Fabrication of a tunable glucose biosensor based on zinc oxide/chitosan-graft-poly (vinyl alcohol) core-shell nanocomposite

S. K. Shukla, Swapneel R. Deshpande, Sudheesh K. Shukla and Ashutosh Tiwari

Linköping University Post Print

N.B.: When citing this work, cite the original article.

Original Publication:

S. K. Shukla, Swapneel R. Deshpande, Sudheesh K. Shukla and Ashutosh Tiwari, Fabrication of a tunable glucose biosensor based on zinc oxide/chitosan-graft-poly (vinyl alcohol) core-shell nanocomposite, 2012, Talanta, (99), 283-287.

<http://dx.doi.org/10.1016/j.talanta.2012.05.052>

Copyright: Elsevier

<http://www.elsevier.com/>

Postprint available at: Linköping University Electronic Press

<http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-85582>

Fabrication of a tunable glucose biosensor based on zinc oxide/chitosan-*graft*-poly(vinyl alcohol) core-shell nanocomposite

S.K. Shukla¹, Swapneel R. Deshpande², Sudheesh K. Shukla², and Ashutosh Tiwari^{2,3*}

¹*Department of Polymer Science, Bhaskaracharya College of Applied Sciences, University of Delhi, New Delhi 110 075, India*

²*Biosensors and Bioelectronics, IFM-Linköpings universitet, 581 83 LINKÖPING, Sweden*

³*Linköping Integrative Regenerative Medicine (IGEN) Centre, IKE-Linköping University, S-581 83 Linköping, Sweden*

*Corresponding author. Tel.: (+46) 13 2823 95; Fax: (+46) 13 13 75 68; E-mail address: ashutosh.tiwari@liu.se.

Abstract

A potentiometrically tuned-glucose biosensor was fabricated using core-shell nanocomposite based on zinc oxide encapsulated chitosan-*graft*-poly(vinyl alcohol) (ZnO/CHIT-*g*-PVAL). The glucose oxidase (GOD) was immobilized onto ZnO/CHIT-*g*-PVAL coated indium-tin oxide (ITO) glass substrate. The electrostatic interaction between GOD and ZnO based core-shell nanocomposite provided the resulting tuned enzyme electrode (GOD/ZnO/CHIT-*g*-PVAL) with a high level of enzyme immobilization and excellent lifetime stability. The response studies were carried out as a function of GOD concentration with potentiometric measurement. The GOD/ZnO/CHIT-*g*-PVAL/ITO bioelectrode has showed a linear potential response to the glucose concentration ranging from 2 μM to 1.2 mM. The glucose biosensor exhibited a sensitivity of $>0.04 \text{ V}/\mu\text{M}$ with a response time within three sec.

Keywords: Chitosan; poly(vinyl alcohol); zinc oxide; core-shell nanocomposite; bioelectrode; glucose biosensor.

1. Introduction

Chitosan (CHIT) is a high molecular weight cationic polysaccharide with potential applications in food, agriculture, cosmetics, pharmaceuticals and biosensor industries [1- 4]. Typically; CHIT is an inexpensive, biocompatible, biodegradable, nonconducting, electroactive biopolymer. These basic properties render it suitable for the fabrication of biosensors; *viz.*, immobilization of enzymes, DNA, RNA, antigens and antibodies. However, CHIT has a few drawbacks; for example, specific solubility, high viscoelastic value and modest electrical/ionic conductivity that can be overcome by graft copolymerization [5, 6]

On the other hand, polyvinyl alcohol (PVAL) is a promising water-soluble polymer for biomedical applications [7]. It was reported that PVAL provides a stable surface for the immobilization of GOD; and, GOD shows excellent enzyme activity even after gamma radiation [8, 9]. Moreover, nanomaterials such as gold nanoparticles [10], carbon nanotubes [11], calcium carbonate nanoparticle [12], cupric oxide [13] and cupric sulphide [14] are adopted for fabrication of various glucose biosensors. However, among these zinc oxide (ZnO) nanoparticles are more versatile nanomaterials for glucose sensing; *viz.* a significant biocompatibility and high value of isoelectric points difference. Thereby, ZnO nanoparticles can help to accommodate GOD through strong electrostatic forces and a good shelf life. In addition, ZnO possesses good electrical conductivity, which enhances the direct electron transfer between the active site of GOD to the sensitive layer suitable for electrochemical sensing [15, 16]. In this context, the use of core-shell nanocomposites to fabricate biosensors is one of the current moving approaches because metal oxide *viz.* ZnO core nanoparticle coated with a polymeric layer would provide the hybrid structure with an additional function on the top of core, which can synergistically emerge the performance of the biosensor. For example, Li *et al.* have used a hollow nanonickel

