



Hollow spherical nanostructured polydiphenylamine for direct electrochemistry and glucose biosensor

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ABSTRACT

Nanostructured, hollow spheres of polydiphenylamine (HS-PDPA) are prepared through a “soft template assisted self-assembly” approach. An enzymatic glucose biosensor is fabricated through immobilizing glucose oxidase (GOx) into HS-PDPA matrix. The HS-PDPA-GOx electrode exhibits a pair of well-defined reversible redox peaks with a fast heterogeneous electron transfer rate. At an applied potential of +0.65 V, HS-PDPA-GOx electrode possesses high sensitivity ($1.77 \mu\text{A mM}^{-1} \text{cm}^{-2}$), stability and reproducibility towards glucose. The amperometric current response of HS-PDPA-GOx to glucose is linear in the concentration range between 1 and 28 mM with a detection limit of 0.05 mM ($S/N=3$). Also, HS-PDPA-GOx electrode shows high selectivity towards glucose in the presence of ascorbic acid, uric acid and acetaminophen at their maximum physiological concentrations.

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1. Introduction

Conducting polymers (CPs) receive extensive research attention owing to their intriguing properties and high application potentials in diversified areas (Janata and Josowicz, 2003; Ramanathan et al., 2004; Manesh et al., 2007). However, the properties of CPs depend mostly on the synthetic conditions. Since, morphology is decisive in controlling the properties of materials, synthesis of CPs with special morphologies has become the focus of attention in recent years. Various nanostructured forms of CPs have been used in the fabrication of chemical sensors and biosensors. Typically, nanotubes (Miao et al., 1999), nanowires (Gao et al., 2003) and films (Wiziack et al., 2007) of CPs have been used as sensor materials. Considerable efforts have been focused on the synthesis of nanostructured CPs (Li and Kaner, 2007; Yang et al., 2006; Lee et al., 2005). Various synthetic strategies have been adapted to nanostructure CPs, that include template and templateless synthesis (Martin, 1994; Liu et al., 2003) scanning probe electrochemical polymerization (Kranz et al., 1996), electrospinning (Gopalan et al., 2008), etc.

The utilities of CPs in monitoring and diagnosing metabolites such as glucose, hormones, neurotransmitters, antibodies,

antigens, etc. have been demonstrated. Most of these investigations were focused mainly on polypyrrole (Wang et al., 2006; Ramanavicius et al., 2005; Ekanayake et al., 2007), polythiophene (Yang et al., 2007) and polyaniline (Wang et al., 2006; Forzani et al., 2007). However, reports on other CPs are scarce. More recently, polydiphenylamine (PDPA) has received much attention owing to its better solubility and processability and finds numerous applications that includes pH and iron sensors (Tsai et al., 2003; Suganandam et al., 2005), corrosion inhibitors (Jeyaprabha et al., 2005), support for electrocatalyst in fuel cell (Santhosh et al., 2006), etc. Nevertheless, to the best of our knowledge, bio-sensing application of nanodimensional PDPA has not been attempted so far.

Hollow spheres (HSs) of polymers have the potential for promising applications such as confined reaction vessels, controlled release and delivery, separation systems, and biosensors because of their advantageous properties that include high specific surface area and low effective density. Polymer hollow spheres are prepared from spherical-particle templates, such as silica colloids (Han and Foulger, 2004), polystyrene beads (Yang et al., 2005; Niu et al., 2003; Marinakos et al., 1999) as hard templates followed by the removal of the sacrificial core through calcination or solvent etching. Hollow microspheres of polyaniline have been prepared by a self-assembled method using different dopants (Wei and Wan, 2002; Zhu et al., 2007).

We have recently prepared hollow spherical nanostructured PDPA (HS-PDPA) by performing in-situ polymerization of diphenylamine (DPA) within the galleries of montmorillonite clay through

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self-assembly approach using β -naphthalene sulfonic acid (β -NSA) as the 'soft template' to induce spherical micelles formation (Gopalan et al., 2006). The HS-PDPA showed several interesting characteristics, which include electronic properties that are different from the bulk PDPA. Importantly, HS-PDPA is soluble in most of the common organic solvents, electrochemically active and stable in neutral pH. This motivates us to investigate on the utilities of HS-PDPA towards biosensor applications. In the present investigation, a glucose biosensor is fabricated by incorporating the enzyme, glucose oxidase (GOx) into HS-PDPA matrix. The electrochemical signal transduction ability of HS-PDPA–GOx biosensor towards glucose is evaluated.

