Synthetic Metals 220 (2016) 551–559

Contents lists available at ScienceDirect

Synthetic Metals

journal homepage: www.elsevier.com/locate/synmet

# Study of structural and electro-catalytic behaviour of amperometric biosensor based on chitosan/polypyrrole nanotubes-gold nanoparticles nanocomposites

Arati Sharma, A. Kumar*

*Materials Research Laboratory, Department of Physics, Tezpur University, Tezpur 784028, Assam, India*

ARTICLE INFO

*Article history:*
Received 26 May 2016
Received in revised form 4 July 2016
Accepted 8 July 2016
Available online 30 July 2016

*Keywords:*
Polypyrrole nanotubes
Gold nanoparticles
Biocompatibility
Bioelectrode
Biosensor

ABSTRACT

In the present work a novel enzyme based biosensor has been developed by immobilizing glucose oxidase onto ITO electrode modified with biocompatible composite consisting of chitosan/polypyrrole-nanotubes and gold nanoparticles nanocomposites. Polypyrrole nanotubes and gold nanoparticles nanocomposites have been synthesized by self degradation method followed by in-situ reduction of auric chloride. The formation of PPy nanotubes and uniform decoration of Au nanoparticles over the nanotubes have been confirmed by HRTEM studies and SEM (Scanning Electron Microscopy). XRD results confirm the formation of PPy-NTs/Au-NPs nanocomposites. The enzyme electrode exhibited a well defined redox peak with formal potential ($E^0$) of −0.45 V. The response of modified electrode showed a quasi reversible behaviour and a surface confined electrode process. A response time of less than 40 s has been found for glucose determination using the modified electrode. The bioelectrode depicted good linearity of 3–230 μM ($R^2 = 0.98$) towards the oxidation of glucose with a sensitivity of ca. 149 μA $\mu M^{-1}$ mL and detection limit of 3.10 μM. The calculated value of heterogeneous electron transfer rate constant ($k_s$) was determined to be 2.54 $s^{-1}$. The pH dependence of $E^0$ suggests that the direct electron transfer of GOx was a two electron and two proton transfer reaction process.



## 1. Introduction

The most fundamental method employed for designing biosensors and enzymatic bioreactors is modification of electrodes with enzymes. The most challenging task in the fabrication of enzyme based biosensors is the efficient immobilization of the biomolecules while retaining their bioactivity. Therefore, a suitable matrix for immobilization is a vital factor in order to obtain their electro catalytic reactions [1]. If an enzyme is immobilized in a suitable matrix onto an electrode which can directly transfer the electrons it may be used in designing biosensors. However, the three dimensional structure of enzymes hinders the accessibility for direct electron transfer [1]. In the recent years, considerable work has been done to enhance the direct transfer of electrons between the entrapped enzymes and the surface of electrode [2]. In the terms of applicability, the electrochemical biosensors based upon nanomaterials have recently attracted wide attention [3]. Electrochemical method has attracted tremendous interest in the development of glucose sensors due to its ease in experimental design and mass fabrication of sensor element [4]. Noble materials like gold nanoparticles (Au NPs)[5], carbon nanotubes [6] and metallic oxides [7] are gaining much interest in designing of biosensors for medical analysis, food quality control etc. due to their special physical and chemical properties. The signal transduction can be improved by using nanomaterials having different morphologies such as nanoparticles, nanotubes, nanofibers, nanowires and nanocomposites in the fabrication of biosensors. Metal, metal oxide, and semiconductors nanoparticles exhibit unique physico-chemical and electronic properties and can be used for fabrication electrochemical biosensors with improved properties [8]. Among them gold nanoparticles are considered as suitable matrix for enzyme immobilization and facilitate the direct electron transfer by providing the essential microenvironment for the biomolecules [5]. Amino acids and proteins can be immobilized on Au NPs which show excellent catalytic activity [9]. Dong et al. [10] developed horseradish peroxidase biosensor by self-assembling Au NPs into three-dimensional sol–gel network. However there are certain drawbacks like poor reusability due to the difficulty in separating the bioconjugate material from the reaction medium. Moreover, aggregation of metal nanoparticles takes place

* Corresponding author.
*E-mail addresses:* ask@tezu.ernet.in, ask_tezu@yahoo.co.in (A. Kumar).

http://dx.doi.org/10.1016/j.synthmet.2016.07.012
0379-6779/

due to their high surface free energy, which limits their applications. In order to overcome these problems most of work has been done to make nanocomposites of gold nanoparticles with promising carbon nanomaterials like graphene [11], carbon nanotubes [12,13]. These applied materials showed good performance but the methodology used may reduce the biocatalytic activity of the biomolecules [14].