oxide (NiO)/CHIT fiber-modified glassy carbon bioelectrode (CHIT/GOD/NiO/GC) for glucose sensing [17].

Compared to conventional analytical methods, biosensors can provide improved specificity, detection limits and speed of response for use in decentralized locations such as home, physician's office or factory environment. Medical diagnostics, biotechnology and food industry is always accompanied with fast and reliable glucose monitoring biosensor. Table 1 shows the different types of reported core-shell nanocomposite based glucose biosensors with their limits of operational flexibility such as selectivity, reproducibility and response time as shown.

Table 1. The different types of glucose sensors with operational flexibility.

Matrix	Transducer	Response time	Linearity range	Reference
ZnO nanowire	Metal-oxide-semiconductor field-effect transistor	5s	1-100 μm	[18]
$\text{Fe}_3\text{O}_4/\text{CHIT}$	Amperometric	5s	10-400 mg	[19]
$\text{Fe}_3\text{O}_4/\text{SiO}_2$	Electrical type	10s	10^{-5} M	[20]
Sol-gel CHIT	Amperometric	30s	2-4 mM	[21]
CHIT/ ZrO_2	Amperometric	10s	10^{-5} - 10^{-3} M	[22]

In general, electrochemical biosensors offer various advantages such as less complexity, robustness and high range of detection limit [23]. Recently, organic-inorganic hybrid nanomaterials offer new dimensions in the field of biosensors and molecular recognitions research [24]. Therefore, the focus of the recent research is to develop a glucose biosensor with

improved properties using a combination of the biologically active site of ZnO nanoparticles and graft copolymer matrix.

The present paper reports the fabrication of ZnO/CHIT-*g*-PVAL core-shell nanocomposite for tunable glucose biosensing. The ability to entrap GOD onto ZnO/CHIT-*g*-PVAL core-shell nanocomposite is a key element in the design of proposed glucose biosensor. We have harnessed GOD/ZnO/CHIT-*g*-PVAL nanomaterials and its extraordinary potentiometric behavior to create a new generation of highly sensitive and reproducible glucose biosensor to a maximum concentration of 1.2 mM in an aqueous media. Thus the prepared glucose biosensor provides relatively good shelf life, high sensitivity and fast response time.

2. Experimental

2.1. Materials

The chemicals, polyvinyl alcohol (Sigma, 99.5%), CHIT (Aldrich, >85% deacetylated), zinc oxide nanoparticles (Aldrich, <25 nm particle size, <20 nm avg. part. size) glucose oxidase (GOD, from *Aspergillus niger*, EC 1.1.3.4. 150,000 unit/g), glucose (Sigma, 99%), L-serine (Sigma, 99%), L-threonine (Sigma-Aldrich, 98%), α -ketoglutaric acid (Sigma, 98.5%), L-alanine (Sigma, 98%), uric acid (Sigma, 99%), L-cystine (Sigma, 97%), L (+)-glutamic acid (Sigma, 98.5%), sodium pyruvate (Sigma-Aldrich, 99%), and L-ascorbic acid (Sigma, 99%), were purchased and used without further purification. All supplementary chemicals were of analytical grades and solutions were prepared with 18.2 M Ω deionized water. Indium-tin-oxide (ITO) coated glass sheets (Balzers) with a resistance of 15 Ω/cm^2 were used as substrates for the deposition of electrodes.

2.2. Preparation of CHIT and PVAL solutions

To prepare CHIT solution, CHIT flakes (1.0 g) was added to acetic acid (100 mL, 1%). The chitosan solution was stirred on magnetic stirrer till a clear solution was obtained. To prepare PVAL solution, PVAL (2 g) granules were dissolved in distilled water (200 mL) in a 500 mL beaker at 50 °C with constant magnetic stirring. The solutions were kept in a refrigerator at 4 °C.