2. Experimental

2.1. Materials

Diphenylamine, ammonium persulfate (APS), β -NSA, D-glucose, Glucose oxidase (GOx) from *Aspergillus niger* (EC.1.1.3.4), glutaraldehyde, Nafion, hydrogen peroxide, ascorbic acid, uric acid and acetaminophen were of analytical grades from Aldrich. Montmorillonite clay, MMT (modified with organoammonium cations) was obtained from Southern Clay Products, Inc., Korea. All the other chemicals were of reagent grades. Aqueous solutions of glucose were prepared in 0.1 M phosphate buffered saline (PBS) afresh at the time of experiments.

2.2. Apparatus and measurements

The morphology of the electrode was examined by field emission transmission electron microscopy; JOEL TEM-2000EX. All electrochemical experiments were carried out using EG&G PAR Potentiostat/Galvanostat with FRA 1025 with a conventional three-electrode system. A platinum (Pt) disc was used for fabricating sensor electrode and used as working electrode. Before modification, Pt electrode was cleaned electrochemically by cycling the potential between -0.5 and $+1.3$ V at a scan rate of 100 mVs^{-1} in $0.5 \text{ M H}_2\text{SO}_4$. A platinum wire as a counter and Ag/AgCl (saturated with NaCl) as reference electrodes were used for all measurements. Potentials notified in the present work are against Ag/AgCl.

Quartz crystal microbalance (QCM) measurements were made using AT-cut quartz crystals (area: 0.196 cm^2) with quartz crystal analyzer (SEIKO EG & G, Model QCA 917). For the rotating disk electrode (RDE) studies, a Pt ring and HS-PDPA–GOx-modified Pt as disc electrode were used. In the case of amperometric measurements, the potential of the electrode was poised for instant at the operating value, allowing the background current to decay to a steady state and the output current was measured as aliquots of glucose were added to a well-stirred PBS. For flow analysis, current vs. time of addition of glucose was recorded for series of concentrations of glucose to the PBS. All the experiments were performed at room temperature ($25 \pm 1^\circ \text{C}$), unless and otherwise stated.

2.3. Preparation of HS-PDPA

The preparation details of HS-PDPA are presented elsewhere (Gopalan et al., 2006). Typically, about 0.5 g of MMT was dispersed in a 10 mM solution of diphenylamine (dissolved in 100 mM β -NSA). The mixture was sonicated for 24 h . After sonication, the solid material (DPA-loaded MMT) was filtered, washed several times with β -NSA and dried. Molecules were thus self-assembled inside the galleries of MMT (the mechanism of self-assembly inside the

galleries of MMT and the characterization have been reported earlier (Gopalan et al., 2006)). The self-assembled DPA loaded MMT powder was then re-dispersed in 50 mL β -NSA. To this, 20 mL of 0.5 M APS was added slowly with constant stirring for 2 h at 5°C . A dark-green colored precipitate, PDPA loaded MMT nanocomposite (in which PDPA exists in the interior galleries of MMT) was obtained. The composite was filtered, washed with distilled water and dried at 60°C in a vacuum oven. In order to extract the PDPA from the galleries of MMT, the following methodology was pursued. The composite was mildly stirred (50 rpm) in DMF (5 mL) for 5 h and filtered. A white mass, probably MMT, was removed. The green-colored filtrate that contains HS-PDPA ($\sim 100 \text{ mg}$) was separated out.

2.4. Fabrication of HS-PDPA–GOx electrode

About $5 \mu\text{L}$ of DMF solution containing HS-PDPA was placed on the Pt electrode and dried at 40°C under vacuum. GOx was immobilized into HS-PDPA. The Pt/HS-PDPA with GOx was cross-linked using glutaraldehyde (Glu). At first instant, $5 \mu\text{L}$ of 0.5% Glu was sprayed over the HS-PDPA film and was allowed to dry. GOx of defined amount was dropped onto the surface of HS-PDPA film electrode and was again allowed to dry further for about 3 h . The HS-PDPA–GOx electrode was washed several times with deionized water to remove the unbound GOx. Further, 1% ($3 \mu\text{L}$) Nafion solution was placed on the surface of the electrode to form a protective film. The electrode was stored at 4°C in PBS and used for further experiments. The steps involved in the fabrication of HS-PDPA–GOx electrode are presented in Scheme 1.

For a comparative purpose, a similar kind of electrode modification was performed using PDPA prepared by the conventional method (Nagarajan et al., 2005). About $5 \mu\text{L}$ of PDPA solution (100 mg of PDPA dissolved in 5 mL DMF) was placed on the Pt electrode and dried at 40°C under vacuum. Immobilization of GOx into PDPA film, cross-linking with Glu and formation of a Nafion layer were done sequentially as detailed above.

