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

S.K. Shukla [a], Swapneel R. Deshpande [b], Sudheesh K. Shukla [b], Ashutosh Tiwari [b,c,*]

[a] Department of Polymer Science, Bhaskaracharya College of Applied Sciences, University of Delhi, New Delhi 110 075, India
[b] Biosensors and Bioelectronics, IFM-Linköpings universitet, 581 83 Linköping, Sweden
[c] Linköping Integrative Regenerative Medicine (IGEN) Centre, IKE-Linköping University, 581 83 Linköping, Sweden

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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). In a typical experiment, ZnO/CHIT-*g*-PVAL core-shell nanocomposite containing <20 nm ZnO nanoparticles was synthesized using wet-chemical method. The glucose responsive bio-electrode, i.e., glucose oxidase/ZnO/chitosan-*graft*-poly(vinyl alcohol) (GOD/ZnO/CHIT-*g*-PVAL/ITO) was obtained by immobilization of glucose oxidase (GOD) onto the electrode made of resulting ZnO core-shell nanocomposite coated on the indium-tin oxide (ITO) glass substrate. The ZnO/CHIT-*g*-PVAL/ITO and GOD/ZnO/CHIT-*g*-PVAL electrodes were characterized with Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM), whereas ZnO/CHIT-*g*-PVAL size of core-shell nanoparticles were measured using transmission electron microscopy (TEM). The electrostatic interaction between GOD and ZnO/CHIT-*g*-PVAL provided the resulting tuned enzyme electrode with a high degree of enzyme immobilization and excellent lifetime stability. The response studies were carried out as a function of glucose 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 fast surface-controlled redox biochemistry with a detection limit of 0.2 μM, a sensitivity of >0.04 V/ μM and a response time of three sec. ZnO/CHIT-*g*-PVAL core-shell nanocomposite could be a promising nanomaterials for a range of enzymic biosensors.

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 viscoelestic value and modest electrical/ionic conductivity that can be overcome by graft copolymerization [5,6]

On 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 point difference (ΔIEP). Thereby, ZnO nanoparticles can offer tunable potentiometric accommodation of GOD through strong electrostatic forces with a good shelf life. In addition, ZnO possess 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 emerged 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].

* Corresponding author at: Linköping Integrative Regenerative Medicine (IGEN) Centre, IKE-Linköping University, S-581 83 Linköping, Sweden.
Tel.: +46 13 2823 95; fax: +46 13 13 75 68.
E-mail address: ashutosh.tiwari@liu.se (A. Tiwari).

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Table 1
The different types of sensors with operational flexibility.

Matrix	Transducer	Response time (s)	Linearity range	Reference
ZnO nanowire	Potentiometric	30	25 μM–125 mM	[18]
Fe_3O_4/CHIT	Amperometric	12	0.2–12 mM	[19]
Fe_3O_4/SiO_2	Amperometric	6	2.03 μM–4.05 μM	[20]
Sol-gel CHIT	Amperometric	10	10–500 mg/dL	[21]
CHIT/ZrO_2	Amperometric	6	8 μM–3 mM	[22]

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 are always accompanied with fast and reliable glucose monitoring biosensor. Table 1 shows the different types of reported core-shell nanocomposite based sensors with their limits of operational flexibility such as selectivity, reproducibility and response time as shown.

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, <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 MΩ Milli-Q 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. Whereas, PVAL solution was prepare by dissolving PVAL (2 g) granules in distilled water (200 mL) in a 500 mL beaker at 50° C with constant magnetic stirring. The both 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 ZnO core-shell CHIT-*g*-PVAL nanocomposite solution was spin-cast on 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 μg/mL) 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 ($\sim$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 1 M 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 the following equation:

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

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:

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

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-s; 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 by wet chemistry viz the graft copolymerization of the CHIT and PVAL followed by self-assembly upon ZnO nanoparticles. 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. Fig. 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.

The FTIR spectra of ZnO/CHIT-*g*-PVAL, and GOD/ZnO/CHIT-*g*-PVAL core-shell nanocomposite electrodes are given in Fig. 2. FTIR spectrum of ZnO/CHIT-*g*-PVAL (Fig. 2a) showed the typical absorption bands at: (i) 3490 (N–H stretching), (ii) 2840 (C–H stretching of aliphatic $-CH_2$ groups), (iii) 1732 cm^{-1} (absorption peak of ester group), (iv) 1654 and 1563 cm^{-1} (C=O stretching of amide I and II groups), (v) 1100 cm^{-1} and 1080 cm^{-1} (C=O stretching of carbonyl group) and (vi) 954, 772, 688 (bending vibrations of C–H groups in the graft copolymer of PVAL chain). The O-H vibrations was observed in ZnO ranging from 3220 to 3650 cm^{-1}, depending on the configuration and number of hydrogen atoms in the core-shell ZnO/CHIT-*g*-PVAL matrix. The broad absorption band at 3450 cm^{-1} embraces the frequencies of O–H on the surface, in the anti-bonding configuration (3360 cm^{-1}), and associated with zinc vacancies (3220 and 3230 cm^{-1}). The FTIR spectrum of the GOD/ZnO/CHIT-*g*-PVAL bioelectrode (Fig. 2b) is shown absorption broadening at (1) 2550 to 3550 cm^{-1} (addition of C–H and N–H stretching vibrations); and (2) 1628 cm^{-1} due to the attachment of GOD enzymes on the electrode. Hence, FTIR spectra confirm (a) formation of ZnO/CHIT-*g*-PVAL nanocomposite and (b) immobilization of GOD on the ZnO/CHIT-*g*-PVAL/ITO electrode.