2.3. Fabrication of ZnO/CHIT-g-PVAL core-shell nanocomposite electrode

In a typical experiment process, PVAL (50 mL), CHIT (150 mL), and ZnO nanoparticles (10 mg) were mixed in a 250 mL flask. The mixture was magnetically stirred at 40 °C for 4-h. The resulting colorless core-shell nanocomposite solution was spin-cast on a ITO glass plate at the speed of 2000 rpm to make core-shell nanocomposite film. The core-shell nanocomposite film was washed with deionized water followed by phosphate buffer solution (PBS, pH 7.4) to remove unbound particles. The core-shell nanocomposite-coated ITO glass plate was used for characterization and glucose sensing.

2.4. Immobilization of GOD

A fresh GOD solution (10 ppm) was prepared in a phosphate buffer solution at pH 7.4. The GOD solution was spread onto ZnO/CHIT-g-PVAL/ITO thin film using spin-casting equipment at a speed of 500 rpm. The resulting GOD immobilized core-shell nanocomposite film was kept overnight at room temperature, then washed with PBS to leach out unbound GOD enzyme from the bioelectrode surface.

2.5. Characterization of electrode

Spectroscopic analysis was carried out using a PerkinElmer (RK-1310) FTIR spectrometer. Field emission scanning electron microscopy was carried out with an Hitachi S-

4800 operated at 5 kV. The specimens were sputter-coated with a thin layer of iridium (~5 nm) prior to examination. The morphology of ZnO encapsulated core-shell nanocomposite was further studied by transmission electron microscopy (TEM, Hitachi H-600) operated at 75 kV. A TEM sample was prepared by depositing 6 μL solution of the core-shell nanocomposite (ultrasonically dispersed in THF) on a copper grid.

The physico-mechanical properties; e.g., solvent content, thickness, swelling and porosity of the prepared core-shell nanocomposite film, were determined by a literature method [25]. Thus, the film was first soaked in a saturated 1M NaCl solution for 24-h, then blotted quickly with a filter paper to remove the surface solvent and weighed immediately. The electrode was further dried to a constant weight in a vacuum over silica gel. The solvent content (% total wet weight) was calculated by Eq. 1:

$$S = \frac{W_w - W_d}{W_w} \times 100 \quad (1)$$

where, W_w is the weight of wet film and W_d is the weight of dry film. The degree of swelling was measured as a difference between average thickness of the wet film equilibrated in NaCl solution for 24-h and the dry film.

The Porosity (E) of core-shell nanocomposite was determined as a volume of a solvent incorporated in the cavities per unit electrode volume; Eq. 2:

$$E = \frac{W_s - W_d}{AL\delta_w} \quad (2)$$

where, W_s is the soaked weight, W_d is the dry weight, A is area of the film, L is thickness of film and δ_w is density of water.

The prepared GOD/ZnO/CHIT-*g*-PVAL core-shell nanocomposite film was used as a glucose sensing electrode against saturated calomel electrode (SCE) in all potentiometric

measurements. The potentiometric sensitivity toward glucose was measured in a freshly prepared aqueous solution at different concentration (i.e., 2×10^{-6} to 1.2×10^{-3} M) at 20 °C. The solutions were stirred for 60-sec; and, the potential readings were taken after stabilization of the potential.

3. Results and discussion

3.1. Electrode fabrication and characterizations

The ZnO/CHIT-g-PVAL core-shell nanocomposite was prepared via the graft copolymerization reaction between the CHIT and PVAL followed by self-assembly upon ZnO nanoparticles periphery. The resulting ZnO/CHIT-g-PVAL core-shell nanocomposite was deposited onto an ITO coated glass substrate to form a uniform film. The ZnO present in the core of nanocomposite was supported to create a high value of isoelectric points difference (ΔIEP ; ZnO nanoparticle = 9.5 and GOD = 4.2) for electrostatic immobilization of GOD onto ZnO based core-shell nanocomposite. Figure 1 shows the overall steps for preparing ZnO/CHIT-g-PVAL core-shell nanocomposite and fabricating the ZnO/CHIT-g-PVAL core-shell nanocomposite/ITO and GOD/ZnO/CHIT-g-PVAL/ITO electrodes.

