



A simple sonochemical approach to fabricate a urea biosensor based on zinc phthalocyanine/graphene oxide/urease bioelectrode

Sekar Selvarajan^a, Ayyadurai Suganthi^{a,b,*}, Muthuramalingam Rajarajan^{c,*}

^a PG & Research Department of Chemistry, Thiagarajar College, Madurai 625 009, Tamil Nadu, India

^b Mother Teresa Women's University, Kodaikanal 624 102, Tamil Nadu, India

^c Directorate of Distance Education, Madurai Kamaraj University, Madurai 625 021, Tamil Nadu, India

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ABSTRACT

A novel zinc phthalocyanine/graphene oxide (ZnPh/GO) nanocomposite modified glassy carbon electrode (GCE) was prepared by using sonochemical approach and simple drop casting method. Urease (Urs) was used as the specific enzyme for urea detection and was physically immobilized onto the surface of ZnPh/GO nanocomposite. The fabricated ZnPh/GO/Urs matrix was successfully characterized by UV-vis-spectroscopy, FT-IR spectroscopy, scanning electron microscopy (SEM), raman spectrum, thermogravimetric analysis, cyclic voltammetric (CV) and amperometric techniques. The electrocatalytic performance of the ZnPh/GO/Urs electrode was investigated by urea biosensor. Our results demonstrate that the modified electrode has excellent electrocatalytic activity towards the sensing of urea in 0.1 M phosphate buffer solution (PBS, pH 7.2). The biosensor tolerated a wide linear concentration range for urea from 0.4 to 22 μ M ($R^2 = 0.991$), with a detection limit of 0.034 μ M (S/N = 3). The ZnPh/GO/Urs bioelectrode has several excellent properties, including a fast response time, high reproducibility and stability.

1. Introduction

Fertilizer plants and animal as well as human urine are supposed to be responsible for empowering urea in aqueous environment [1]. Higher urea level poses several adverse effect in the body including, renal failure, obstruction of urinary tract, bleeding issue in stomach and intestine, dehydration and shock burns while the lower urea level is responsible for nephritic syndrome, cachexia and hepatic failure [2]. In view of biological and environmental importance it is highly demanding to investigate urea concentration in clinical and environmental samples. Several diagnostic methods have been reported regarding urea determination in various types of samples including potentiometry [3,4], piezoelectricity [5], surface plasmon resonance [6] and molecularly imprinted polymer [7]. All these mentioned methods for urea determinations are faced with certain problems like use of several chemicals, less sensitivity, limited selectivity and expensive nature of material utilized for sensing application.

Phthalocyanines are aromatic macrocycles with a high electronic delocalization and an intense absorption in the red to near-IR zone [8]. They possess a great thermal, photochemical stability, electrocatalytic properties and are very versatile, since it is possible to change the central atom and to introduce substituents in the peripheral and axial

positions, thus bringing the possibility to tune their physicochemical and optical properties [9]. In addition to their applications as photosensitizers for photodynamic therapy [10], as liquid crystals, [11] sensor [12] and in nonlinear optics [13] phthalocyanines have emerged with force in the field of photovoltaic devices.

Over the past few years, graphene (a one-atom-thick planar sheet of sp^2 -bonded carbon atoms that are densely packed in a honeycomb crystal lattice) has attracted considerable research interest owing to its unique physical and chemical properties [14–16]. These properties suggest a wide-range of applications for graphene based materials such as adsorbents [17], catalyst supports [18], thermal transport media [19], electrode materials for electrochemical energy-storage devices (batteries/supercapacitors) [20] and components of biosensors [21]. Furthermore, ultrasound is accomplished due to the distinctive mechanisms and reaction pathways leading to a significant tool for chemistry became serious conditions. Recently, sonochemistry has been applied in the synthesis of functional nanoparticles and nanostructured materials. In this technique, the amount of energy is provided to the system via irradiation of a liquid with elevated intensity ultrasonic waves to facilitate produce regions of extreme temperature and pressure. Indeed, the sonochemical approach has allowed generating carbon supported nanoparticles with great uniformity whereas

* Corresponding authors at: Mother Teresa Women's University, Kodaikanal 624 102, Tamil Nadu, India (A. Suganthi).
E-mail addresses: suganthiph09@gmail.com (A. Suganthi), rajarajan_1962@yahoo.com (M. Rajarajan).

conventional preparation techniques do not provide sufficient control [22].

