Designing a Urease Biosensor Using Modified Electrode with Pb doped Al_2O_3 Nanoparticles

P. D. Kale, A.B.Bodade, P.R.Padole and G. N. Chaudhari
Nanotechnology Research Laboratory , Department of Chemistry, ShriShivaji Science College, Amravati- 444602 (M.S), India.
gnchaudhari@gmail.com

Abstract

Chitosan (CH) -Pb doped Al_2O_3nanoparticles-nanocomposite film has been fabricated onto gold plate to immobilize urease (Ur) via physiosorption for urea detection. Both CH-Pb doped Al_2O_3/gold electrode and Ur/ CH- Pb doped Al_2O_3 /gold biocelectrode have been characterized using cyclic voltametry (CV) and electrochemical impedance spectroscopy (EIS). Cyclic voltametry shows the persence of Pb doped Al_2O_3 nanoparticles results in increased electroactive surface area of CH-Pb doped Al_2O_3nanobiocomposite for immobilization of enzyme (Ur), enhanced electron transfer between Ur and electrode and electrochemical impedance spectroscopy (EIS) shows the conductivity of composite electrode.

Keywords: Biosensor; Pb doped Al_2O_3; Urease (Ur); Nanoparticles; Chitosan (CH)

Introduction:

In the past few years, substantial research effort has been devoted to the development of organic biosensors, since they combine general merits of electronic sensors, like speed, size and system integration, with the ones of organic semiconductors, such as low cost production, facile integration with flexible substrates, and biocompatibility [1,2]. According to the fact, the design of modern biosensors is strictly combined with achievements of the nanotechnology. Nanostructures and nanotechnology are connected with design and production of material and devices in range 1-100 nm. In this context, using of different type of nanoparticles or nanosized materials could improve i.e. sensitivity of device on the step of this construction already [3-5].

The increasing demand for clinical diagnostics relating to kidney and liver diseases has necessitated evolution of new methods for faster and accurate estimation of urea in desired samples including urine and blood samples. The increased urea concentration (normal level in serum is 8-20 mg/dL) causes renal failure (acute or chronic), urinary tract obstruction, dehydration, shock, burns and gastrointestinal bleeding. Moreover, decreased urea concentration causes hepatic failure, nephritic syndrome, cachexia (low-protein and high-carbohydrate diets) [6-8]. The conventional methods for urea detection including gas chromatography, calorimetry and fluorimetric analysis suffer from complicated sample pretreatment and are unsuitable for on-line monitoring. Electrochemical biosensors have been considered to provide interesting alternatives due to their simplicity, low cost and high sensitivity [8, 9].

More recently, biosensors have emerged as a promising technology, especially for applications requiring rapid and continuous monitoring. Biosensors are being applied to a wide variety of analytical problems such as in medicine [10-

12], environment [13–15], food and process industries [16,17], security and defence [18].Although urea biosensors emphasising on better sensitivity or higher response range [19]are reported, not much effort has been made in resolving the drawbacks of enzyme instability, difficulty in storage and handling, and fragility of the immobilization matrix.

Immobilization of Ur onto a suitable matrix is a crucial for the development of an electrochemical urea sensor [1-4]. In hybrid nanobiocomposites, surface fuctionalization of nanoparticles allows their covalent attachment and self-assembly on surfaces that can be used for loading of desired biomolecules in a favorable microenvironment for development of a biosensor [20, 21]. In this context, metal oxide nanoparticles-chitosan (CH) based hybrid composites have attracted much interest for development of a desired biosensor [20].

In this manuscript, we report results of studies relating to the immobilization of Ur onto CH-Pb doped Al_2O_3 nanobiocomposite film.

Experimental details

Material:

All chemicals and solvents were analytical grade and purchased from commercial sources.

Preparation of Pb doped Al_2O_3 nanoparticles

The Pb doped Al_2O_3nanoparticles prepared by using sol-gel citrate method. The stiochiometry mixture of lead nitrate ,aluminium nitrate magnetically stirred with citric acid and ethanol at 80° C for 3hrs to get homogeneous and transferrent solution. The solution was further heated at about 130° for 12 hrs. in pressure vessel. The prepared product was subjected to 3hrs heat treatment at 350°C in muffle furnance and then milled to a fine powder. The dried powder then calcinated in range of 350°-650°C in order to improve the crystalinity, sensitvity and selectivity of material.

