Analytical Biochemistry 551 (2018) 29–36

Contents lists available at ScienceDirect

Analytical Biochemistry

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

Enhanced amperometric detection of paracetamol by immobilized cobalt ion on functionalized MWCNTs - Chitosan thin film

Shamima Akhter[a,**], Wan Jefrey Basirun[a,b,*], Yatimah Alias[a,c], Mohd Rafie Johan[b], Samira Bagheri[d], Md. Shalauddin[b], Magaji Ladan[e], Nadzirah Sofia Anuar[a]

[a] *Department of Chemistry, Faculty of Science, University Malaya, 50603, Kuala Lumpur, Malaysia*
[b] *Nanotechnology and Catalysis Research Center (NANOCAT), University Malaya, 50603, Kuala Lumpur, Malaysia*
[c] *University Malaya Centre for Ionic Liquids (UMCiL), University Malaya, 50603, Kuala Lumpur, Malaysia*
[d] *Department of Mechanical Engineering, Tufts University, 200 College Ave, Medford, MA, 02155, USA*
[e] *Department of Chemistry, Bayero University, Kano, Nigeria*

ARTICLE INFO

Keywords:
Paracetamol
Electrochemical sensor
f-Multi-wall carbon nanotube
Chitosan
Cobalt

ABSTRACT

In the present study, a nanocomposite of *f*-MWCNTs-chitosan-Co was prepared by the immobilization of Co(II) on *f*-MWCNTs-chitosan by a self-assembly method and used for the quantitative determination of paracetamol (PR). The composite was characterized by field emission scanning electron microscopy (FESEM) and energy dispersive x-ray analysis (EDX). The electroactivity of cobalt immobilized on *f*-MWCNTs-chitosan was assessed during the electro-oxidation of paracetamol. The prepared GCE modified *f*-MWCNTs/CTS-Co showed strong electrocatalytic activity towards the oxidation of PR. The electrochemical performances were investigated by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV). Under favorable experimental conditions, differential pulse voltammetry showed a linear dynamic range between 0.1 and 400 μmol L^{-1} with a detection limit of 0.01 μmol L^{-1} for the PR solution. The fabricated sensor exhibited significant selectivity towards PR detection. The fabricated sensor was successfully applied for the determination of PR in commercial tablets and human serum sample.

Introduction

The consumption of prescribed drugs and medicines without knowledge of the proper dosage, toxicity and side-effects are a common problem these days. Paracetamol (PR) is one of the most frequently used medicines which falls into this category. Paracetamol (N-acetyl-P-aminophenol) or acetaminophen (electronic supporting information ESI, Fig. S1) is one of the most widely used ‘over the counter’ (OTC) drugs in the world. In addition, it is the most popular analgesic and anti-pyretic drug. Generally, it is considered as a safe and effective drug in regular therapeutic doses [1,2]. The PR activity as a painkiller is based on the inhibition of prostaglandin synthesis in the CNS (central nervous system), while the antipyretic effect is through the sedation of heat regulation in the hypothalamic region [3,4]. The pain relief effect of PR includes arthralgia, neuralgia, migraine, joint pain, general pain, cancer pain, headache, toothache, backache and postoperative pain [5]. However, the excessive ingestion of PR could result in life threatening and serious health hazards such as liver damage, kidney failure and severe hypersensitivity reaction [6]. Such overdose could lead to gastrointestinal bleeding, nausea and vomiting. The misadministration of paracetamol in the younger children especially to 6–7 year olds could lead to asthma, rhino conjunctivitis and eczema [7].

