



## Regular Article

# Glucose oxidase enzyme immobilized porous silica for improved performance of a glucose biosensor



Anees Y. Khan, Santosh B. Noronha \*\*, Rajdip Bandyopadhyaya \*

Department of Chemical Engineering, Indian Institute of Technology Bombay, Powai, Mumbai 400076, India

## ARTICLE INFO

## Article history:

Received 15 March 2014

Received in revised form 7 June 2014

Accepted 11 July 2014

Available online 19 July 2014

## Keywords:

Biocatalysis

Biosensors

Enzyme activity

Immobilized enzymes

Mesoporous silica

Glucose oxidase

## ABSTRACT

High activity of glucose oxidase (GOD) enzyme (immobilized in porous silica particles) is desirable for a better glucose biosensor. In this work, effect of pore diameter of two porous hosts on enzyme immobilization, activity and glucose sensing was compared. The hosts were amine functionalized: (i) microporous silica (NH<sub>2</sub>-MS) and (ii) mesoporous silica (NH<sub>2</sub>-SBA-15). Based on whether the dimension of GOD is either larger or smaller than the pore diameter, GOD was immobilized on either external or internal surface of NH<sub>2</sub>-MS and NH<sub>2</sub>-SBA-15, with loadings of 512.5 and 634 mg/g, respectively. However, GOD in NH<sub>2</sub>-SBA-15 gave a higher normalized absolute activity (NAA), which led to an amperometric sensor with a larger linear range of 0.4–13.0 mM glucose. In comparison, GOD in NH<sub>2</sub>-MS had a lower NAA and a smaller linear range of 0.4–3.1 mM. In fact, the present GOD-NH<sub>2</sub>-SBA-15 electrode based sensor was better than other MS and SBA-15 based electrodes reported in literature. Thus, achieving only a high GOD loading (as in NH<sub>2</sub>-MS) does not necessarily give a good sensor performance. Instead, a host with a relatively larger pore than enzyme, together with optimized electrode composition ensures the sensor to be functional in both hyper- and hypoglycemic range.

© 2014 Elsevier B.V. All rights reserved.

## 1. Introduction

Immobilization of glucose oxidase (GOD, EC number 1.1.3.4) enzyme has drawn significant attention for glucose biosensing [1]. Enzyme immobilization on a solid host has always been favoured due to ease of separation, stability and re-usability of the immobilized enzyme [2,3]. Therefore, better glucose biosensors need knowledge of both immobilization methods and host properties. The most common techniques for enzyme immobilization are physical adsorption [4,5], chemical crosslinking [6,7] and encapsulation in gels [8]. Physical adsorption is preferred as enzyme conformation is preserved and higher enzyme activity is achieved. Crosslinking gives better stability against leaching; however, enzyme activity may decrease due to possible conformational change of enzyme. Gel entrapment leads to better enzyme activity, but works only for small size of substrate and product molecules. Immobilization of GOD by physical adsorption was preferred in the present work, as it preserves the native conformation of enzyme.

\* Corresponding author. Tel.: +91 22 2576 7209; fax: +91 22 2572 6895.

\*\* Corresponding author. Tel.: +91 22 2576 7238; fax: +91 22 2572 6895.

E-mail addresses: [noronha@iitb.ac.in](mailto:noronha@iitb.ac.in) (S.B. Noronha), [rajdip@che.iitb.ac.in](mailto:rajdip@che.iitb.ac.in) (R. Bandyopadhyaya).

Porous silica based solid materials are promising hosts as their properties can be varied to achieve high enzyme loadings. In glucose sensing applications, the performance of glucose sensors improves with increase in GOD loading [9]. In general, host pore diameter is used to achieve high enzyme loadings. For example, microporous silica (MS, pore diameter <2 nm) was used as a host for immobilization of lysozyme (molecular size 3 nm × 3 nm × 4.3 nm) [10]. However, a loading of only 40 mg/g was achieved [11], due to the smaller pore diameter of MS compared to the size of lysozyme, resulting in immobilization only on the available small external surface of MS. A much higher loading (400 mg/g) was achieved by a mesoporous silica host, MCM-41 (pore diameter 3 nm), where immobilization took place inside pores, the latter with pore size comparable to lysozyme size [12]. However, when MCM-41 was used for immobilization of bigger enzyme molecules such as GOD (6 nm × 5.2 nm × 7.8 nm [13]), it resulted in a loading of only 100 mg/g [14]. As expected, this lower loading was a result of immobilization on the external surface, as GOD molecules were prevented from accessing the internal surface area, due to the larger size, compared to the pore diameter of MCM-41.

