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Amperometric Biosensor: Increased Sensitivity Using Enzyme Nanoparticles

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***Abstract*—Here we present a comparative study of amperometric biosensor developed by using nanoparticles of Glucose oxidase enzyme itself *(GOx np)* with that of enzyme immobilized on to gold nanoparticles *(Au np–GOx)*. The biosensor developed by immobilizing the *GOx np* (45-70 nm in size) directly onto the Pt. electrode could detect glucose concentrations as low as 1 mM while the one with *Au np–GOx (Au np* 10-16 nm in size) showed a detection limit up to 5 mM only. Current response showed linearity up to 35 mM in both the cases. To the best of our knowledge the glucose biosensors prepared using various metal nanoparticles, Au, Ni, Ag (as reported in literature) showed a linear range from 2.7-30 mM glucose concentration. We are in the process of optimizing the immobilization methods and functionalization of the enzyme nanoparticles so as to have a wider temperature and pH stability of the biosensor. We feel the direct immobilization of enzyme nanoparticles, with proper choice of functionalization seems to be a promising strategy for biosensor development with higher sensitivities.**

I. INTRODUCTION

Presently available industrial biosensors are based on membrane or hydrogel technology with response time ranging in minutes[1-2]. Researchers have shown that the response time could be decreased to few seconds (4 -10 sec) by using enzyme immobilized onto nanoparticles rather than membrane or hydrogel[3-4]. The small response time, high senstivity, and stability are attributed to the unique properties of nanostructures-like the small size (normally in the range of 1-100nm), high surface-to-bulk ratio leading to interesting optical, electric, chemical, mechanical, physical and catalytic properties which are not present in their respective bulk material[9]. Furthermore, method of immobilization of enzyme onto these nanomaterials, *i.e.* adsorption, cross linking or covalent binding etc., has profound effect on activity of the enzyme as well as shelf life of the biosensor. In the present work we intend to increase the sensitivity of the sensor by using the nanoparticles of enzyme itself rather than immobilizing enzyme onto nanoparticles and then onto electrode. We further intend to optimize the biosensor efficiency in terms of the cross linking or covalent binding agents.

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II. MATERIALS AND METHODS

A. Materials

Glucose *oxidase,* Gold (III) chloride trihydrate, Chitosan, *D*-(+)- Glucose, cysteamine dihydrochloride were purchased from Sigma Aldrich whereas Ethanol, Na_2HPO_4 , NaH_2PO_4 , Glutamic acid and glutaraldehyde (25% solution) were commercially supplied by CDH.

B. Gold and enzyme nanoparticles Synthesis

Gold nanoparticles were synthesized by chloroauric acid ($HAuCl_4$) using glutamic acid as a reducing agent. 75 mM glutamic acid was added slowly at a rate of 0.1-0.2 ml/min with constant stirring to a boiling solution of 0.1mM $HAuCl_4$. The onset of nanoparticle formation was marked by a color change from colorless to pale pink. This was followed by addition of 0.1% chitosan solution while the mixture was cooled down using an ice bath[5]. The particles thus formed were subjected to sonication for 15 minutes. The color of the colloidal gold solution changed from pale pink to red. These chitosan stabilized and amino functionalized gold nanoparticles were then separated by centrifugation at 9,000 rpm for 30 minutes.

The *glucose oxidase* nanoparticles were synthesized by addition of glucose *oxidase enzyme* to ethanol (1mg/ml) at a rate of 0.1-0.2 ml/min while the solution was continuously stirred at a speed of 500 rpm[6]. Here, the desolvating agent encouraged the protein to aggregate into small particles. This was followed by addition of 1% glutaraldehyde solution while stirring at 500 rpm for 24 hrs at 4^0C to ensure complete cross linking of the particles. The enzyme nanoparticles thus formed were amino functionalized by adding 0.02g/ml of cystamine dihydrochloride with constant stirring for 5-6 hours. Enzyme particles were separated from free enzyme by centrifugation. Particle solution was centrifuged at 12000 rpm for 10 minutes at 4^0C followed by redispersion in 0.1 M phosphate buffer (pH 7.4) and sonication for 5 minutes. The amino functionalized enzyme nanoparticles were stored at 4^0C till further use.