Recent studies showed that the conducting polymer nanostructures such as nanotubes, nanoparticles, nanofibers and microstructured films enhance the sensitivity of the sensors [15]. Particularly, the properties like high effective surface area, low density, along with unique chemical and physical properties offer the advantages of using conducting polymer nanostructures over conventional bulk conducting polymers [16]. Among the various conducting polymers, Polypyrrole (PPy) is considered as the most promising matrix for biosensing application owing to its unique properties like high electrical conductivity, long lasting stability at ambient condition, high surface to volume ratio for ramified polymer network along with long term electrochemical stability and electroactivity in phosphate buffer (pH 7.4) [16] makes it well-matched for the integration with redox enzyme. Moreover, the conducting polymers like polypyrrole are non-toxic and the enzymes are easily immobilized in the high surface area of the conducting polymer nanostructures. Nanocomposites of PPy/CNT/GOx, Au/SWCNT/GOx–HRP/PPy, GOx–MLV–PPy, PPy/polyacrylamide microparticles based glucose biosensors have been reported [17–20]. In the present work we have developed conducting polymer nanostructures and gold nanoparticles nanocomposites. High conductivity ($10^4\,S\,cm^{-1}$) and porous structure of the conducting polymers and the large surface area, size and quantum effect of metal nanoparticles enhance the properties like fast response time of the biosensors. The combination of Au-NPs with these conservative matrices may result in irreproducibility of the nanocomposite films. In order to overcome this drawback Chitosan has been used in the present work. Chitosan (Chi) has an excellent film-forming ability and remarkable biocompatibility [20]. As a biocompatible polymer it has other advantages such as low cost, hydrophilicity, non-toxicity. These characteristics have promoted its application as one of the most promising matrix for enzyme immobilization. Chitosan has been incorporated with conducting polymers, ILs, metal nanoparticles, CNTs as a matrix for biosensing application [20–24]. It is reported that each of the matrices has its advantages and disadvantages [25–27]. Hence, thorough research activities are being continuously pursued in the direction of developing new matrices.

In the present work, a new multi-component matrix consisting of (i) Chitosan (Chi) a biopolymer (ii) Polypyrrole nanotubes (PPy-NTs) and (iii) Gold nanoparticles (Au-NPs) has been developed for the immobilization of glucose oxidase (GOx) for fabrication of a glucose biosensor by using an easy methodology for preparation of Chi/PPy-NTs/Au-NPs matrix. The bioelectrode has been designed with a view to increase the charge transfer between the electrode and enzyme in order to enhance the sensitivity and linearity. The existence of Chi adds stability and reproducibility to the biosensor. The GOx/Chi/PPy-NTs/Au-NPs electrode exhibits a remarkable linear response to glucose for a wide concentration range of glucose with excellent selectivity and sensitivity.

## 2. Experimental

### 2.1. Materials and methods

GOx (EC 1.1.3.6), Pyrrole, methyl orange, ferric chloride and gold chloride were obtained from Sigma-Aldrich. Chitosan, glucose and

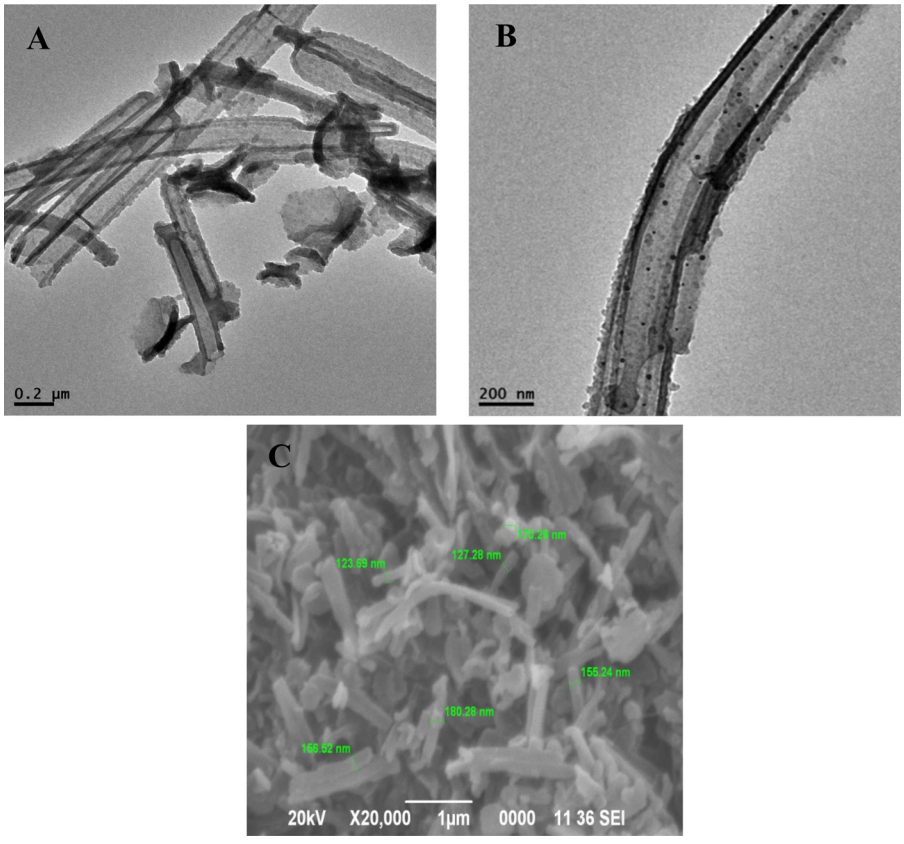


**Fig. 1.** HRTEM micrograph of (a) PPy nanotubes(PPy-NTs) (b) PPy-NTs/Au-NPs nanocomposites. Inset Fig. 1(b) is SEM micrograph of PPy-NTs/Au-NPs nanocomposites.