Electrochimica Acta 246 (2017) 303–314

Contents lists available at ScienceDirect

Electrochimica Acta

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

Non-enzymatic hydrogen peroxide sensor based on graphene quantum dots-chitosan/methylene blue hybrid nanostructures

Fariba Mollarasouli[a,b], Karim Asadpour-Zeynali[a,*], Susana Campuzano[b], Paloma Yáñez-Sedeño[b], José M. Pingarrón[b,c,*]

[a] Department of Analytical Chemistry, Faculty of Chemistry, University of Tabriz, Tabriz 51666-16471, Iran
[b] Departamento de Química Analítica, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, 28040 Madrid, Spain
[c] IMDEA Nanoscience, Ciudad Universitaria de Cantoblanco, 28049 Madrid, Spain

ARTICLE INFO

Article history:
Received 29 March 2017
Received in revised form 29 May 2017
Accepted 1 June 2017
Available online 8 June 2017

Keywords:
Graphene quantum dots (GQDs)
Methylene blue (MB)
Chitosan (CS)
Non-enzymatic electrochemical sensor
H_2O_2

ABSTRACT

Graphene quantum dots (GQDs) functionalized with chitosan (GQDs-CS) were used for the first time as a suitable nanostructured sensing film for efficient immobilization of methylene blue (MB) through amino-hydroxyl reaction to prepare a novel non-enzymatic hydrogen peroxide sensor using a glassy carbon electrode (GCE). The synthesized hybrid nanostructures were characterized by X-ray diffraction, field emission scanning electron microscopy, cyclic voltammetry, FT-IR, UV–vis, photoluminescence, and energy dispersive X-ray spectroscopy techniques. Cyclic voltammograms showed that the GQDs-CS/MB/GCE exhibited a significant electrocatalytic activity for the reduction of H_2O_2. The calculated k_{cat} is $4.45 \times 10^4\ cm^3 mol^{-1} s^{-1}$. The calibration graph for H_2O_2 constructed by amperometry (−0.6 V vs. SCE) at the modified electrode showed two different linear ranges (1.0×10^{-6}–2.9×10^{-3} M and 2.9–11.78 mM) with a sensitivity of 10.115 μA/mM for the lower linear range and a calculated detection limit of 0.7 μM (S/N = 3). The response time of the sensor for H_2O_2 detection was 3s. The electrochemical response of GQDs-CS/MB/GCE is not influenced by potential interferents (ascorbic and uric acids, dopamine, caffeine, glucose, and various inorganic salts). This modified electrode exhibited suitability for the non-enzymatic H_2O_2 sensing in food and water samples.

1. Introduction

Graphene, a two-dimensional (2D) form of sp^2-hybridized carbon atoms with honeycomb network structure, possesses important advantages facilitating such as heterogeneous electron transfer between electrode substrates and biomolecules [1]. Unique graphene properties such as high electrical conductivity, good biocompatibility, huge specific surface area, and mechanical flexibility have largely contributed to the great attention that this nanomaterial has attracted for sensing applications [2]. Graphene quantum dots (GQDs), with diameter below 20 nm, are derived from both carbon dots (CDs) and graphene [3]. This 0D nanomaterial exhibits stable luminescence, high solubility in many solvents, good biocompatibility, possibility of edge functionalization and suitable conductivity which make it an excellent probe for both high contrast imaging and sensing applications [4,5]. In addition, GQDs have been proposed as a less toxic alternative to other quantum dots (CdS, CdSe, ZnS, ZnSe, PbS, HgTe, Ag_2Se, Ag_2S, $CuInSe_2$, $CuInS_2$, InAs and InP), which cannot be used in biological systems due to the presence of toxic metals. Moreover, in order to enhance their hydrophility and biocompatibility, GQDs can be easily functionalized with oxygen containing groups such as hydroxyl, carboxyl and epoxy groups [6].

In recent years, the use of natural polymers such as agar, carrageenan, chitosan, and chitin has attracted considerable attention as an immobilization matrix for enzyme and nanomaterials. In particular, the attractive properties of chitosan (CS) such as good film forming ability, non-toxicity, chemically inert, biocompatibility, high mechanical strength, good stability, adhesion capability, and high hydrophilicity make this material a relevant immobilization matrix to be used in electrode surface modification for (bio)sensing applications [6,7]. Moreover, CS has demonstrated also to facilitate the electron transfer after its swelling in the reaction mixture due to its hydrophilic nature [6]. CS has been used recently as a dispersant to modify GQDs and

* Corresponding authors.
E-mail addresses: asadpour@tabrizu.ac.ir, k.zeynali@gmail.com (K. Asadpour-Zeynali), pingarro@quim.ucm.es (J.M. Pingarrón).

http://dx.doi.org/10.1016/j.electacta.2017.06.003
0013-4686/

prepare GQDs-CS nanocatalysts for forming stable composite films on electrode surfaces. This nanocomposite material demonstrated efficient electrocatalysis for vitamin C [6] and methanol oxidation [7].

Hydrogen peroxide (H_2O_2) is an important reactive oxygen species (ROS) in living organisms generated as the products of normal aerobic metabolism and enhanced under stress conditions. H_2O_2 can be used as mediator in various fields such as food processing, environmental protection, pharmaceutical, chemical industry, biology, and biomedicine diagnosis [8,9]. Therefore, the sensitive detection of H_2O_2 is an important concern and many analytical methods involving spectroscopy [10–12], chemiluminescence [13,14], fluorimetry [15,16], chromatography [17], and electrochemistry [18–20] have been reported for such purpose. Among these, electrochemical methods are a suitable alternative because of their low cost, high sensitivity, quick response, simple instrumentation, and possibility of miniaturization. Electrochemical sensors for the determination of H_2O_2 can be classified in two major classes: enzymatic and non-enzymatic sensors. In the first category, direct electron transfer occurs between enzyme and electrode giving rise to mediatorless H_2O_2 sensors. In the second category, a mediator such as hexacyanoferrates, ferrocene derivatives, or poly (vinyl pyridine) polymer is used to shuttle the electron between H_2O_2 and the electrode. Limitations of enzyme electrochemical sensors such as reduced catalytic activity and sensitivity after repeated measurements, high cost, low electron transfer rate and dependence with pH and temperature, lead to the development of non-enzymatic H_2O_2 sensors. Methylene blue (MB), a redox dye belonging to the phenothiazines family, can be used as a mediator for these purposes: taking advantage of its electrochemical reversibility, high electron transfer rate, chemical stability and adsorption ability on carbon electrode surfaces.