On the other hand, metallophthalocyanine (MPh) and metalloporphyrins belong to the classes of organometallic macrocycles, in particular zinc phthalocyanine has been well studied and showed an excellent electrocatalytic materials towards several important molecules such as red grapes, carbamate, organophosphate, and various sensor [23–25]. Moreover, CoPh has also been used as redox mediators for enzyme based glucose sensors [26]. The ZnPh is not stable on the electrode surface due to its low conductivity along with poor electrochemical activity [27]. Hence the carbon nanomaterials [28], such as carbon nanotubes (CNTs) and graphene have been used with MPh to enhance the electrochemical conductivity and electron transfer of MPh and thus used for many potential applications including electrochemical sensors and biosensors [29–32]. Earlier studies revealed that GO is an ideal carbon nanomaterial to combine with MPh; more stable on the electrode surface due to the strong π - π interaction between MPh and GO [32]. The main aim of the present work is utilization of the special properties of the ZnPh/GO/Urs and used as an electrocatalyst for the sensing of urea.

Herein, we propose the sonochemical synthesis of ZnPh/GO nanocomposite modified GCE fabricated by the simple sonochemical and drop casting technique. This modified glassy carbon electrode have been further used to immobilize urease for urea detection via cyclic voltammetry technique. The fabricated bioelectrode showed good stability, specificity as well as reproducibility. The proposed ZnPh/GO/Urs biosensor showed a high sensitivity and a good detection limit compared to many other reports.

2. Experimental

2.1. Materials

Graphite powder (1–2 μm , synthetic) was obtained from Aldrich. Urease (urease enzyme, E.C.3.5.1.5 from Jack Bean 100 U mg^{-1}), Urea (ACS reagent 99.9%), zinc phthalocyanine and all chemicals used in this project were of analytical grade and purchased from Sigma Aldrich. The phosphate buffer solution (PBS) 0.1 M with a pH of 7.2 was prepared from Na_2HPO_4 and NaH_2PO_4 . A stock solution of urea was prepared in 0.1 M PBS (pH of 7.2), and stored at 4 °C ultra-pure water was obtained from a Milli-Q water system.

2.2. Synthesis of graphene oxide

Graphene oxide was prepared using graphite according to the modified hummer's method [33] whereby 1 g of graphite powder was suspended in 2.5 g of $\text{K}_2\text{S}_2\text{O}_8$ and 46 ml of H_2SO_4 and then stirred in a round bottom flask at 0 °C for 15 min. Next, 2.5 g of P_2O_5 was added into the mixture and stirred vigorously for 6 h at 20 °C. On completion, the mixture was then poured and diluted with 1 L of water and filtered. Afterward, a 6 g of portion of KMnO_4 and 1 g of NaNO_3 in 31.2 ml of distilled water were added gradually while being stirred. The temperature of the mixture was controlled to below 20 °C. The reaction occurred as the mixture was stirred at 35 °C for 2 h, after which 500 ml of distilled water was added slowly to keep the temperature below 50 °C. After that further reaction was allowed to proceed for 2 h, and then 250 ml of water and 6 ml of H_2O_2 (30% weight) were added. The color of the mixture changed to brilliant yellow. Finally, the solid suspension was washed with a 2 M HCl solution and then washed 3–4 times with ethanol and dried in a vacuum at 60 °C overnight. The graphite oxide slurry was then dried in vacuum at 60 °C for 48 h before use.

