Enzyme and Microbial Technology 66 (2014) 48–55

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

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

Zirconia-poly(propylene imine) dendrimer nanocomposite based electrochemical urea biosensor

Sudheesh K. Shukla, Ajay K. Mishra, Bhekie B. Mamba, Omotayo A. Arotiba*

Department of Applied Chemistry, Doornfontein Campus 2028, University of Johannesburg, Johannesburg, South Africa

ARTICLE INFO

Article history:
Received 9 March 2014
Received in revised form 13 August 2014
Accepted 14 August 2014
Available online 27 August 2014

Keywords:
Electrochemical biosensor
Urea
Urease enzyme
ZrO_2 nanoparticle
Poly (propylene imine) dendrimer
Nanocomposite
Electro-co-deposition

ABSTRACT

In this article we report a selective urea electrochemical biosensor based on electro-co-deposited zirconia-polypropylene imine dendrimer (ZrO_2-PPI) nanocomposite modified screen printed carbon electrode (SPCE). ZrO_2 nanoparticles, prepared by modified sol–gel method were dispersed in PPI solution, and electro-co-deposited by cyclic voltammetry onto a SPCE surface. The material and the modified electrodes were characterised using FTIR, electron microscopy and electrochemistry. The synergistic effect of the high active surface area of both materials, i.e. PPI and ZrO_2 nanoparticles, gave rise to a remarkable improvement in the electrocatalytic properties of the biosensor and aided the immobilisation of the urease enzyme. The biosensor has an ampereometric response time of ~4 s in urea concentration ranging from 0.01 mM to 2.99 mM with a correlation coefficient of 0.9985 and sensitivity of 3.89 $\mu A\,mM^{-1}\,cm^{-2}$. The biosensor was selective in the presence of interferences. Photochemical study of the immobilised enzyme revealed high stability and reactivity.

1. Introduction

The estimation of urea concentration in serum or urine is essential for the diagnosis of kidney and liver diseases [1,2]. Abnormally high urea levels (the normal range in the blood being 8 mg dL^{-1}–20 mg dL^{-1}) can cause the obstruction of the urinary tract, shock, burns, gastrointestinal bleeding, dehydration, and renal failure. On the other hand, a decrease in the level of urea can cause nephritic syndrome, cachexia, and hepatic failure. Since both high and low concentrations of urine pose health risks, the estimation of urea levels in the blood/serum/urine is therefore important for the assessment of kidney functions and the diagnosis of different kidney related diseases [3,4]. In addition to medical diagnoses, the estimation of urea is an important parameter in other fields such as the food industry, pharmaceuticals, as well as in environmental protection and monitoring.

