International Journal of Biological Macromolecules 104 (2017) 672–680

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Electrochemical behavior of Pb (II) on a heparin modified chitosan/graphene nanocomposite film coated glassy carbon electrode and its sensitive detection

T. Priya, N. Dhanalakshmi, N. Thinakaran*

Environmental Research Lab, PG and Research Department of Chemistry, Alagappa Government Arts College, Karaikudi, 630 003, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 21 April 2017
Received in revised form 10 June 2017
Accepted 19 June 2017
Available online 20 June 2017

Keywords:
Heparin
Chitosan
Reduced graphene oxide
Metal ion
Simultaneous detection

ABSTRACT

In this study, we developed a novel composite material containing biological macromolecules like heparin and chitosan coated on reduced graphene oxide (rGO) for the modification of glassy carbon electrode (hep/CS-rGO/GC). It can be applied for the sensitive electrochemical detection of Pb^{2+} by square wave anodic stripping voltammetry (SWASV). The physicochemical analysis such as XRD, FTIR, FESEM and Raman spectroscopy techniques revealed that an effective functionalization occurred at the rGO surface. The consequence of deposition and stripping of metal ions by various electrochemical parameters such as supporting electrolytes, pH value, deposition potential, and deposition time were carefully studied and optimized. Under the optimized conditions, the linear calibration curve was calculated to be from 1.125 to 8.25 $\mu g\,L^{-1}$ for Pb^{2+} with the correlation coefficient (R^2) 0.9988. The detection limit and sensitivity achieved for the modified electrode were 0.03 $\mu g\,L^{-1}$ and 1.34 μA/nM respectively. Furthermore, the electrochemical investigation indicates that the hep/CS-rGO composite electrode exhibits high selectivity, strong adherence to the electrode surface, good stability and reproducibility towards the detection of Pb^{2+}. Finally, hep/CS-rGO/GC electrode was assessed by the quantity of Pb^{2+} present in the practical samples, and the determined results were consistent with that of AAS.

1. Introduction

Graphene is a two dimensional one atom thick allotrope of carbon with sp^2 carbon atoms in a honey comb like lattice [1]. It is usually called as a creator of all advanced carbon materials and it has been used now a days in almost all engineering and scientific fields. These materials possess outstanding properties such as very high active surface area, electron transfer rate, excellent stability, mechanical and thermal properties which can be exploited to measure trace concentration of metal ions, organic compounds, biomolecules, and drugs [2–5]. Besides, an ideal graphene sheet provides a good substrate for chemical tailoring of carbon nanomaterials which will further facilitate the surface suitability and electronic properties in order to hike the sensitivity of detection [6]. Thus, suitable surface functionalities can be introduced to bestow the graphene sheets to develop a novel composite for specific applications.

As for as heavy metal contamination to our environment is concerned, lead ions (Pb^{2+}) have a long history with respect to usage and human health. Because of the ill effects of these on humans, World Health Organization (WHO) reduced the limit of lead ion in drinking water from 50 ppb to 10 ppb. Various industrial and household necessities are dumping Pb^{2+} into the aquatic environment. This shows an urgency to quantify the amount of Pb^{2+} present in drinking water sources to ensure public health and environmental protection [7]. Hence, the quest for the development of rapid and sensitive detection Pb^{2+} is significant. The conventional way of Pb^{2+} determination includes atomic absorption spectroscopy, inductively coupled plasma spectroscopy, and atomic fluorescence spectroscopy [8]. However, complicated analytical procedures, costly instrumentation and a long sample processing time hamper the in-situ applicability of these methods [9]. But electrochemical methods offer encouraging access [10] to detect the metal ions with high sensitivity, environment friendly, simple and efficient operating procedures when square wave anodic stripping voltammetry (SWASV) is applied.

One major disadvantage of graphene is the characteristic poor dispersion due to agglomeration. In order to overcome this dispersion problem, it is essential to functionalize graphene [11].

---

* Corresponding author.
E-mail addresses: priyathangamani8@gmail.com (P. T.), dhanalakshmi92.n@gmail.com (D. N.), thinakaran2k@yahoo.com (T. N.).

http://dx.doi.org/10.1016/j.ijbiomac.2017.06.082
0141-8130/

The natural polysaccharides containing nitrogen and oxygen, such as chitosan provide a hydrophilic surface to the carbon nanostructures for covalent, absorptive or ionic linkages with bioactive molecules which have also been utilized for the superior sensing of target metal ions. Moreover, adhesive coating on electrode surface, biocompatibility, high electrical conductivity, excellent chemical stability and film forming abilities are the chief advantages of using chitosan [12,13]. A variety of materials based on graphene chitosan have been explored in various metal ion detection and electrochemical sensors [14]. However heparin modified chitosan for heavy metal detection and the interaction mechanism has not been reported. Heparin is a complex and high negatively charged sulphated mucopolysaccharides called glycosaminoglycans, having anticoagulant properties of blood. Heparin has the highest negative charge density due to the presence of sulfonate (-SO^{3-}) and corboxylate (COO^-) groups among any known biological molecules and also exhibiting a wide range of biological functions by electrostatic interaction with various proteins, inhibitors and drugs [3,6,15].

In the present work attempts are made to unify the advantages of graphene, chitosan and heparin in a potential approach for the sensitive detection of Pb^{2+} by the electrochemical method. During the electrochemical detection of Pb^{2+} with hep/CS-rGO/GC electrode, heparin forms a sensitive interface with Pb^{2+} through metal–ligand interaction. Experimental parameters, such as the deposition potential, deposition time, supporting electrolyte and the effect of pH value were optimized by SWASV. Finally, the electrochemical sensor was used to determine the concentration of Pb^{2+} in real samples with a satisfactory result.