In this work, a non-enzymatic hydrogen peroxide electrochemical sensor is reported using GQDs-CS/MB composites as GCE modifier. GQDs-CS were used as a suitable scaffold for the electrodeposition of MB. The electrochemical behavior and electrocatalytic properties of this new sensor toward H_2O_2 were characterized by cyclic voltammetry and chronoamperometry. The obtained results demonstrated the great potential of GQDs-CS/MB/GCE as a sensitive and reliable amperometric sensor for direct determination H_2O_2 in spiked food and water samples after minimal sample treatment.

2. Experimental

2.1. Apparatus and electrodes

Electrochemical experiments were performed on an AUTOLAB potentiostat (model PGSTAT30) equipped with a three-electrode cell. Three–electrode system employed includes a GQDs-CS/MB modified GCE (ϕ = 2.0 mm purchased from Azar electrode company, Urmia, Iran) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum wire electrode as the counter electrode. The pH values of solutions were adjusted with a pH/mV/ion meter (model 827 Metrohm, Utrecht, The Netherlands). Field emission scanning electron microscopy (FESEM) of GQDs-CS/MB were carried out with a Tescan Mira3 FESEM equipped with gold coating and an EDX system. X-ray powder diffraction (XRD) was done by a Rigaku D/max-2200X-Ray Diffractometer (Bruker AXS) using a Cu Kα source (λ=0.154056 nm). Fourier transform infrared (FTIR) analysis was carried out using a Bruker FTIR spectrometer at room temperature (25 ± 1 °C). The photoluminescence (PL) spectra were recorded by a Shimadzu RF–540S fluorescence spectrometer. UV–visible studies were made with an Avantes diode array spectrophotometer. In order to prepare TEM samples, one drop of diluted nanoparticle solution was casted on the Cu-TEM grid coated with the amorphous carbon thin film and left to dry in air. The TEM characterization was performed using a Phillips CM 12 microscope operating at an accelerating voltage of 120 kV.

2.2. Reagents and solutions

Methylene blue (MB) was purchased from Sigma-Aldrich. Chitosan (M_w = 600,000-800,000, code: 428855000 with purity of $\geq$90) was purchased from Acros Organics. Hydrogen peroxide (H_2O_2), glucose, glacial acetic acid, K_3 [Fe (CN)$_6$] ($\geq$ 99.5%) and ascorbic acid were purchased from Merck (Darmstadt, Germany). Phosphate buffer solution (PBS, 0.1 M) containing Na_2SO_4 0.1 M with adjusted pH at 7.4 was used as supporting electrolyte for determination of H_2O_2. All chemical reagents used in the experiment were of analytical grade and were used directly without further purification. All aqueous solutions were prepared with deionized water.

2.3. Synthesis of graphene quantum dots-chitosan (GQDs-CS) nanocomposites

GQDs were synthesized by the hydrothermal method. Briefly, 0.7 g of glucose was weighed and dissolved in 50 mL water to obtain an aqueous solution of glucose as carbon source. The solution was transferred into a 100 mL Teflon equipped stainless steel autoclaved and sealed. After heating at 180 °C for 4 h in a muffle furnace, and further cooling at air, a yellowish brown solution was obtained. The color change from colorless to yellowish brown implies the formation of GQDs. To remove larger GQDs nanoparticles, the solution was dialyzed in a dialysis bag (molecular weight: 2000 Da). Synthesis of GQDs-CS was carried out by dissolving 0.1 g of chitosan in 10 mL of 1% (v/v) acetic acid and mixing with the GQDs. The mixture was heated with a conventional heater at 60 °C for 30 min under stirring. The as prepared GQDs-CS nanoparticles were stored in a dark place at 4 °C to maintain stability for more than 1 month.

2.4. Preparation of GQDs-CS/MB modified GCE

Before modification, the surface of GC electrode was polished on polishing paper with 0.3 μm alumina powder to mirror-like, and subsequently rinsed with deionized water. Then, the electrode was sonicated in a mixture solution of ethanol and deionized water (1:1, v:v) for 10 min to remove the remaining alumina particles. 10 μL of the GQDs-CS solution was casted on the GCE surface and allowed drying at room temperature to obtain a homogeneous film (GQDs-CS) on the GCE. The modified electrode needs to be electrochemically activated by applying a constant potential of +1.7 mV versus SCE for 600 s. Electrodeposition of MB on the activated GQDs-CS/GCE was carried out potentiostatically at −1.2 V for 600 s in an aqueous solution of MB 13 μM prepared in 0.1 mol L^{-1} phosphate buffer and 0.1 mol L^{-1} Na_2SO_4 (pH 8.5). After rinsing the electrode surface with doubly distilled water, the GQDs-CS/MB/GCE is ready to be used.

2.5. Determination in honey, fruit juice and water samples

In order to evaluate the practical applicability of the developed sensor, the determination of H_2O_2 was carried out in four different spiked samples: honey, fruit juice, spring water and tap water. The samples were stored in a dark place at room temperature prior to