Preparation of CH-Pb doped Al_2O_3/Gold nanobiocomposite electrode

Pb doped Al_2O_3 nanoparticles prepared using sol-gel method are dispersed into 10 mL of CH (0.5 mg/mL) solution in acetate buffer of 0.05M at pH 4.2 under continuous stirring at room temperature . Finally, viscous solution of CH with uniformly dispersed Pb doped Al_2O_3 nanoparticles is obtained. CH-Pb doped Al_2O_3 hybrid nanobiocomposite films have been fabricated by uniformly dispersing 10 µL solution of CH-Pb doped Al_2O_3 for 12 h. These solution cast CH-Pb doped Al_2O_3 hybrid nanobiocomposite films are washed repeatedly with deionized water to remove any unbound particles.

Immobilization of Ur onto CH-Pb doped Al_2O_3 nanobiocomposite film

10 µL of bienzyme solution containing Ur (10 mg/ml) [prepared in phosphate buffer buffer (0.1mol),ph 7] is immobilized onto CH-Pb doped Al_2O_3/gold electrode. The Ur/CH-Pb doped Al_2O_3nanobiocomposite/gold bioelectrodes are keptundisturbed for about 12 h at 4^{o}c. Finally, the dry bioelectrode is immersed in

50 mM PBS(pH 7.0) in order to wash out any unbound enzymes from the electrode surface.

Results and Discussions:

Cyclic Voltammetry Studies

Figure. 1. Showsthe cyclic voltamogrammes response of the prepared CH-Pb doped Al_2O_3/gold electrode and Ur/Pb doped Al_2O_3 nanobiocomposite electrode recorded in KCL solution containing 5M $[Fe(CN)_6]^{3-/4-}$ in the potential range of -0.3 to 0.6 V at 10mV/s rate.The magnitude of the current response for CH–Pb doped Al_2O_3 electrode increases in comparison to that of Ur/CH-Pb doped Al_2O_3nanobiocomposite electrode. These results suggest that the presence of Pb doped Al_2O_3 nanoparticles results in increased electroactive surface area of electrode resulting in enhanced electron transport between electrolyte medium and the electrode. However, magnitude of the current response decreases after immobilization of urease onto CH–Pb doped Al_2O_3/gold electrode. This may perhaps be due to insulating characteristics of urease.

Electrochemical impedance spectroscopy (EIS) can provide useful information on the impedance changes of the electrode surface during the fabrication process.**Figure. 2.** Shows the variation of ac conductivity of the prepared CH-Pb doped Al_2O_3/gold electrode and Ur/Pb doped Al_2O_3 nanobiocomposite electrode recorded in KCL solution containing 5M $[Fe(CN)_6]^{3-/4-}$ in log-log scale. In the Figure.ure two different curve of CH-Pb doped Al_2O_3/gold electrode and Ur/Pb doped Al_2O_3 nanobiocomposite electrodes frequency appears to merge at high frequency side. The ac conductivity increases with immobilization of urease on the surface of CH-Pb doped Al_2O_3/gold electrode.