2. Materials and methods

2.1. Materials

Heparin (Porcine intestinal mucosa) and graphite flakes (+100 mesh) were purchased from sigma Aldrich India. Lead nitrate ($Pb(NO_3)_2$), Copper nitrate ($Cu(NO_3)_2$ $3H_2O$), Zinc nitrate ($Zn(NO_3)_2$ $4H_2O$) and cadmium nitrate ($Cd(NO_3)_2$ $4H_2O$) were purchased from Merck, Mumbai, India. The prawn shells (PenaeusIndica or Indian Prawn) were collected from Thondi fishing harbor, Tamil Nadu, India. The other common chemicals used in this study were of analytical reagent grade obtained from CDH, India and used without further purification. Ultrapure water (Milli-Q-Millipore 18.2 MΩ/cm, Millipore simplicity) was used throughout the studies.

2.2. Preparation of chitosan and graphene oxide

The Chitosan was prepared from Indian Prawn shells [16]. The preparation process was briefly discussed in the supporting document (S1). Graphene oxide (GO) was successfully prepared by modified Hummer's method [17]. Briefly, 2 g of graphite powder was mixed with 100 mL of con. H_2SO_4 and it was cooled under ice water bath. After cooling, 8 g of potassium permanganate was added slowly into the mixture with constant stirring for 1 h at room temperature. The resultant mixture was diluted with 250 mL of water and the stirring continued for 30 min. When the temperature of the mixture reached 90 °C, the progress of the reaction was terminated by adding 300 mL water followed by 3 mL of H_2O_2 (30%) solution. During this step, the color of the solution was gradually changing from dark brown to greenish yellow. The solid graphene oxide was separated by centrifugation, washed repeatedly with ultrapure water and finally it was dried under vacuum.

2.3. Preparation of chitosan –reduced graphene oxide nanocomposite (CS-rGO)

Homogeneous dispersion of CS-GO was prepared by dissolving 5 mg of GO and 10 mg of CS into 10 mL of 0.1 M acetic acid followed by ultrasonication for 30 min. Then the contents were heated in an oil bath maintained at 70 °C for 4 h. At this time, the color of solution was continuously changing from yellow to black. The final solid mass is separated, washed with distilled water and dried at 50 °C in hot air oven. The role of CS is that it is a reducing agent cum stabilizer for the effective conversion of GO into rGO. [18].

2.4. Preparation of modified electrodes

The bare Glassy carbon electrode was polished with alumina powder (1.0, 0.3, 0.05 μm) sequentially, then cleaned with ethanol, ultra pure water and dried in nitrogen atmosphere. 5 mg of GO was dispersed in 5 mL of ultrapure water and it was sonicated for 30 min. 5 μL of GO suspension was drop casted on the polished GCE and it was dried under an IR heating lamp. The obtained electrode was denoted as GO/GCsimilarly CS-rGO/GC and hep/CS-rGO/GC were prepared by dispersing 5 mg CS-rGO in 5 mL of water and 5 mg of hep + 5 mg of CS-rGO in 5 mL of water respectively. Increase or decrease of 1 mg/mL heparin concentration in CS-rGO mixture has affected the homogeneous dispersion of nanocomposite. Fig. S1 shows the photograph of GO and CS-rGO dispersion in water.

2.5. Instrumentation

Surface morphology of the prepared nanocomposite materials were investigated by Field Emission Scanning Electron Microscopy (FE-SEM, Carl Zeiss Supra 55VP/41/46). X-ray diffraction (XRD) patterns were obtained on X-ray Diffractometer D8 (Bruker) operating at 1.6 kW and using CuKα radiation. Raman spectra were collected using a Renishaw 2000 system with an Argon ion laser (514.5 nm) and charge coupled device detector. A Fourier transform infrared (FTIR) spectrum of the prepared materials and its composites were recorded using JASCO 4600 FT-IR spectrometer. Atomic absorption spectrometric measurements were conducted with AAS (AA6300 SHIMADZU, Japan). All the electrochemical measurements were carried out with a Bio-Logic (SP-150, EC-Lab software version 11.01, France) workstation and typical three electrode arrangement with Ag/AgCl (3 M NaCl) as reference electrode, Platinum wire (1 mm diameter) was the counter electrode and modified glassy carbon electrode was the working electrode. All the experiments were performed at ambient temperature. The pH of the supporting electrolyte was determined with a Eutech, pH meter.

3. Result and discussion

3.1. Characterization of CS, GO, CS-rGo and hep/CS-rGO

3.1.1. XRD analysis

Fig. 1 shows the X-ray powder diffraction patterns of graphite, GO and CS-rGO were measured using a PANalytical X'Pert PRO MPD X-ray diffraction systems using Cu K radiation (λ = 1.5418 Å, 40 kV, 40 mA) between 10^0 and 80^0 for wide angle Xray diffraction. The (0 0 2) crystal plane of graphite with a diffraction peak at $2\ \theta = 26.5^\circ$ indicates an interlayer spacing of 0.35 nm. After oxidation, the XRD peak of graphene oxide (GO) is shifted to 10.3° indicating that the interlayer spacing increases to 0.73 nm. After chemical reduction with chitosan, the sharp peak of GO disappeared while another broad peak of around 28° appeared. The disappearance of the sharp peak can be attributed to the exfoliation of layered structures of GO.