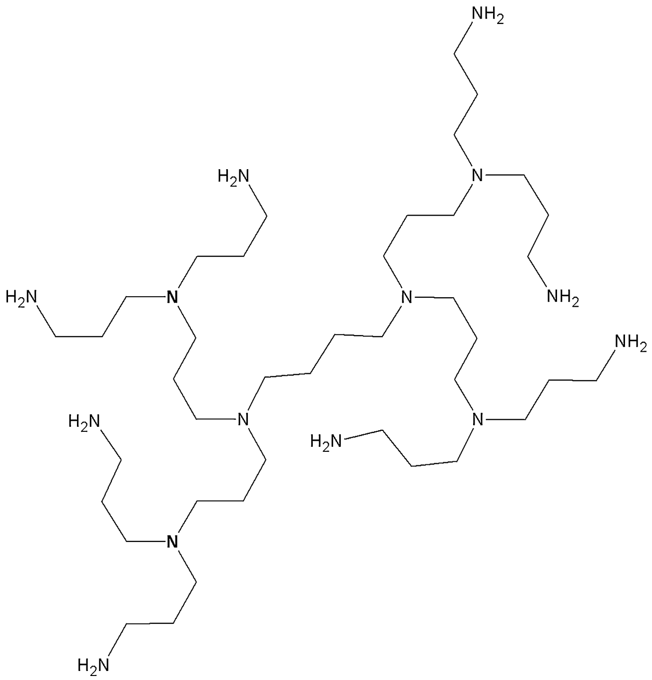
Several conventional methods such as fluorometric and chromatographic analyses have been reported for urea detection in tissue and body fluids. These detection techniques can sometimes be complicated with extensive sample pre-treatment and may be unsuitable for on-line monitoring. In response to these challenges, electrochemical biosensors have gained increasing interest as an alternative to conventional detection methods due to their comparative low cost, high sensitivity and simplicity [5,6]. The performances of electrochemical biosensors however need to be improved for more real life and versatile applications. One of the approaches used in the optimisation of biosensors is the incorporation of nanomaterials especially as immobilisation layers where they are expected to increase surface area for better biomolecule (e.g. enzyme) adsorption and loading; and promote faster electron transfer between the electrode and the active site of the enzyme [7,8]. Various inorganic nanomaterials such as Au, Ag, Fe_3O_4, ZnO, ZrO_2 [7–12] and organic polymer nanostructures such as dendrimer [13–15] have been used in the development of electrochemical biosensors. Dendrimers [16,17] are an exciting new class of three dimensional, highly branched, nanoscale, globular shaped and macromolecular architecture that emanates from a central core, and has a definite number of generations and functional end groups. A typical example is poly(propylene imine) (PPI) which has a tertiary inner amine branch and a peripheral primary amine (Scheme 1).

Depending on the properties sought, a nanomaterial can be combined with other materials (which may or may not be in the nanoscale) to form nanocomposites which can greatly improve the immobilisation layer in a biosensor. For example, a polymer such as chitosan has been combined with ZrO_2 in a glucose biosensor as an inorganic–organic nanocomposite (ION) immobilisation matrix where the polymer was used to entrap the metal

---

* Corresponding author. Tel.: +27 11 559 6200; fax: +27 11 559 6425.
E-mail addresses: oarotiba@uj.ac.za, omotayoarotiba@gmail.com (O.A. Arotiba).

http://dx.doi.org/10.1016/j.enzmictec.2014.08.003
0141-0229/© 2014 Elsevier Inc. All rights reserved.



Scheme 1. Chemical structure of generation 1 Poly(propylene imine) dendrimer.

oxide nanoparticle [11]. Owing to their host–guest abilities, dendrimers are therefore nanomaterials of choice in the preparation of ION and such nanocomposites have been reported in literature either for biosensors [18] or other catalytic purposes [19]. In a dendrimer nanocomposite, the dendrimers can serve as the biocompatible layer, nano-encapsulator and covalent attachment site for the immobilisation of bioreceptors and modification of the electrode as well [20].

This article focuses on harnessing the properties of ZrO_2 nanoparticle and poly(propylene imine) dendrimers by preparing a novel inorganic-organic nanocomposite matrix based on ZrO_2/PPI for development of an electrochemical urease enzyme biosensor.

2. Materials and methods

2.1. Reagents and preparation of solutions

The following were purchased from Sigma–Aldrich (St. Louis, Montana, USA) and were used without further purification: urease (Aldrich, from *Canvalia ensiformis*), urea, Generation 2 (G2) poly(propylene imine) (PPI) dendrimer (SyMO-Chem, Eindhoven, Netherland), glutaraldehyde, uric acid, zirconium (IV) nitrate [$Zr(NO_3)_4{\cdot}3H_2O$], ethylene glycol, HNO_3, ammonia solution, potassium chloride, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, potassium ferricyanide, potassium ferrocyanide. Phosphate buffer solution (PBS) (10 mM, pH 7.0) and ferri/ferro cyanide solution were used as supporting electrolytes for all amperometric measurements. Milli-Q water (18.2 $M\Omega/cm^2$ at 25 °C) deionized water was used to prepare all the aqueous solutions. All the glassware and solutions were autoclaved prior to being used.