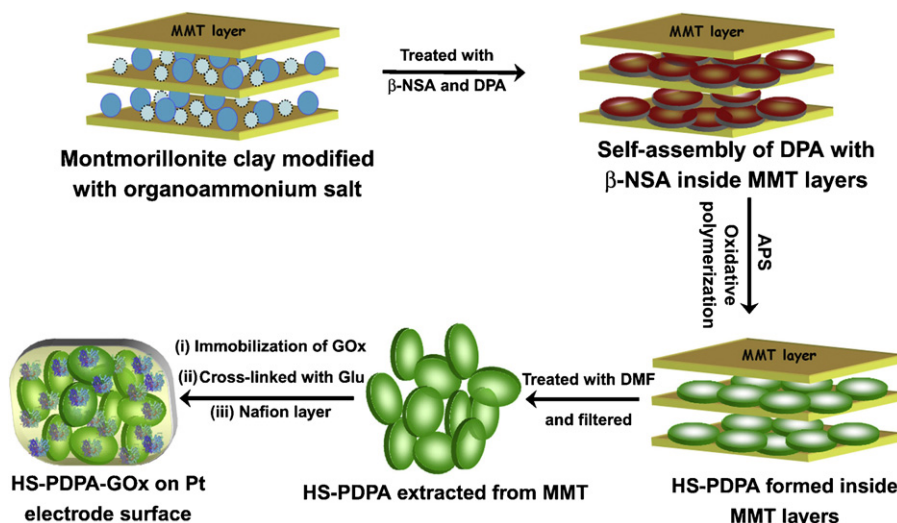
3. Results and discussion

3.1. GOx immobilization

Quartz crystal microbalance (QCM) was used to monitor the direct immobilization of GOx onto the HS-PDPA electrode. Fig. S1 (see Supplemental information) shows the QCM spectrum for the immobilization process. The spectrum reveals a sudden change in the frequency upon addition of $1 \mu\text{g mL}^{-1}$ GOx in PBS. The change in frequency (Δf) indicates the successful immobilization of GOx to HS-PDPA electrode. The Δf reached a saturation value of 106.65 Hz at higher frequency (beyond 100 Hz). The change in mass (Δm) was calculated by

$$\Delta m = \Delta f \times 5.608 \text{ (ng cm}^{-2}\text{)/Hz}$$

where $5.608 \text{ (ng cm}^{-2}\text{)/Hz}$ is the sensitivity factor. QCM analysis revealed that 598.1 ng cm^{-2} of GOx was immobilized into the HS-PDPA film. Using electrode surface area as 0.196 cm^2 , the amount of GOx immobilized into HS-PDPA was estimated as 117.2 ng . GOx used in this work contains 256 catalytic units per mg protein. And, one catalytic unit would oxidize $1 \mu\text{M}$ of D-glucose per minute at 25°C . The activity of immobilized enzyme on the electrode is estimated to be $3.0 \times 10^{-2} \text{ U}$. The enzyme activity of GOx was also determined using o-dianisidine method from the amount of H_2O_2 (Sigma Technical Bulletin, 1983) and found to be $2.81 \times 10^{-2} \text{ U}$. A slightly higher activity of GOx was noticed as compared to the theoretical value.



Scheme 1. Fabrication methodology of HS-PDPA-GOx electrode.

3.2. Morphology

Morphology of the HS-PDPA-GOx was observed by FETEM (Fig. 1). Hollow spheres of PDPA with inner diameter in the range of 40–90 nm and an outer diameter of about 60–110 nm can be seen. However, we could not observe any evidence for the adsorption of GOx at HS-PDPA electrode. The formation of hollow spherical morphology of PDPA is assisted by the soft template, β -NSA. Prior to polymerization, β -NSA molecules were self-assembled with DPA molecules to result spherical micellar structure (Gopalan et al., 2006). Upon polymerization of β -NSA/DPA self-assembled structure, PDPA with hollow spherical morphology was formed.