2.3. Fabrication of urease immobilize ZnPh/GO modified GCE

ZnPh/GO nanocomposite modified electrodes was prepared by

using a mixture of a slurry of ZnPh (4.3 mg) in ultra-pure Milli-Q water (1.5 mL) and 3.3 mg/mL suspension of GO that was ultrasonicated (irradiation power 75 W) for 30 min. 2 μL of this mixture was dropped on the GCE electrode and kept to dry for 30 min at 80 °C. The freshly Urs solutions was prepared in PBS (phosphate buffer solution) and stored at 4 °C when not in use. Schematic illustration 1 provides an overview of the fabrication process of the urea sensor. Prior to use, covalently immobilize Urs on the as-prepared ZnPh/GO composite modified GCE, about 3 μL Urs was drop casted and allowed to dry at room temperature. For the one-step reaction, enzyme specific activity of 620 min^{-1} on membrane supports was seen to be comparable to solution enzyme specific activity of 10 min^{-1} . Thus, immobilization of Urs on the ZnPh/GO is highly favored through the electrostatic interactions. The obtained GC bioelectrode was then few times gently rinsed with doubly distilled water to remove the loosely bound Urs. The fabricated ZnPh/GO/Urs bioelectrode was used for further electrochemical experiments. All the electrochemical experiments were carried out at room temperature. Preparation of GC/GO electrodes without ZnPh was obtained according to the same procedure, using only functionalized GO (without mixing with ZnPh). The overall experimental synthesis mechanisms are clearly documented in Scheme 1.

2.4. Apparatus

Electrochemical experiments were carried out using a CH-660E Instruments electrochemical work station (CH Instruments, Texas, USA). The Three – electrode system consisted of a bare GCE 0.079 cm^2 geometrical surface area) or ZnPh/GO/Urs bioelectrode as working electrode, an Ag/AgCl as a reference electrode (saturated KCl) and platinum wire as a counter electrode. The potentials mentioned in all experimental results were referred to standard Ag/AgCl (saturated KCl) reference electrode. SEM measurements were carried out at VEGA3 TESCAN, USA. Raman spectra were measured with a Raman spectrometer (Dong woo 500i, Korea) equipped with a charge-coupled detector. TGA of samples was carried out with a TGA-50, SHIMADZU thermogravimetric analyzer at a heating rate of 10 °C min^{-1} up to 800 °C under nitrogen. Fourier transform infrared (FT-IR) measurements were performed using a Perkin Elmer spectrophotometer RXI. Hitachi U-3300 spectrophotometer was used to carry out UV–visible absorption spectroscopy measurements. The crystalline structure of the nanoparticles was studied by an X-ray diffraction (XRD; XPERT PRO X-RAY) with Cu K α radiation at 25 °C and the structural assignments were made with reference to the JCPDS power diffraction files (Scheme 2).

3. Results and discussion

3.1. UV–vis-spectroscopy

The UV–vis absorption spectra of GO, ZnPh, ZnPh/GO, and urease immobilize/ZnPh/GO are displayed in Fig. 1. Absorption spectra of GO (Fig. 7a) indicated the characteristics absorption band near to 230 nm due to $\pi \rightarrow \pi^*$ transition of aromatic ring electrons and a small hump near to 300 nm due to $n \rightarrow \pi^*$ of carbonyl groups [33]. As shown in Fig. 7b, ZnPh exhibit three main characteristic absorption peaks, such as B band at 320 nm in ultraviolet band, Q bands at 612 nm and 676 nm in visible region, respectively. This is confirms the formation of ZnPh [34]. After the addition of ZnPh onto the surface of GO, the absorbance was shifted from 328 to 678 nm, which is due to Q band absorption of ZnPh was (Fig. 7c) confirmed by on the surface of GO. Finally, Urs was further added to on the surface of ZnPh/GO hybrid shows three characteristic absorption bands are observed, such as 322 nm, 621 nm and 682 nm respectively. It was confirmed by successfully fabricate Urs on the surface of ZnPh/GO nanocomposite (Fig. 7d).

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