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Hollow spherical nanostructured polydiphenylamine for direct electrochemistry and glucose biosensor

P. Santhosh [a], K.M. Manesh [a], S. Uthayakumar [b], A.I. Gopalan [a,c], K.-P. Lee [a,c,*]

[a] Department of Chemistry Graduate School, Kyungpook National University, Daegu 702-701, South Korea
[b] Max-Planck-Institute for Solid State Research, Heisenbergstrasse 1, D-70569 Stuttgart, Germany
[c] Nano Practical Application Center, Daegu 704-230, South Korea

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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 $\mu A\,mM^{-1}\,cm^{-2}$), 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 scare. More recently, polydiphenylamine (PDPA) has received much attention owing to its better solubility and processibility 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

* Corresponding author at: Department of Chemistry Graduate School, Kyungpook National University, Daegu 702-701, South Korea. Tel.: +82 53 950 5901; fax: +82 53 95 28104.
E-mail address: kplee@knu.ac.kr (K.-P. Lee).

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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 mV s^{-1} in 0.5 M H_2SO_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 ± 1 °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 (∼100 mg) was separated out.

2.4. Fabrication of HS-PDPA–GOx electrode

About 5 μL 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 μL 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 μL) 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 μL 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 μg 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 (ng cm^{-2})/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 μM of D-glucose per minute at 25 °C. The activity of immobilized enzyme on the electrode is estimated to be 3.0×10^{-2} 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 2.81×10^{-2} U. A slightly higher activity of GOx was noticed as compared to the theoretical value.

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