3.3. Direct electron transfer of GOx

The electrochemical characteristics of GOx immobilized at HS-PDPA electrode was studied using cyclic voltammetry. Fig. 2 shows the cyclic voltammograms (CVs) of the HS-PDPA electrode before (a) and after (b) immobilization of GOx in PBS (pH 7) saturated

with N_2 at a scan rate of 10 mV s^{-1} . CV of HS-PDPA-GOx electrode reveals the presence of a pair of well-defined, nearly symmetrical redox peaks ($E_p^c = -0.44 \text{ V}$, $E_p^a = -0.39 \text{ V}$, $\Delta E_p = 0.05 \text{ V}$). These peaks are ascribed to the redox reaction of the prosthetic flavin adenine dinucleotide (FAD) group bound to GOx. Free FAD may have dissociated away from GOx due to conformational changes during immobilization and hence not expected to contribute for charge transfer process. Thus, it is clear that the immobilized GOx retains its biochemical activity in HS-PDPA matrix. In the nanostructured form, GOx molecules are expected to align with PDPA chains to result in direct electron transfer (DET) from protein.

Studies were performed to understand the influence of pH and scan rate on the voltammetric characteristics (potential and current) of HS-PDPA-GOx electrodes (Fig. S2 (see Supplemental information)). With increasing pH between 5.0 and 8.0, shifts in the value of formal redox potential (E°) towards negative direction were witnessed. The variation in E° with pH was estimated as 0.056 V per pH . This signifies that the redox reactions of GOx involve two protons and two electrons:

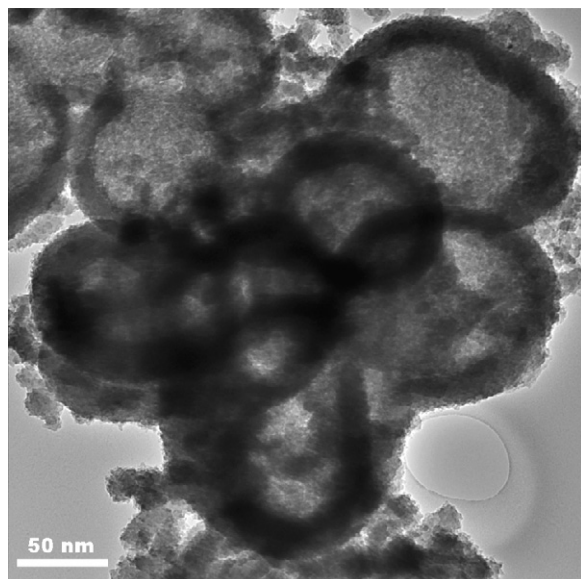
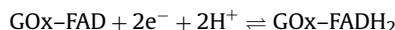


Fig. 1. FETEM image of HS-PDPA-GOx.

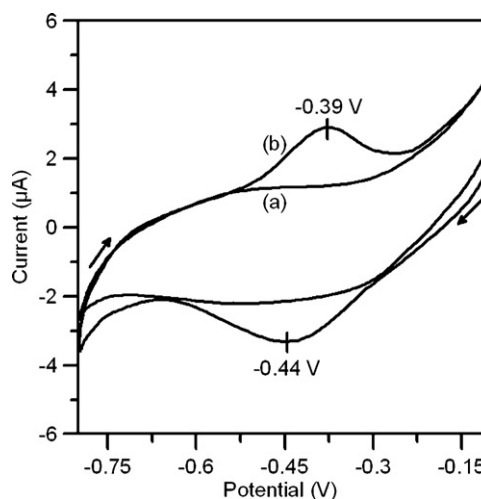


Fig. 2. Cyclic voltammograms of (a) HS-PDPA electrode and (b) HS-PDPA-GOx electrode in PBS (pH 7) saturated with N_2 ; scan rate 10 mV s^{-1} .

It was also found that the redox processes occurring at HS-PDPA-GOx electrode were significantly influenced by scan rate. The cathodic (i_p^c) and anodic peak (i_p^a) currents were found to increase with scan rates (up to 400 mV s^{-1}). The values of I_p^c and I_p^a were found to be close to each other. The values of E_p^c and E_p^a , shifted to negative and positive directions, respectively, with increasing scan rates. Beyond the scan rate of 400 mV s^{-1} the voltammetric wave distorted severely. On summarizing these informations, it is concluded that DET at HS-PDPA-GOx electrode is a surface-controlled and quasi-reversible process. The apparent heterogeneous electron transfer rate constant, k_s , was calculated to be 2.25 s^{-1} from the dependence of ΔE_p ($E_p^a - E_p^c$) on the scan rates. The value of k_s is larger than that observed for GOx immobilized at carbon nanostructures (typically, $k_s < 2.0$) (Elie et al., 2002; Cai and Chen, 2004; Liu et al., 2005; Jia et al., 2005).