Other studies have reported a higher GOD loading (210–487 mg/g), either with mesoporous silica of pore diameter bigger than molecular size of GOD [15], or by further functionalization of bigger pores in mesoporous silica [15–17]. However, just high GOD loading is not enough; immobilized GOD need to

show high activity too. Therefore, a favourable choice of host would be the one having high GOD loading with least diffusional resistance to glucose, resulting in high GOD activity. In this regard, MS could be a better choice, as it does not provide any internal pore-diffusional resistance. However, efforts in immobilization of GOD on MS have been neglected due to much lower loading of 60 mg/g observed so far [18], in comparison to mesoporous silica. This is because low loading results in less number of active centres of immobilized GOD, leading to lower activity. Hence, a comparison of GOD activity is required when immobilized at high loading in both MS and mesoporous silica. Furthermore, this will help in understanding the effect of enzyme attachment location on glucose sensing performance. Therefore, in the present work, amine functional groups on both MS and SBA-15 were introduced, in order to vary the surface potential (i.e. surface charge) on silica, which amplify the potential difference between silica and GOD, leading to high loading [17]. Thus, we systematically immobilized high quantities of GOD, both on the external and internal surfaces of amine functionalized microporous silica (NH<sub>2</sub>-MS, pore diameter 0.9 nm) and amine functionalized mesoporous silica (NH<sub>2</sub>-SBA-15, pore diameter 11.4 nm), respectively. This will help in obtaining better biosensor performance with respect to pore diameter and location of enzyme attachment on the surface of the host.

## 2. Materials and methods

### 2.1. Materials

Tetraethyl orthosilicate (TEOS, Fluka), Pluronic 123 (P123, Sigma–Aldrich), decane (C<sub>10</sub>H<sub>22</sub>, Sigma–Aldrich), hydrochloric acid (HCl, Merck, 35%), and ammonium fluoride (NH<sub>4</sub>F, HiMedia) were used for SBA-15 synthesis. 3-Aminopropyltriethoxysilane (APTES, Sigma–Aldrich) was used as an amine source. Glucose oxidase (GOD, EC number 1.1.3.4) from *Aspergillus niger* and horseradish peroxidase (HRP, EC number 1.11.1.7) were purchased from Sigma–Aldrich. Sodium dihydrogen phosphate (Merck), disodium hydrogen phosphate (Merck), acetic acid (Merck) and sodium acetate (Qualigens) were used for buffer solution preparation. Sodium azide (S. D. Fine Chemicals) was used as a preservative. Phenol (Merck), dye 4-aminoantipyrine (AAP, Spectrochem), and D-glucose (HiMedia) were used for GOD assay. Ferrocene (Sigma–Aldrich, 98%) was used as an electron mediator. Nafion (Sigma–Aldrich, 5 wt.% in lower aliphatic alcohol and water) was diluted to 0.5 wt.% with water before use. Deionized water was used in all experiments. All chemicals were used as received without further purification.

### 2.2. Synthesis of amine functionalized microporous (NH<sub>2</sub>-MS) and mesoporous silica (NH<sub>2</sub>-SBA-15)

NH<sub>2</sub>-MS was synthesized by co-condensation method reported by Li et al. [19] with the modification that no surfactant was used and the mole ratios of APTES to TEOS ( $M_{AT}$ ) were optimized in order to get isolated, spherical silica particles. In brief, 1.215 ml of sodium hydroxide solution (2 M) was added to a polypropylene reactor, containing 168 ml water maintained at 80 °C, followed by simultaneous drop-wise addition of TEOS (1.75 ml) and APTES (0.37 ml) under stirring at 400–500 rpm. The mixture was reacted for 2 h, and the resulting white product was centrifuged, washed with water and ethanol, and finally dried at 100 °C.