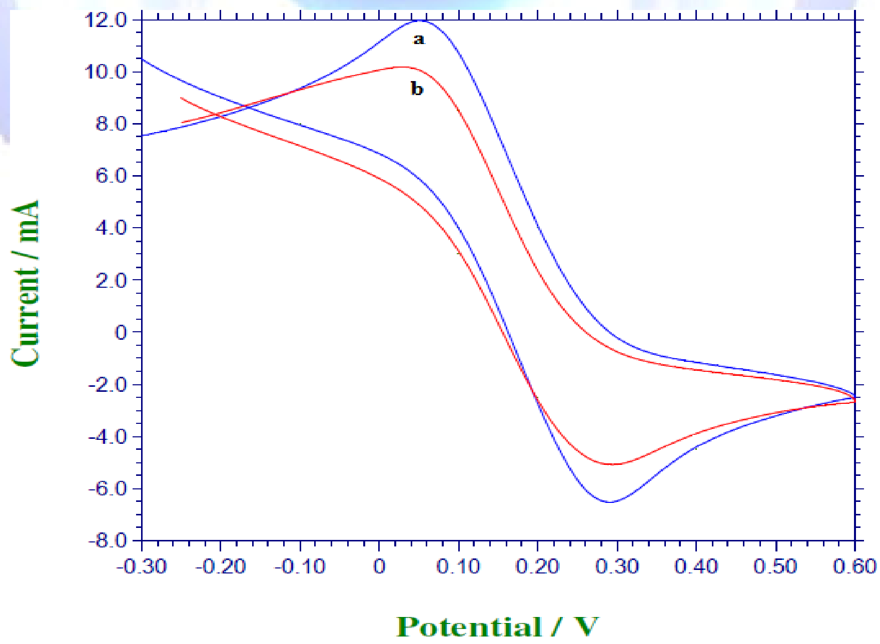

Figure. 1- Cyclic voltamogrammes of (a) CH–Pb doped Al_2O_3/goldelectrode (b) Ur/ CH–Pb doped Al_2O_3/goldbioelectrode in KCL (0.1m) containing $[Fe(CN)_6]^{3-/4-}$ at $10mVs^{-1}$

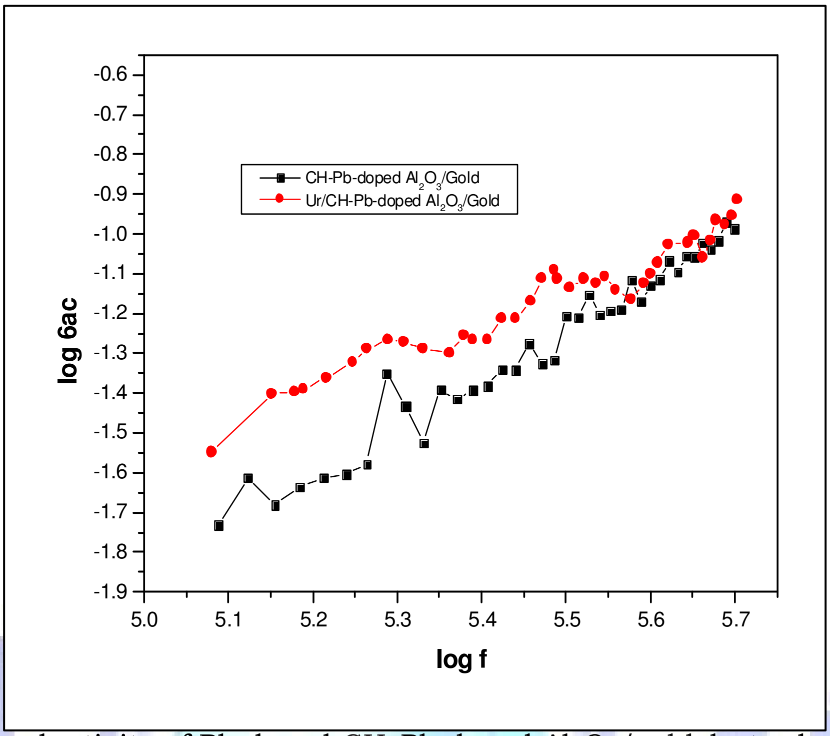

Figure. 2- Ac conductivity of Pb doped CH–Pb doped Al_2O_3/goldelectrode; Ur/ CH–Pb doped Al_2O_3/goldnanobiocomposite electrode in KCL (0.1m) containing $[Fe(CN)_6]^{3-/4-}$

Conclusions:

In this summery CH–Pb doped Al_2O_3/goldnanoparticles was synthesized by sol-gel method. Finally results of our research work lead to design new biosensor for determination of urea. The thin film of the preparedCH–Pb doped Al_2O_3/gold/gold electrode and Ur/CH-CH–Pb doped Al_2O_3/goldnanobiocomposite electrode is form by physical adsorption. Ur immobilized on CH-CH–Pb doped Al_2O_3/goldnanobiocomposite electrodewas studied by cyclic voltammtery which confirm the immobilization. And electrochemical impedance spectroscopy shows the ac conductivity increases with immobilization of urease on the surface of CH-Pb doped Al_2O_3/gold electrode.

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