3.4. Electrocatalytic behavior of HS-PDPA-GOx

The electrocatalytic activity of the HS-PDPA-GOx electrode towards the oxidation of hydrogen peroxide (H_2O_2) in the PBS (pH

7) was investigated using rotating disc electrode measurement. The potential corresponding to electro oxidation of H_2O_2 and rate constant of electrooxidation processes were evaluated. The current responses for various applied potentials between -1.0 and $+1.0 \text{ V}$, for (i) various concentrations of H_2O_2 (ii) and at different electrode rotation rates were monitored (Fig. 3). The sigmoidal shape of voltammograms thus observed is similar to nano-electrode assembly (Lin et al., 2004). Further, the transduced current was found to be dependent on potentials between the limits: -1.0 and $+1.0 \text{ V}$. Hence, potential corresponding to electrocatalytic oxidation of H_2O_2 (E_{cat}) was determined from the first derivative of the sum of scans ($n=5$) and found to be $+0.632 \text{ V}$ (Fig. 3(i)-inset). Also, the Faradaic limiting current (i_L) showed an increasing trend with rotation rate between 100 and 3700 rpm (Fig. 3(ii)). The lack of linearity in the typical Levich plot indicates that the oxidation of H_2O_2 at HS-PDPA-GOx electrode is a kinetic control process. The rate constant of electrooxidation of H_2O_2 (k°) was determined to be 0.083 cm s^{-1} from the Koutecky-Levich plot.

3.5. Hydrodynamic voltammetry

The most appropriate working potential suited for the detection of glucose at the HS-PDPA-GOx electrode was further ascertained by hydrodynamic voltammetric measurements. Toward this purpose, constant potential chronoamperometry was performed by varying the potential (in 0.05 V steps) of the HS-PDPA-GOx electrode between -1.0 and $+1.0 \text{ V}$ and steady-state current values were recorded (Fig. 4). The difference between the Faradaic current and the background current in 25 mM glucose (in PBS) was plotted against applied potentials. A substantial rise in current was observed at potentials higher than $+0.65 \text{ V}$, presumably due the oxidation of the enzymatically formed H_2O_2 (line a). Hence, $+0.65 \text{ V}$ was considered to be the best working potential for the detection of glucose. It is important to note that this value ($+0.65$) is nearer to the value determined by rotating ring disc method ($+0.632 \text{ V}$). On the contrary, the current response at the PDPA (conventional)-GOx electrode (line b) was comparatively much lower than noticed at the HS-PDPA-GOx. Typically, at $+0.65 \text{ V}$, the transduced current observed at HS-PDPA-GOx is about $40.1 \mu\text{A cm}^{-2}$ as compared to $1.85 \mu\text{A cm}^{-2}$ at PDPA (conventional)-GOx electrode. The high surface area of nanosized HS-PDPA provides more sites for the biocatalytic reaction and caused an augmented electron transfer rate.

3.6. Factors influencing the catalytic current response at HS-PDPA-GOx electrode

The performance characteristics of HS-PDPA-GOx electrode towards oxidation of glucose were investigated under various conditions such as amount of GOx, different pH and temperatures