Figure 1. Schematic illustration of (a) fabrication of ZnO/CHIT-g-PVAL core-shell nanocomposite electrode, and (b) immobilization of GOD onto core-shell nanocomposite electrode.

The FTIR spectra of PVAL, CHIT and core-shell nanocomposite materials are given in Figure 2. The C=O peaks in pure PVAL and CHIT were observed at 1100 cm^{-1} and 1080 cm^{-1} , respectively. In case of the synthesized core-shell nanocomposite, the C=O peak was observed at 1021 cm^{-1} , which indicates a shift towards lower wavenumber of about 60 cm^{-1} . Likewise, the CHIT gives a peak for the amide group at 1654 cm^{-1} and 1599 cm^{-1} ; for the ZnO/CHIT-g-PVAL core-shell nanocomposite, a broad peak occurs at 1623 cm^{-1} . The hydrogen bonding peaks between the -OH groups in CHIT, PVAL and ZnO/CHIT-g-PVAL are observed at 3473 cm^{-1} , 3532 cm^{-1} and 3429 cm^{-1} , respectively, and shifts towards again lower wavenumbers. These lowering of IR frequencies may be attributed to the interaction between the functional groups such as -OH and -NH₂ of PVAL and CHIT.

Figure 2. FTIR spectra of (a) ZnO/CHIT-g-PVAL core-shell nanocomposite, and (b) GOD/ZnO/CHIT-g-PVAL electrode.

The spectra of 1M HCl-treated and untreated ZnO/CHIT-g-PVAL core-shell nanocomposite have shown a shift to higher wavelength in the treated materials. This is probably due to the weakening of the bonds and/or partial ionization, which is consistent with a conduction of electric impulse and pH responsive behavior of the core-shell nanomaterial. The GOD-based glucose sensor works on the principle of monitoring hydronium ion generation after oxidation of glucose. Thus, the pH responsive nature of the ZnO/CHIT-g-PVAL core-shell nanocomposite should be favorable for glucose sensing.

Moreover, SEM measurements of ZnO/CHIT-g-PVAL core-shell nanocomposite film before and after immobilization of GOD are shown in Figure 3 (a and b). The representative topology confirms that before immobilization of GOD onto core-shell nanocomposite film was observed to have a homogenous porous multi-component surface, whereas after immobilization of GOD enzyme resulted in a smooth surface. The uniform smooth surface may be formed due to the binding of GOD molecules over the CHIT-g-PVAL core-shell nanocomposite. To further understand the morphology of the ZnO/CHIT-g-PVAL nanoparticles, TEM analysis was conducted using PTA as a staining agent (Figure 3c). As demonstrated in Figure 3c, CHIT-g-PVAL graft copolymer may be rapped over ZnO nanoparticle. The TEM result reveals that the average size of the resulting ZnO/CHIT-g-PVAL nanocomposite particles were ranging from 30 to 80 nm. The PTA stain clearly showed the light contrast of CHIT-g-PVAL copolymer periphery in the nanocomposite particles and the dark contrast of ZnO nanoparticles with an average size of 20 and 35 nm towards core, respectively. The TEM image of the ZnO/CHIT-g-PVAL nanoparticles clearly showed the capping of CHIT-g-PVAL onto ZnO nanoparticles in Figure 3.

Figure 3. SEM micrographs of core-shell nanocomposite films (a) before, and (b) after immobilization of GOD, and (c) TEM image of ZnO/CHIT-g-PVAL core-shell nanocomposite with a zoom graphical nanoparticles illustration.

3.2. Glucose biosensing and nano-biocatalysis

The potential variation of induced glucose sensing is shown in Figure 4, i.e., via GOD immobilization onto CHIT-*g*-PVAL matrix (with and without ZnO nanoparticles) vs. glucose concentration. The plots indicate linear potential response with glucose concentration ranging from 2×10^{-6} to 1.2×10^{-3} M. The linearly increase in glucose concentration were observed through potential differences in bioelectrode. The change in potential may be observed due to enzymic oxidation of glucose onto bioelectrode surface, *via*; more generation of H^+ ions as end product. The produced hydrogen ions are directly proportional to glucose concentration. Thus, effect of pH on bioelectrode surface was studied with the objective to notice the solvation effect. The results indicate the comparable values of potential which obtained at in between 6 to 8 pH. The result supports the dissolution effects of CHIT in slight acid and basic medium [26].