Synthesis of SBA-15, in brief, was as follows [20]: 4.7 mM of P123 was made in 1.3 M HCl solution at 30 °C in a polypropylene reactor. Then, NH<sub>4</sub>F (0.027 g) and decane (11.762 ml) were added and stirred for 5 h, followed by drop-wise addition of

TEOS (3.464 ml). After 20 h of reaction, the contents were transferred into a closed vessel and kept under static conditions at 100 °C for 48 h. The molar ratio of reactants used was as follows: P123:HCl:NH<sub>4</sub>F:C<sub>10</sub>H<sub>22</sub>:H<sub>2</sub>O:TEOS = 1:261:1.8:135:11,278:60. The solid product was filtered, washed, dried at ambient condition and calcined at 540 °C for 6 h.

SBA-15 was amine functionalized by post synthesis grafting method [21], using APTES as the amine source. Briefly, in a round bottom flask, dry toluene (50 ml), 1.2 g SBA-15 and 4 ml APTES were reacted in nitrogen atmosphere at 110 °C in reflux condition for 12 h, under stirring. The resulting powder was then filtered, washed with ethanol and water, and dried in air.

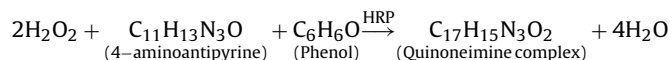
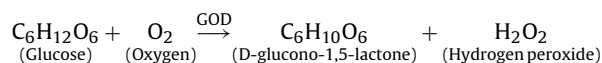
### 2.3. Immobilization of GOD

GOD solutions of known concentrations were prepared in 0.2 M acetate buffer of pH 4.0, and calibrated against their absorbance using the Bradford assay [22]. This is a colorimetric assay for protein quantification, based on binding of the Bradford reagent with GOD at acidic pH. Therefore, it can detect very low GOD concentrations, down to 2 µg/ml. Typically, 100 µl GOD solution was mixed with 1 ml Bradford reagent and allowed to stand for 5 min, before measuring its absorbance at 595 nm.

For immobilization of GOD, NH<sub>2</sub>-MS (or NH<sub>2</sub>-SBA-15) particles were dispersed in 0.2 M acetate buffer (pH 4.0) of a known concentration of GOD. The mixture was then kept at 4 °C in a circular cell mixer at 70 rpm for 24 h. Thereafter, it was centrifuged at 13,000 rpm (approximately 16,060 g) for 10 min to separate GOD immobilized solids. The latter was washed three times with pH 7 buffer, to remove loosely adsorbed GOD. The quantity of immobilized GOD was found by subtracting its mass present in the supernatant, from what was present before immobilization (determined from Bradford assay).

### 2.4. GOD assay

Glucose reacts following the scheme below and produces a quinoneimine complex [23].



A known mass of GOD immobilized NH<sub>2</sub>-MS or (NH<sub>2</sub>-SBA-15) was reacted with 100 mM glucose in 0.2 M phosphate buffer (pH 7), for 10 min at 26 °C. Next, the supernatant was obtained on centrifugation at 13,000 rpm for 5 min. A known aliquot of the supernatant was mixed with an assay mixture (containing 10 units of HRP, 0.2 mM AAP, and 2.5 mM phenol). The concentration of the resultant pink quinoneimine complex, as determined spectrophotometrically (at 510 nm) was obtained by multiplying the absorbance with the dilution factor and a calibration plot. This gives activity of the immobilized GOD.

The step involving separate addition of the supernatant (which contains enzymatically generated hydrogen peroxide product) to the assay mixture was required. There could otherwise be an underestimation of the true activity due to adsorption of pink quinoneimine complex on silica particles. Free GOD activity (in absence of silica) was measured under the same condition, as that of immobilization conditions, for proper comparison. All experiments were carried out under sterile conditions.

As activity of GOD is affected by GOD loading, hence, mass of GOD immobilized in silica ( $m_i$  measured in mg) is defined in three ways. (i) GOD loading ( $G_i$ ) which is  $m_i$  per g silica. This is used to

Download English Version:

<https://daneshyari.com/en/article/3114>

Download Persian Version:

<https://daneshyari.com/article/3114>

[Daneshyari.com](https://daneshyari.com)