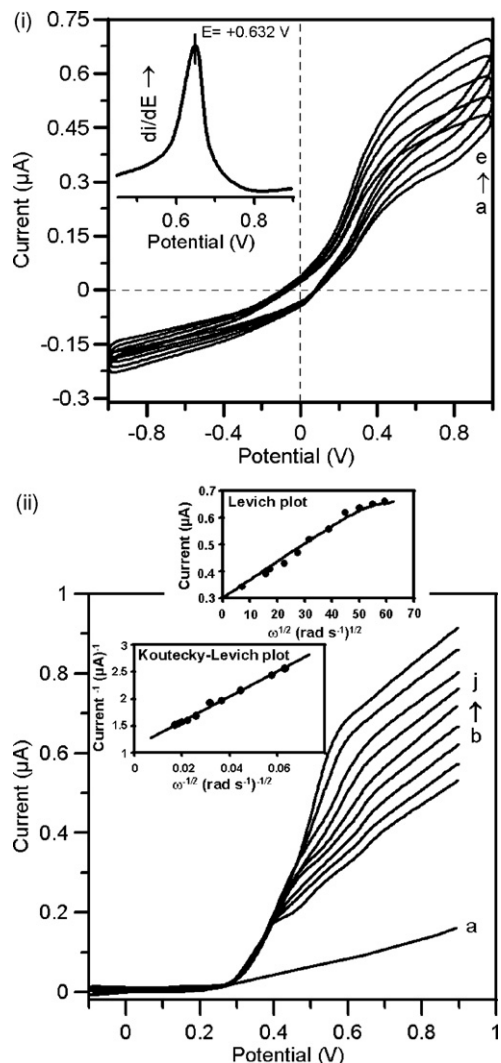


Fig. 3. (i) Current vs. potential profile for various concentration of H_2O_2 (0.1 – 0.5 mM ; a–e, respectively) in PBS (pH 7) at a rotating Pt disk electrode modified with HS-PDPA-GOx (at 1500 rpm). Inset shows the first derivative of the sum of scans ($n=5$). (ii) Current vs. potential profile recorded at various rotation rates between 100 and 3700 rpm in absence (a) and in presence of H_2O_2 (0.2 mM) (b–j) at a rotating Pt disk electrode modified with HS-PDPA-GOx. Typical Levich and Koutecky-Levich plots are shown.

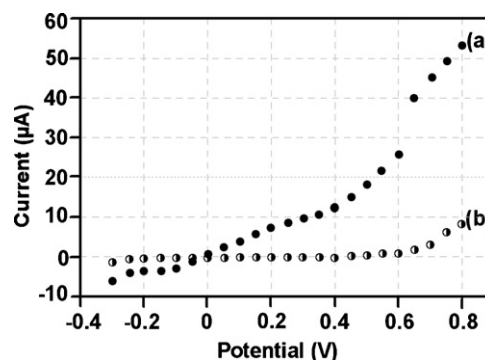


Fig. 4. Hydrodynamic voltammograms recorded at HS-PDPA-GOx (a) and PDPA (conventional)-GOx (b) electrodes in PBS (pH 7) containing 25 mM glucose.

(Fig. S3 (see Supplemental information)). The changes in electrode sensitivity (current response) with the amount of GOx used for the construction of the electrodes were investigated. Nine electrodes were prepared with different amount of GOx in the range between 1 and 5 mg mL⁻¹ and the sensitivity towards glucose was measured. The current response increases with amount of enzyme. Nevertheless, by considering the cost, the GOx of about 3 mg mL⁻¹ was used to construct the HS-PDPA-GOx electrodes.

Generally, electrochemical and bio-catalytic enzymatic reactions of glucose are influenced by changes in pH. To understand the influence of pHs, chronoamperograms were recorded at HS-PDPA-GOx electrode with the applied potential of +0.65 V between pH 4 and 9. The current response increased instantaneously from pH 4 to 7 and reached a maximum at 7.5 and then decreased up to pH 9. The decrease in current beyond the pH 7.5 is due to deactivation of GOx in alkaline pH. Hence, measurements were made at pH 7.5. At this juncture, it is pertinent to note that the presence of β -NSA as a dopant ensures the conductivity and the electroactivity of HS-PDPA at pH (7.5).

Temperature also plays a key role in deciding the performance of the sensor. Generally, rate of reactions increases with temperature (Arrhenius law). However, in the cases of enzyme catalyzed reactions, the loss of activity of the enzyme (denaturation of enzyme beyond certain temperature (generally $\sim 50^\circ$) must be considered. The current responses of HS-PDPA-GOx electrode towards glucose oxidation were determined at various temperatures. A rapid increase in current up to a temperature of 40 °C was noticed. Only a slight increase in current response was observed beyond 40 °C. By considering the convenience of the practical application, measurements were performed at 25 °C.

3.7. Constant potential amperometry

From the above experiments, the optimal conditions for the glucose detection at HS-PDPA electrode were determined. Fig. 5 shows the current–time profile of the HS-PDPA-GOx electrode to the successive additions of 4 mM glucose under the optimized experimental conditions ($E = +0.65$ V; rpm = 400). The current response at HS-PDPA-GOx electrode reached a steady state by 5 s upon the addition of glucose. The electrode exhibited a linear current response to the glucose between 1 and 28 mM. The linear plot between concentration of glucose and current response has a slope (sensitivity) of $1.77 \mu\text{A mM}^{-1}$, with a correlation coefficient of 0.9931 ($n=5$, R.S.D. = 3.84%). The sensitivity observed at HS-PDPA-GOx electrode is higher than that observed for glucose at polypyr-