All over again, the potential change was also recorded of GOD/CHIT-*g*-PVAL without ZnO nanoparticles. Figure 4b clearly indicates that ZnO nanoparticles enhance about 3 times sensitivity of core-shell nanocomposite bioelectrode for glucose sensing. It may be understood that ZnO nanoparticles probably acts as electrocatalytic center for glucose oxidation and it enhances the bioelectrode sensitivity for glucose. The performance of the sensing bioelectrode was investigated in non-aqueous media using water-acetic acid and water-ethanol up to 12% v/v. With solvent mixtures, the slope of curve remains more or less the same. However, slope of curve decreases at higher alcohol concentration.

Figure 4. Potential of bioelectrode vs. glucose concentration (a) with and (b) without ZnO nanoparticles in CHIT-g-PVAL matrix.

3.3. Tunable glucose biosensing

The high swelling value of ZnO/CHIT-g-PVAL core-shell nanocomposite film, i.e., 76 % favors for glucose sensing. Since, the diffusion of glucose solution is better in swellable film which generates the instantaneous osmotic pressure gradient across interface between sensitive films (cf., ZnO/CHIT-g-PVAL) and surrounding analyte (glucose) solution. The osmotic pressure gradient reversibly affects the distribution of (H^+) ions [27, 28], viz.; generated after glucose oxidation. Thus, swelling behavior of ZnO/CHIT-g-PVAL core-shell nanocomposite

film may also support GOD efficiency through allowing the quick diffusion of analyte and providing small surface reaction zones like molecular cavities to generate H^+ ion after oxidation of glucose. Moreover, the swelling behavior of core-shell nanocomposite film was also observed at different pH. The best result has been observed at pH 6.5. CHIT-g-PVAL may be moderately created stable surface near to neutral pH.

The interference effects of L-serine, L-threonine, α -ketoglutaric acid, L-alanine, uric acid, L-cystine, L(+)-glutamic acid, sodium pyruvate, and L-ascorbic acid were investigated by measuring the potentiometric response of the GOD immobilized ZnO/CHIT-g-PVAL film in the presence of 250 μ M glucose. The interference chemicals were added into the reaction solution at 25 μ M concentration, i.e., 1/10 of glucose concentration. It was observed that in the presence of interferents, a relative error about 3.6% was calculated in the potential differences. Hence, this bioelectrode can detect glucose with negligible interference effects. The static response time (i.e., 3 sec) and recovery time (i.e., 5 sec) with detection limit of >0.04 mV/ μ M glucose was observed during sensing experiment. The enhanced sensitivity may be due to the presence of ZnO nanoparticles catalytic nature. It can be also be attributing the excellent adsorption ability of GOD onto biocompatible ZnO/CHIT-g-PVAL core-shell nanocomposite.

To demonstrate the feasibility of the GOD/ZnO/CHIT-g-PVAL/ITO biosensor for glucose detection, samples of fresh blood serum and urine from a healthy person were analyzed. The value of glucose concentration in blood serum and urine obtained with GOD/ZnO/CHIT-g-PVAL/ITO biosensor was ~10-15% higher than those obtained from the standard photometric method, most likely due to the interferences of the electroactive species present in blood plasma and urine. Overall, a good agreement of the glucose concentration in both cases was observed.

4. Conclusion

A tunable ZnO/CHIT-g-PVAL core-shell nanocomposite film was fabricated by physiochemical technique. The characterization of core-shell nanocomposite was revealed by the formation CHIT-g-PVAL encapsulated ZnO nanoparticles having excellent glucose biosensing ability. The GOD immobilized ZnO/CHIT-g-PVAL core-shell nanocomposite bioelectrode was used for glucose monitoring effectively in aqueous and/or moderately non-aqueous media in the concentration range from μM to mM level. The results made two inferences: i) ZnO nanoparticles enhances the sensitivity of bioelectrode, ii) pH responsive, high swelling behavior of core-shell nanocomposite film which provides small surface reaction zone and good impulse propagating materials for glucose biosensing. Thus, the present research provides a platform to use GOD/ZnO/CHIT-g-PVAL core-shell nanocomposite bioelectrode to formulate an efficient tunable glucose biosensor. The proposed biosensor system could broadly be explored with more wide application for the determination of cholesterol, triglycerides, etc. in micro/nano molar concentrations.