Moreover, the spectra of 1 M HCl-treated and untreated ZnO/CHIT-*g*-PVAL core-shell nanocomposite have shown a shift to higher wavelength in the treated material. 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.

Further, SEM measurements of ZnO/CHIT-*g*-PVAL core-shell nanocomposite film before and after immobilization of GOD are shown in Fig. 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

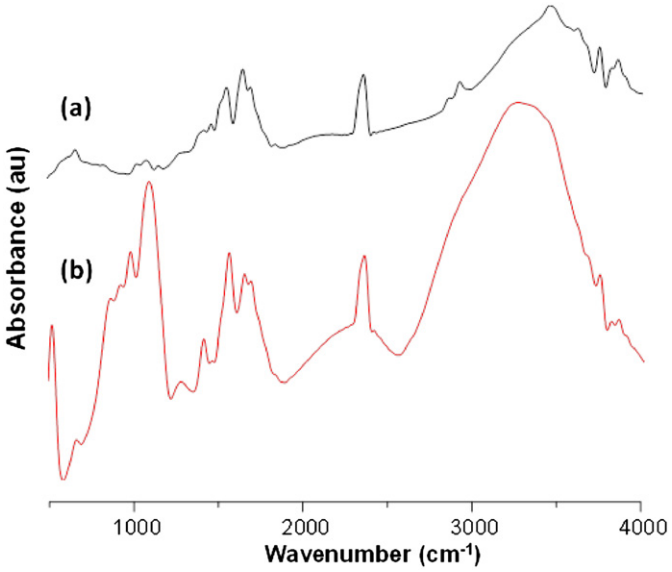

Fig. 2. FTIR spectra of (a) ZnO/CHIT-*g*-PVAL, and (b) GOD/ZnO/CHIT-*g*-PVAL core-shell nanocomposite electrodes.

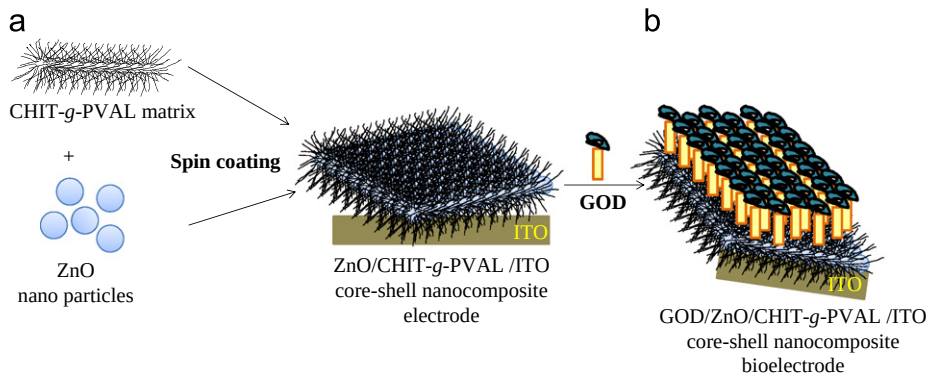

Fig. 1. Schematic illustration of (a) fabrication of ZnO/CHIT-*g*-PVAL core-shell nanocomposite electrode, and (b) immobilization of GOD on core-shell nanocomposite electrode.