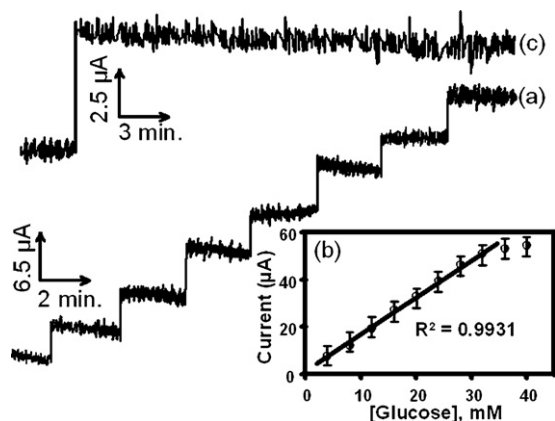


Fig. 5. (a) Current vs. time profile recorded at HS-PDPA-GOx electrode when the potential was held at +0.65 V to the successive additions of 4 mM glucose, (b) calibration plot and (c) stability of the current response of 4 mM glucose at HS-PDPA-GOx electrode in PBS (pH 7.5); $E = +0.65$ V.

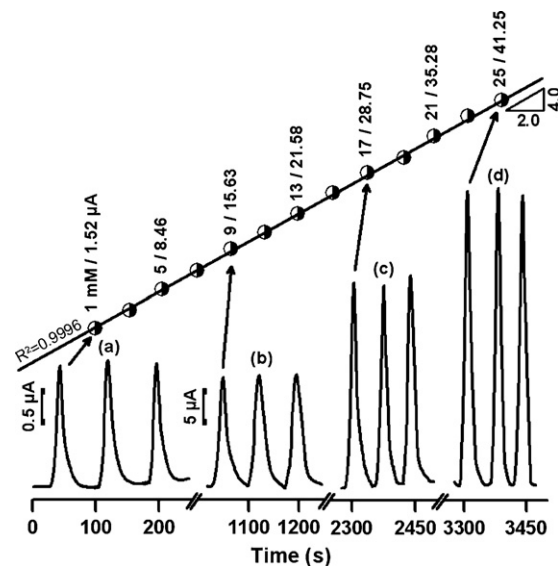


Fig. 6. Flow injection amperometric (FIA) response at HS-PDPA-GOx electrode with increasing concentration of glucose in PBS (pH 7.5); $E = +0.65$ V; flow rate: 1.5 mL min^{-1} . (a), (b), (c) and (d) are the FIA responses with glucose concentration of about 1, 9, 17 and 25 mM, respectively with scale bar of $0.5 \mu\text{A}$ for (a) and $5 \mu\text{A}$ for (b), (c) and (d). Calibration plot is also shown.

role nanoelectrode ensembles ($0.08 \mu\text{A mM}^{-1}$) (Liu et al., 2007) and at carbon nanotubes modified electrodes (Zhao and Ju, 2006; Salimi et al., 2004). Beyond 28 mM of glucose, the current response reached a saturation, typically follows the Michaelis-Menten kinetics (Manesh et al., 2008a).

Additionally, a stable amperometric signal could be witnessed for glucose (4 mM) at HS-PDPA-GOx electrode over the entire period of operation (30 min). A key feature of any analytical method is its detection limit; the smallest concentration of the analyte that can be detected to a specified degree of certainty. Hence, the detection limit of glucose at HS-PDPA-GOx electrode is estimated to be 0.05 mM ($S/N = 3$).

3.8. Flow injection analysis

The utility of HS-PDPA-GOx for the determination of glucose in flowing stream was also explored. The operating parameters such as flow rate and applied potential were optimized to have better sensitivity at HS-PDPA-GOx electrode. Fig. 6 shows the FIA response with increasing concentration of glucose (1–25 mM) in PBS (pH 7.5) at an applied potential of +0.65 V and at a flow rate of 1.5 mL min^{-1} . The calibration plot was linear up to 25 mM glucose ($R^2 = 0.9996$) with high sensitivity. Repeated injection of glucose yielded a R.S.D. value of 0.52%; $n = 3$ indicating good reproducibility of HS-PDPA-GOx electrode under flow conditions. Furthermore, no significant decrease in the peak current values was observed, which informs the absence of any surface fouling or leaching of materials from the electrode.

3.9. Influence of interferences

In the present study, an applied potential of +0.65 V is used to monitor the glucose concentration and this potential is high enough to oxidize the other electroactive species such as ascorbic acid (AA), uric acid (UA) and acetaminophen (AP) present in the sensing solution. Moreover, these species have higher electron transfer rates than glucose. As a result, the transduced net current would contain the interference signal. Thus, concentration of glucose will be overestimated. Hence, it is essential to eliminate the