C. Characterization of nanoparticles

The morphological analysis of synthesized gold and enzyme nanoparticles was done by transmission electron microscope (TEM). Morgagini 268 transmission electron microscope with magnification of 8900 x was used for size estimation. 10µl of the sample solution was placed onto a

carbon-coated copper grid and air dried before viewing it under transmission electron microscope.

The nanoparticle samples were further characterized by optical studies (absorbance) using a cuvette free spectrophotometer ND 1000 UV-VIS Spectrophotometer from Nanodrop technologies operated at 12 V.

D. *Functionalization of electrode*

1) Preparation of Au nanoparticles immobilized enzyme electrode : The platinum electrode was cleaned before immobilizing the nanopartciles. This was achieved by dipping the electrode in boiling H_2SO_4 for 30 minutes followed by washing with distilled water. The electrode was amino functionalized by dipping the cleaned electrode into aqueous cystamine dihydrochloride solution (10 mg/ml) in darkness for 2 hrs. The electrode was then immersed in 10% (v/v) glutaraldehyde for 20 minutes followed by 4 hr incubation in eppendorf containing redispersed gold nanoparticles at 4^oC. Glucose oxidase enzyme was immobilized onto the electrode precoated with nanoparticles by leaving it overnight in the enzyme solution. This Au np–GOx electrode was rinsed with distilled water and stored in 0.1 M phosphate buffer (pH-7.4) at 4^0C for further use [7-9].

2) Preparation of enzyme nanoparticles based electrode : The platinum electrode was cleaned and functionalized as per the protocol given above. After dipping the electrode into glutaraldehyde solution, it was rinsed with distilled water and was then immersed into amino functionalized glucose oxidase nanoparticle (GOx np) solution for 10 hours at 4^0C. Finally the electrode was washed with water, dried and then stored in phosphate buffer (pH-7.4) at 4^0C.

E. *Amperometric Measurements*

Amperometric measurements were carried out using Ag/AgCl as reference electrode and the modified platinum electrode as a working electrode. Current measurements were done with the microammeter connected in series at an applied potential of 0.4 V across the two electrodes[10-12]. β-D glucose (1mM to 50 mM) prepared in 0.1M phosphate buffer (pH 7.4) were left overnight for mutarotation at room temeparture. Current was recorded for 1mM-50mM glucose and for water (molecular biology grade water from Hyclone) and 0.1 M phosphate buffer (pH 7.4) which were used as negative control.

III. Results And Discussion

The synthesized *Glucose oxidase* enzyme nanoparticles (***GOx np***) and gold nanoparticles (***Au np***) were characterized using transmission electron microscopy (TEM) and UV-visible spectroscopy. Transmission electron microscope (TEM) micrograph shows spherical *Au np* with an average diameter of 11nm and a narrow size distribution 10-16 nm diameter (as shown in Fig 1a). The micrograph image of 45-70 nm enzyme nanoparticles (*GOx np* - white colored particles) showed an average diameter of 50 nm (Fig1b).

These nanoparticles were further characterized by UV-visible absorption spectroscopy. The *Au np* showed the characteristic absorption peak at 522 nm (Fig. 2) and *GOx np* showed at 235 nm (Fig. 3a). The *GOx np* in comparison with

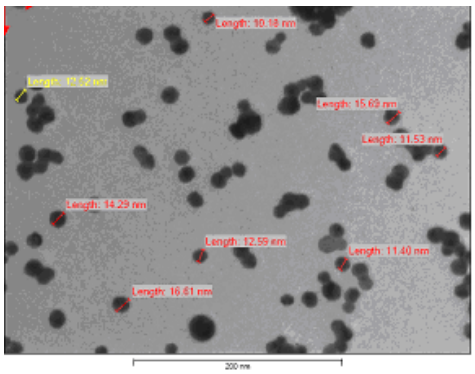

Figure 1. a. Transmission electron micrograph of the Gold nanoparticles (Au np)