With regards to the health effects from the overdose of PR as mentioned earlier, it is very crucial to determine the concentration of PR from different pharmaceutical dosage forms and biological preparations via a simple, rapid and precise quantification method. Until now several techniques have been reported for the detection of PR from pharmaceutical preparations such as high performance liquid chromatography (HPLC) [8,9], UV-spectrophotometry [10], titrimetry [11], capillary electrophoresis [12], HPLC-tandem mass spectrometry [13] and thermogravimetric analysis (TGA) [14]. But these methods have several limitations such as high cost, time consumption, lower sensitivity and poor selectivity. In addition, some of these methods require highly trained personnel to operate the instruments. Recently, electrochemical techniques have gathered the attention of analytical and biomedical researchers as they are effective tools for the detection of certain analytes compared to the methods described earlier. Electrochemical techniques possess advantages such as higher selectivity,

---

* Corresponding author. Department of Chemistry, Faculty of Science, University Malaya, 50603, Kuala Lumpur, Malaysia.
** Corresponding author.
E-mail addresses: shamimaakhter053@yahoo.com (S. Akhter), jeff@um.edu.my (W.J. Basirun).

https://doi.org/10.1016/j.ab.2018.05.004
Received 23 March 2018; Received in revised form 27 April 2018; Accepted 2 May 2018

higher sensitivity, cost effectiveness, simplicity, ease of miniaturization, wider detection limits and quick response [15]. Several researchers have reported the quantitative detection of PR using electrochemical techniques [16–19]. However, PR shows poor electrochemical response on bare glassy carbon electrode (GCE), thus the quantification is quite difficult. Thus GCE electrodes modified with materials such as nanoparticles [20], electro active polymers [21], metal oxides [22] and integrated nanomaterials [23] have been utilized for the quantitative detection of PR. When the GCE surface is modified with these materials, they exhibit outstanding properties such as rapid electron transfer, high surface area, high stability and good biocompatibility. They have been used extensively for the detection of several types of analytes. In the current research field, chemically modified electrodes are a growing interest among analytical researchers because of the wide application for several analyses [24].

Nanocarbons such as mesoporous carbons [25], carbon nanowires [26], carbon nanotubes (CNT) [27] and carbon nanofibers [28] have been frequently utilized for the fabrication of multipurpose electrode in analytical experiments and industrial electrochemistry [29]. They are considered as advanced nanosensors due to advantages such as favorable catalytic response for many redox reactions, wider potential range, chemical inertness and compatibility [30]. CNTs are sp^2 hybridized carbon nanosheets or graphene sheets which are folded into nano-cylinders [31]. The two types of CNTs are single-walled carbon nanotubes (SWCNT) [32] and multi-walled carbon nanotubes (MWCNT) [33]. CNTs are multi-functional nanocarbons and have attracted huge attention because they possess several distinctive properties such as chemical stability, mechanical strength and high electrical conductivity. The axial modulus properties which serve as a support to guest molecules are considered the largest among all materials [34,35]. Compared to SWCNTs, functionalized multi-walled carbon nanotubes (*f*-MWCNTs) have attracted greater attention due to the higher solubility, better adsorption capability, higher chemical stability, enhanced morphological features and electroanalytical properties. Thus, it is hardly surprising that *f*-MWCNTs have achieved wider applications since its discovery [36,37].

Chitosan (CTS) is a linear co-polymer which consists of both amino and hydroxyl groups which facilitates the adsorption of several organic compounds and heavy metal cations. CTS consists of D-glucosamine and N-acetyl-d-glucosamine functional groups. It has been reported that *f*-MWCNTs can be homogeneously dispersed in the CNT matrix, thus CTS can decrease the agglomeration of *f*-MWCNTs. Moreover, CTS possesses outstanding properties such as good biocompatibility, biodegradability, excellent water permeability, non-toxicity, excellent film formation ability and higher mechanical strength [38,39]. Therefore, sensors based on CTS matrix have been utilized for the quantitative determination of PR [40]. The transition metal complexes immobilized on the surface of polymeric matrices are favorable catalysts due to the synergic combination of homogenous and heterogeneous network [41]. The composite of chitosan and metal element provides the annulus chelating configuration and possesses the properties of natural enzymes. For this reason, they show suitable catalytic activity for several chemical reactions [42]. A composite of MWCNTs, chitosan and cobalt was utilized for the quantitative determination of oleanic acid [43]. Azab et al. prepared a composite of MWCNTs, chitosan and cobalt modified electrode for the determination of *Daclatasvir* [44], while a composite containing MWCNT, chitosan and copper was reported for the determination of diclofenac sodium [15]. However, PR is a very common target analyte and several electrochemical sensors based on different types of materials have been developed for the quantitative determination of PR. To the best of our knowledge, electrochemical sensors based on a composite of *f*-MWCNT, chitosan and cobalt has not been developed for the quantitative detection of PR. In this study, we report an electrochemical sensor based on *f*-MWCNTs/CTS-Co complex for the sensitive detection of PR. By applying a self-assembly technique, the sensor was fabricated by modifying a glassy carbon electrode (GCE) with the *f*-MWCNTs/CTS-Co composite. The fabricated sensor was investigated for the quantitative detection of PR in tablet form and biological preparations from human serum.