References

1. V.K. Mourya, N.N. Inamdar, A. Tiwari, Adv. Mat. Lett. 1 (2010) 11.
2. S. Trombotto, C. Ladaviere, F. Delolme, A. Domard, Biomacromolecules. 9 (2008) 1731.
3. Y.C. Tsai, S.Y. Chen, H.W. Liaw, Sens. Actuators B: Chem. 125 (2007) 474.
4. I. A. Sogias, A. C. Williams, V.V. Khutoryanskiy, Biomacromolecules 9 (2008) 1837.
5. A. Tiwari, S. Gong, Electroanalysis 20 (2008) 2119.
6. A. Tiwari, A.P. Mishra, S.R. Dakate, R. Khan, S.K. Shukla, Mater. Lett. 61 (2007) 4587.
7. A. Tiwari, M. Prabakaran, R. R. Pandey, S. Li, J. Inorg. Organomet. Polym. 20 (2010) 380.
8. S.J. Park, J.H. Sung, J.H. Park, H.J. Choi, J.S. Choi, Curr. Appl. Phys. 6 (2006) 636.

9. J. Lee, D. Bhattacharyya, A.J. Easteal, J.B. Metson, *Curr. Appl. Phys.* 8 (2008) 42.
10. S. Zhao, K. Zhang, Y. Bai, W.W. Yang, C. Sun, *Bioelectrochemistry* 69 (2006) 158.
11. Y. Liu, M. Wang, F. Zhao, Z. Xu, S. Dong, *Biosensors & Bioelectronics*. 21 (2005) 984.
12. D. Shan, M. Zhu, H. Xue, S. Cosniers, *Biosensors & Bioelectronics* 22 (2007) 1612.
13. L-C. Jiang, W-D Zhang, *Biosensors & Bioelectronics* 25 (2010) 1402.
14. X. Zhang, G. Wang, A. Gu, Y. Wei and B. Fang, *Chem. Comm.* 5945 (2008).
15. S. Cao, R. Mishra, S. Pilla, S. Tripathi, et al. *Carbohydrate Polymers* 82 (2010) 189.
16. Z.W Zhao, X.J. Chen, B.K Tay, J.S. Chen, Z.H. Han, K.H. Khor, K.H., *Biosensors & Bioelectronics*. 23 (2007) 135.
17. C. Li, Y. Liu, Z. Du, S. Xu, M. Zhang, X.Xin, T.Wang, *Talanta* 77 (2008) 455.
18. S.M.U Ali, Q. Nur, M. Willander, B. Danielsson, *IEEE Transaction of Nanotechnology*, 8 (2009) 678.
19. A. Tiwari, *J. Inorg. Organomet. Polym.* 19 (2009) 361.
20. J. Qiu, H. Peng, R. Liang, *Electrochem. Commun.* 9 (2007) 2734.
21. X. Chen, J. Jia, S. Dong, *Electroanalysis* 15 (2003) 608.
22. Y. Yang, H. Yang, M. Yang, Y. Liu, G. Shen, R. Yu, *Anal. Chim. Acta.* 525 (2004) 213.
23. M.S. Wilson, *Anal. Chem.* 77 (2005) 1496.
24. H. Liu, Z. Zuo, Y. Guo, Y. Li, Y. Li, *Angew Chem. Int. Ed.* 49 (2010) 2705.
25. A.P. Gupta, H. Agrawal, S.K. Shukla, R. Bhardwaj, *J. Ind. Chem. Tech.* 11 (2004) 500.
26. D. Knorr, *J. Food Sci.* 47 (2006) 593.
27. P. M. Wiggins, *J. Biol. Phys. Chem.* 2 (2002) 25.
28. H. Li, R. Luo, *Biosensors & Bioelectronics*. 24 (2009) 3630.