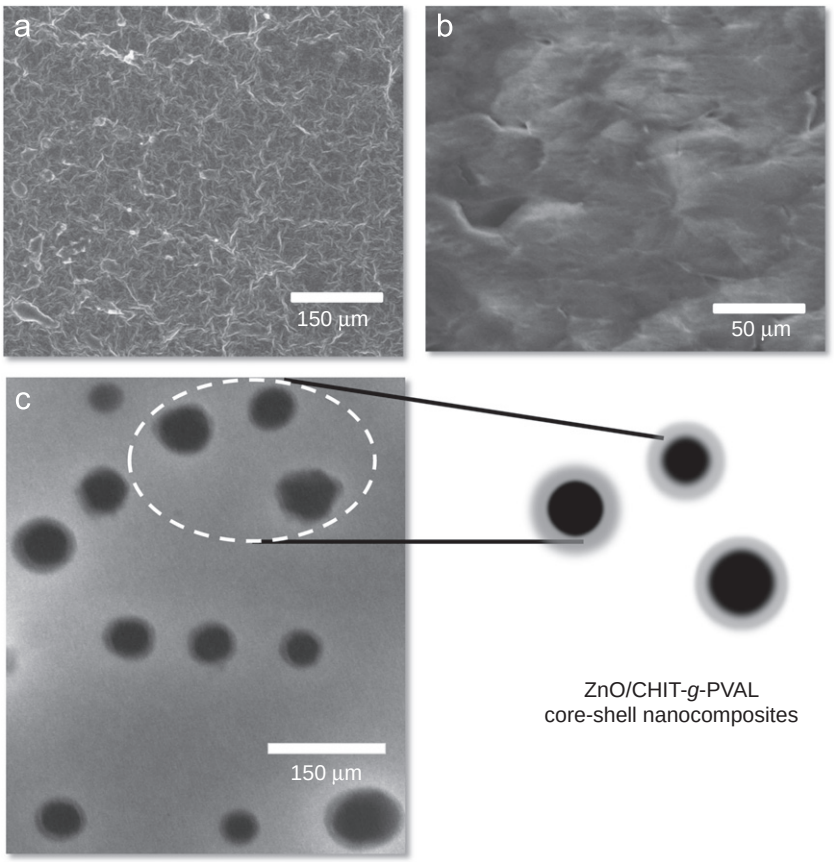

Fig. 3. SEM micrographs of core-shell nanocomposite films (a) before, and (b) after immobilization of GOD, and (c) TEM image of ZnO/CHIT-*g*-PVAL core-shell nanocomposite with zoomed illustration of nanoparticles.

nanocomposite. To further understand the morphology of the ZnO/CHIT-*g*-PVAL nanoparticles, TEM analysis was conducted using PTA as a staining agent (Fig. 3c). As demonstrated in Fig. 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 was 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 in the core, respectively. The TEM image of the ZnO/CHIT-*g*-PVAL nanoparticles clearly showed the capping of CHIT-*g*-PVAL onto ZnO nanoparticles in Fig. 3.

3.2. Glucose biosensing and nano-biocatalysis

The potential variation of induced glucose sensing is shown in Fig. 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 into gluconic acid onto bioelectrode surface, i.e., generation of H^+ and/or H_3O^+ ions as the end product. The produced gluconic acid, i.e., hydrogen ions is 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 and 8 pH. The result supports the dissolution effects of grafted CHIT in slight acid and basic medium [26].

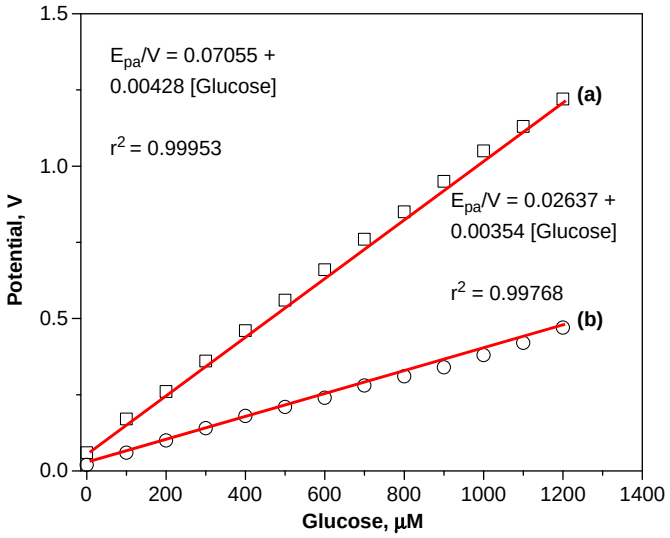

Fig. 4. Potential of bioelectrode vs. glucose concentration (a) with and (b) without ZnO nanoparticles in CHIT-*g*-PVAL matrix. Values of (a) and (b) are mean ± SE; %SD=5.08; $n=5$.

All over again, the potential change was also recorded of GOD/CHIT-*g*-PVAL without ZnO nanoparticles. Fig. 4b clearly indicates that ZnO nanoparticles enhance about three 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, ~1–3.5%. However, slope of curve decreases in a range of 8–12% at higher alcohol concentration.

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, i.e., oxidation of glucose into gluconic acid in the presence of GOD. 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 μM glucose. The interference chemicals were added into the reaction solution at 25 μM 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 s) and recovery time (i.e., 5 s) with a sensitivity of >0.04 mV/μM of biosensor 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. The storage stability of the GOD/CHIT-*g*-PVAL/ITO bioelectrode was measured and found constant potential response up to 28 weeks at 4 °C.

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.

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