Experimental methods

Chemical reagents

Pristine MWCNTs were procured from Baytubes, Germany. Paracetamol, $CoCl_2{\cdot}6H_2O$, chitosan (CTS), K_2HPO_4 and KH_2PO_4 were purchased from Sigma Aldrich. The remaining reagents were of analytical grades and used without further purification. Deionized water was used in the preparation of buffers and standard solutions. Ethanol solution (25%) was used for the dilution and preparation of PR stock solution (0.1 $mol\,L^{-1}$). The preparation of standard solutions was done on a daily basis. A solution of 0.2 $mol\,L^{-1}$ phosphate buffer (PBS) at different pH were prepared by mixing the standard solutions of K_2HPO_4 and KH_2PO_4 at the correct proportions.

Characterizations

Cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV) were performed using a Galvanostat/Potentiostat (Autolab, Metrohm, Model- AUT72609) in an electrochemical cell with the three electrode system. A platinum (Pt) wire and Ag/AgCl were the counter and reference electrodes, respectively. All electrochemical experiments were conducted at room temperature (25 °C).

Purification of *f*-MWCNTs

The purification steps of MWCNTs was performed based on the literature [45]. Pristine MWCNTs was dispersed in a solution mixture of nitric acid and sulfuric acid (1:3) and then refluxed at room temperature for 24 h. The COOH-functionalized MWCNTs product was diluted by adding adequate DD water. Then the obtained *f*-MWCNT was filtered through a Whatman filter paper. The residue was washed several times with DD water until pH 7. Finally the filtered product was dried for 24 h at 80 °C in a vacuum oven.

Modified electrode

Two glassy carbon electrodes (GCE) of 3 mm diameter each were polished to a mirror-like surface on 0.05 μm alumina slurry on polishing cloth. After polishing for two minutes, the electrodes were rinsed with distilled water and sonicated for 5 min, in a mixture of 15 ml ethanol and 10 ml distilled water solution. Finally, the electrodes were air dried at room temperature [46]. A suspension of *f*-MWCNTs was prepared by dissolving 2 mg *f*-MWCNTs in 2 ml distilled water and sonicated for 15 min. The chitosan suspension were prepared by dissolving 0.25 mg of chitosan powder in 50 ml 0.10 $mol\,L^{-1}$ acetic acid and sonicated for 30 min. The cobalt solution was prepared by dissolving 0.1 g of $CoCl_2{\cdot}6H_2O$ in 100 ml distilled water and sonicated for 30 min. The electrodes were dipped into these solutions to prepare the modified surface. The GCE surface modified with the *f*-MWCNT/CTS-Co composite was prepared from the following procedures. A 5 μL of *f*-MWCNTs suspension was drop-casted onto the GCE surface using a micropipette and dried at room temperature. Then the dried electrode was immersed in the chitosan solution for 30 min. The positively charged CTS was self-assembled on the negatively charged carboxylic group of the functionalized MWCNTs surface via electrostatic interaction. Then the electrode was removed from the CTS solution and washed with distilled water to remove any weekly bonded CTS molecules. Finally, the *f*-MWCNTs/CTS/GCE was immersed into a freshly prepared cobalt ion solution for 30 min, where the Co^{2+} were self-assembled onto the CTS surface by a complexation process. Then the modified *f*-MWCNTs/CTS-Co/GCE