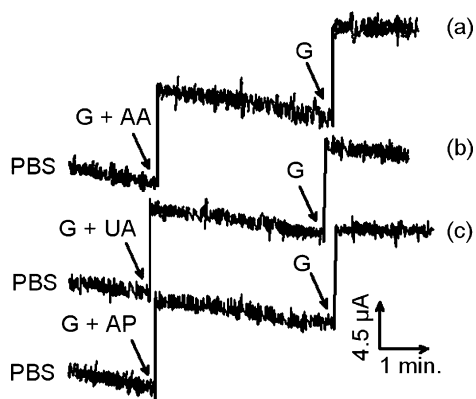


Fig. 7. Effect of interfering signals from AA (a), UA (b) and AP (c) of 5 mM each on the performance of HS-PDPA-GOx electrode towards 5 mM glucose (G) in PBS (pH 7.5); $E = +0.65$ V. AA, UA and AP represent ascorbic acid, uric acid and acetaminophen, respectively.

current contributions from interfering substances. Nafion with its ion-exchange capabilities can minimize the interferences (Manesh et al., 2008b) caused by such electroactive species. Considering this, measurements were made at HS-PDPA-GOx electrode with a coating of thin layer of Nafion (Fig. 7).

Initially, glucose (5 mM) along with AA (5 mM) was introduced into the stirred PBS and the amperometric current responses at HS-PDPA-GOx electrode were recorded. A sudden increase in the current response ($8.87 \mu\text{A}$) was observed after the addition of mixture of glucose and AA (line a). In order to authenticate that the current contribution was only from the oxidation of glucose and not from the combined influence of oxidation of AA, another experiment was performed with a concentration of glucose (5 mM). An increase in current of $8.83 \mu\text{A}$ was observed. This confirms that the current response at HS-PDPA-GOx electrode resulted only from the oxidation of glucose. Similar measurements were performed with UA and AP (Fig. 7, lines b and c, respectively) at HS-PDPA-GOx electrode. The results indicated that the electroactive substances either AA or UA or AP did not cause any observable interference in the current signal at HS-PDPA-GOx electrode in the concentration range (1–25 mM) of glucose.

3.10. Reproducibility, repeatability and life time of HS-PDPA-GOx electrode

The reproducibility of the current responses at the HS-PDPA-GOx electrode was evaluated. A R.S.D. value of 4.23% was noticed for nine electrodes fabricated concurrently. The repeatability of the HS-PDPA-GOx electrode was also checked by performing amperometric measurements ($n = 9$) with 4 mM glucose, the R.S.D. obtained was about 0.8%. The value is indicative of the good repeatability of the HS-PDPA-GOx electrode.

The life time of the HS-PDPA-GOx electrode was investigated by performing triplicate amperometric measurements with 4 mM glucose in PBS every day. The electrode was stored at 4°C in a PBS, when not in use. The mean value of the current responses was noted every day. There was no significant change in mean value observed for first 23 days. However, we could observe a decrease in sensitivity with successive calibrations after 23 days. This could be attributed due to deterioration of the electronic conductivity of HS-PDPA caused by the oxidation products of H_2O_2 or loss/denaturation of GOx.

3.11. Real sample analysis

Monitoring of glucose in blood is important for diagnosis and surveillance of diabetes. Hence measurements were performed

with glucose in serum samples to assess the precision and practical applicability of HS-PDPA-GOx electrode in real samples. The blood samples were diluted with PBS prior to the measurements. For comparison, the glucose content in the serum sample was also determined by the commercially available glucose meter (EZ Smart blood glucose monitoring system; ME-3301). At first, the samples were measured with the glucose meter and then with the HS-PDPA-GOx electrode. The correlation and straight-line fit plot between the two measurements is presented in Fig. S4 (see Supplemental information). The straight-line fit yielded slope of 0.982 with $R^2 = 0.9913$, which demonstrates the good correlation of results obtained between HS-PDPA-GOx and commercial glucose meter.

4. Conclusions

We have established that the modified probe based on nanostructured, hollow spherical-PDPA-GOx can effectively be used for the sensitive and selective detection of glucose. The HS-PDPA-GOx electrode showed high sensitivity ($1.77 \mu\text{A mM}^{-1} \text{cm}^{-2}$), as well as the best repeatability (R.S.D. = 0.8%, $n = 9$) and reproducibility (R.S.D. = 4.23%, $n = 9$) values for glucose detection. These parameters can be directly related to the operational stability, signifying the absence of electrode fouling which is an essential characteristic for biosensors to be used in real systems. In addition, the high selectivity of the biosensor for glucose over the common interferences provides the possibility to use these probes in clinical labs. Further, the strategy elaborated in the present investigation offers scopes for the fabrication of reliable and inexpensive biosensors with other enzymes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2008.10.004.

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