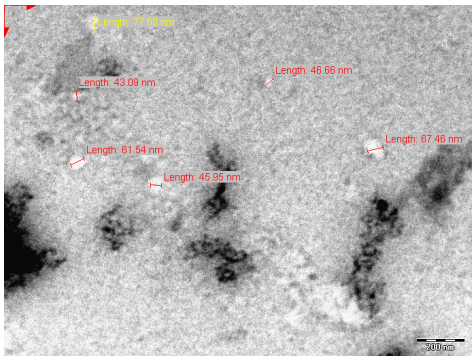

Figure 1. b. Transmission electron micrograph of the Glucose oxidase nanoparticles (GOx np)

the *GOx* enzyme (free enzyme, absorption maxima 267 nm, see Fig. 3b) showed a blue shift confirming the formation of nanoparticles

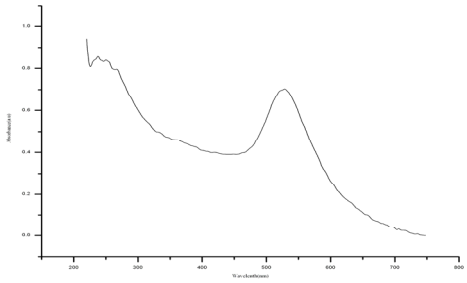

Figure 2. UV-visible absorption spectra of gold nanoparticles

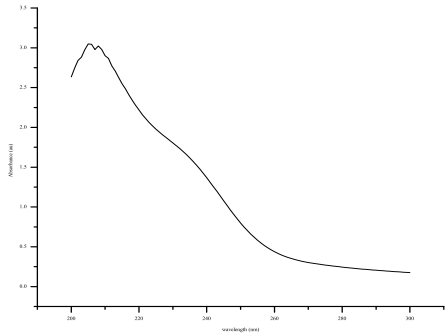

Figure 3. a. UV-visible absorption spectra of Glucose oxidase nanoparticles

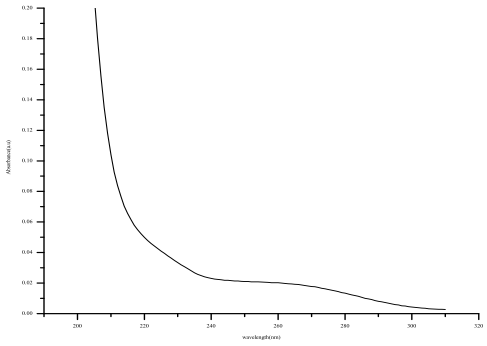

Figure 3. b. UV-visible absorption spectra of Glucose oxidase enzyme

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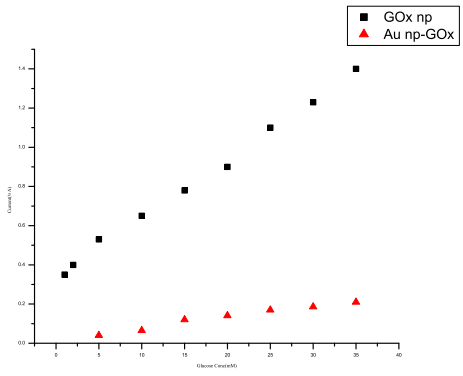

Figure 4. Variation of current as a function of glucose concentration using modified Pt electrode a) *Au-np GOx* Complex (Δ) and b) *GOx-np*(□).

No absorption peak was observed with glutaraldehyde or cysteamine dihydrochloride solutions which were used in nanoparticle synthesis. This confirms the fact that the shift in absorption maxima is because of nanoparticle formation only and not from the traces (if any) of the reactants in the sample.

Glucose *oxidase* enzyme nanoparticles (GOx np) were synthesized and immobilized onto the functionalized electrode in an attempt to improve the sensitivity of glucose biosensor. The sensitivity was measured in terms of current response as a function of glucose concentration.

