg GOD was dissolved in 4 ml of pH 4.0 buffer to obtain the GOD solution, to which 50 mg of SBA-15 particles were dispersed. The contents were stirred at 4 °C for 12 h followed by drying at 37 °C. The dry powder of GOD with SBA-15 (GOD-SBA-15) was obtained. This step resulted in part immobilization of GOD in SBA-15, with some free GOD also. Subsequently, graphite powder and silicone oil were mixed in a mortar with a pestle for 2 h, to which ferrocene and GOD-SBA-15 were added sequentially and mixed for 1 h each. Finally, the modified paste was packed in an open barrel syringe as mentioned before.

2.4. GOD assay

Glucose reacts according to the scheme given below and produces quinoneimine complex. This is measured at 510 nm using a spectrophotometer. The absorbance is proportional to concentration of glucose in the sample [15].

$$\underset{\text{(Glucose)}}{C_6H_{12}O_6} + \underset{\text{(Oxygen)}}{O_2} \xrightarrow{\text{GOD}} \underset{\text{(D-glucono-1,5-lactone)}}{C_6H_{10}O_6} + \underset{\text{(Hydrogen peroxide)}}{H_2O_2}$$

$$2H_2O_2 + \underset{\text{(4-aminoantipyrine)}}{C_{11}H_{13}N_3O} + \underset{\text{(Phenol)}}{C_6H_6O} \xrightarrow{\text{HRP}} \underset{\text{(Quinoneimine complex)}}{C_{17}H_{15}N_3O_2} + 4\,H_2O$$

The protocol for GOD assay is based on [15]; details are given in Section S1 of SI. GOD assay was carried out at pH 7.0, which is close to the physiological pH of 7.3.

2.5. Characterization techniques

Scanning electron microscopic (SEM) and transmission electron microscopic (TEM) images were taken in Hitachi SEM (FEI Quanta 3D, 10–15 kV) and Philips (CM 200, 200 kV), respectively. Nitrogen sorption measurements were conducted at liquid nitrogen temperature (77 K) using a Micromeritics ASAP 2020 apparatus. Samples were degassed at 150 °C for 6 h. Pore diameters were estimated from adsorption branch of the isotherm using the BJH model. Surface areas were calculated using the BET model in the relative pressure range of 0.05–0.3. Total pore volumes were estimated at a relative pressure of 0.995, assuming full surface saturation of

Table 1
Electrode compositions of carbon paste electrode used in this work.

Electrode	Electrode composition (wt.%)				
	GOD	SBA-15	Ferrocene	Silicone oil	Graphite
S0-CPE	0	0	0	20	80
S5-CPE	0	5	0	19	76
S10-CPE	0	10	0	18	72
S15-CPE	0	15	0	17	68
GOD-S15-CPE	2.3	12.7	0	17	68
Fer[a]-GOD-S15-CPE	2.3	12.7	10	15	60

[a] Fer means ferrocene.