2.2. Instrumentation

The particle phase, size, and structure of synthesised ZrO_2 nanoparticles were studied using X-ray powder diffraction patterns on an X-ray diffractometer (Rigagu Ultima IV) with Cu Kα (λ = 1.5405 Å) radiation and transmission electron microscopy (TEM) (Jeol model, JEM-2100F) respectively. Fourier transform infrared (FTIR) spectra were obtained from PerkinElmer spectrum 100 spectrophotometer (PerkinElmer, Waltham, MA, USA). The IR spectra were recorded on an IR universal ATR sampling system accessory transmission mode with an accumulation of 16 scan and a resolution of 4 cm^{-1} in the range of 4000 cm^{-1} to 450 cm^{-1}.

The surface morphology of the electrode was examined by scanning electron microscopy (SEM) TESCAN (Vega 3 XMU) operated at 20 kV and the samples were sputter-coated with thin layer of carbon. Electrodeposition and all electrochemical measurements (CV, impedance, and amperometric measurements) were performed using an IVIUM CompactStat electrochemical workstation (Ivium Technologies B.V., Netherland). Screen printed electrodes (DRP-110) were purchased from DROPSENS. Platinum wire and Ag/AgCl (3 M KCl) were used as auxiliary and reference electrode respectively. Amperometric studies were performed under stirred conditions and all the solutions were purged with high purity argon gas for at least 15 min before electrochemical measurements were done.

2.3. Synthesis of ZrO_2 nanoparticles

ZrO_2 nanoparticles have been prepared by adopting a modified sol-gel methods. An analytical grade of $Zr(NO_3)_4{\cdot}3H_2O$ (0.98 mmol) was dissolved in 0.1 M HNO_3 (10 ml). A 1 mL volume of ethylene glycol was added and the mixture was stirred for 45 min. Then 25% ammonia solution was added to the mixture with continuous stirring by using magnetic stirrer at room temperature to adjust the pH to 8. The suspension was stirred at room temperature for 30 min and the resultant suspension was allowed to dry in an oven at 100 °C for 12 h and further annealed at 600 °C for 2 h.

2.4. Preparation of ZrO_2/PPI dendrimer solution

ZrO_2 nanoparticle was dispersed in 30 mM solution PPI in a mass to volume ratio of 1:100 where a typical mixture was 0.30 g ZrO_2 in 30 mL PPI. The mixture was stirred for one hour at room temperature after which it was sonicated for 20 min. Finally, a highly dispersed colloidal solution was formed.

2.5. Preparation of ZrO_2/PPI dendrimer modified SPE

Prior to the electrode modification, the bare SPCE was electrochemically cleaned by cyclic voltammetry using a potential range of −200 mV to +1500 mV in a solution of 0.5 H_2SO_4 for five consecutive cycles. The cleanliness of the surface was verified in the PBS (10 mM, pH 7.5) by using potential sweep in the range of −0.1 V to 0.65 V at a scan rate of 50 mV s^{-1} where no peak was expected. Finally, the electrode was thoroughly washed with deionized water and dried in an oven at 50 °C for five minutes. ZrO_2/PPI hybrid nanocomposites modified SPCE were then fabricated by using electro-co-electrodeposition methods according to the modified procedure reported by Arotiba et al. [20,21] in the following manner: 50 µL of a viscous mixture of ZrO_2 nanoparticles and 30 mM PPI dendrimer (molar ratio 1:100) was pipetted onto the pre-cleaned SPCE. This was followed by cyclic voltammetry (CV) between potential sweeps −0.3 V to 1.500 V for 20 cycles at a 50 mV s^{-1} scan rate. The preparation of the ZrO_2-PPI dendrimer/SPCE system involved the simultaneous electro-co-deposition of PPI and ZrO_2 on a clean SPCE. These ZrO_2/PPI modified SPCE was rinsed repeatedly with de-ionized water and PBS respectively, to remove the physically unbound (or any weakly bound) particles. A freshly prepared electrode was used in all the respective electrochemical studies.