The amperometric measurements were carried out for Pt-*Au np*-GOx and Pt-GOx np electrodes at an applied potential of 0.4V (Vs Ag/AgCl electrode) at room temperature (27^{o}C). A linearly increasing current response was observed upto 35 mM glucose concentrations only though measurements were done for a range of 1mM – 50 mM. The enzyme nanoparticles biosensor (Pt-*Au np*-GOx Vs Ag/AgCl) could detect glucose upto as low as 1 mM (Fig. 4a) while the *GOx* immobilized onto *Au np* (Pt-GOx np Vs Ag/AgCl) could detect up to 5 mM only (Fig. 4b). Water and PBS acted as negative control which showed no current

IV. CONCLUSIONS

In the present work we have compared the sensitivity of glucose biosensor constructed using *Glucose oxidase* nanoparticles (Pt-GOx np) with the one using enzyme immobilized onto gold nanopartciles (Pt-*Au np*-GOx). *GOx np* with an average nanoparticles diameter of 50 nm were synthesized successively using simple desolvation process and *Au np* of 11 nm using glutamic acid as reducing agent via chemical reduction method. It was observed that the sensitivity of the Pt-GOx np biosensor showed an improved performance not just in terms detection limits but also much larger current response.

We are in the process of optimizing the immobilization methods and functionalization of the enzyme nanoparticles so as to have a wider temperature and pH stability of the biosensor. We feel the direct immobilization of enzyme nanoparticles, with proper choice of functionalization seems to be a promising strategy for biosensor development with higher sensitivities

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REFERENCES

[1] A. Guiseppi-Elie, S. Brahim, D. Narinesingh "Composite hydrogels containing polypyrrole as support membranes for amperometric enzyme biosensors" Journal of Macromolecular Science, Part A, Vol 38, Issue 12 November 2001 , pp 1575 – 1591.

[2] R. J. Russel, M. V. Pishko, C. C. Gefrides, M. J. Mcshane and G. L.Cot "A Fluorescence-Based Glucose Biosensor Using Concanavalin A and Dextran Encapsulated in a Poly(ethylene glycol) Hydrogel" *Anal. Chem.*, *71* (15), 1999, pp 3126–3132.

[3] Lu. Huang, Z. Peng, Y.Guo,L.Porter, "Identifying Emerging Nanoparticle Roles in Biosensors" Journal of Business Chemistry, 2010, p 15.

[4] Y. Zoua,,C. Xiang , Li-Xian Suna,, F. Xu "Glucose biosensor based on electrodeposition of platinum nanoparticles onto carbon nanotubes and immobilizing enzyme with Chitosan- SiO2 sol gel" Biosensors and Bioelectronics 23 ,2008, pp 1010–1016.

[5] A. Sugunan, P. Juilland, C. Thanachayanont, J. Dutta, J.G. Hilborn, Synthesis of bio-compatible gold nanoparticles, Proceedings of International Conference on Smart Materials (SMARTMAT-04), Chiang Mai, Thailand, 2004.

[6] G. Liu, Y. Lin, V. Ostatna and J. Wang "Enzyme nanoparticles-based electronic biosensor" Chem.Commun, 2005, pp.3481-3483.

[7] Y. Sun, F. Yan, W. Yang, S. Zhao, W. Yang and C. Sun,"Effect of silica nanoparticles with different sizes on the catalytic activity of glucose oxidase." Springer-Verlag 2007.

[8] Liang R, Jiang J, Qiu J. "An amperometric glucose biosensor based on titania sol- gel/Prussian Blue composite film." Anal Sci.24 (11):1, 2008 pp 425-430.

[9] S. Zhang, N. Wang, H. Yu, Y. Niu, C. Sun, Covalent attachment of glucose oxidase to an Au electrode modified with gold nanoparticles for use as glucose biosensor, Bioelectrochemistry 67 (2005) pp 15–22Y.

[10] X. Ren, X. Meng, D. Chen, F. Tang and J. Jiao "Using silver nanoparticle to enhance current response of biosensor" Biosensors and Bioelectronics vol 21, 3, 2005 pp 433-437.

[11] TP. Ya´ n ez-Sedeno .J. M. Pingarro´n "Gold nanoparticle-based electrochemical biosensors." Anal Bioanal Chem, 382, ,2005, pp 884–886.

[12] X. Meng, X. Ren, F. Tang, "A Glucose biosensor using Au nano particles modified glucose oxidase." Nanotech, vol 3, 2003,pp-404-407.