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Highly sensitive electrochemical detection of palmatine using a biocompatible multiwalled carbon nanotube/poly-L-lysine composite

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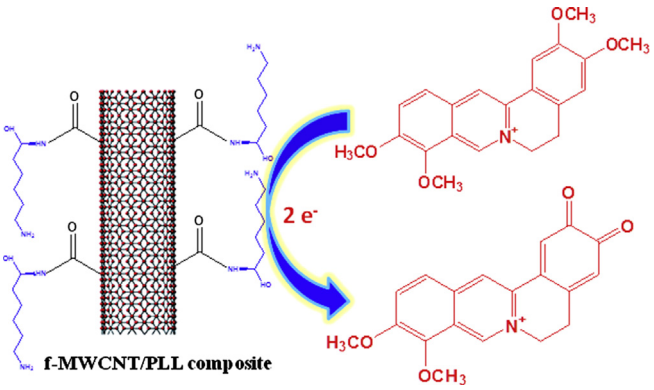
GRAPHICAL ABSTRACT



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ABSTRACT

To date, the natural alkaloids are mostly used in the field of pharmacological applications and the active substance of palmatine was extensively used in cancer therapy and other biomedical applications. Hence, in this study we report a simple preparation of poly-L-lysine (PLL) electro-polymerized on the surface of functionalized multiwalled carbon nanotubes (f-MWCNT) for electrochemical detection of palmatine content in human serum and urine samples. The active amino group of PLL plays a vital role towards the oxidation palmatine and exhibits superior electrocatalytic activity. Under optimum conditions, the prepared f-MWCNT/PLL composite shows a wide linear response range over the palmatine concentration ranging from 0.5 μM to 425 μM, and a detection limit (LOD) of 0.12 μM based on S/N = 3 (signal to noise ratio). The real time monitoring of palmatine content in serum and urine samples displays an appropriate recoveries and excellent performance for the practical analysis. The advantage of this developed system was simple, higher electrocatalytic activity, long-term stability and low cost. We hope that the prepared composite opens a new way for the fabrication of different biosensors in the field of biomedical application.

1. Introduction

Alkaloids are most abundant natural product and have been widely used in the filed of therapeutic applications due to their remarkable biological relevance and utilities [1–3]. Palmatine is an 8-substituted derivative of protoberberine and/or isoquinoline

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alkaloid, and mostly found in various plant species such as phellodendron amurense, rhizoma coptidis/coptis chinensis and corydalis yanhusuo [4]. In addition, palmatine has been exposed to display a significant role in anticancer activities, also exhibits a variety of pharmacological activities, including antimicrobial activity against fungi, anti-inflammatory, antinociceptive and antipyretic, viruses and bacteria, vasodilatory and sedative [5–8]. The superior nature of palmatine has been widely focused on various clinical applications and received much attention in jaundice, dysentery, hypertension, liver related diseases, gynaecological inflammation, respiratory tract infection, urinary infection, etc [9,10]. Moreover, the pharmacology substance of palmatine could be applied in the field of cancer therapy, especially alleviating GaIN/LPS-induced liver injury by modulating the cytokine response and also inhibiting the apoptosis, emphasizing its antineoplastic effects [11,12]. Thus, there is a demand to identify and quantify the trace level amount of palmatine in analytical research, particularly in the field of pharmaceutical and clinical analysis. Meanwhile, the quantification of palmatine is more important in traditional Chinese medicines for the evaluation of the quality of the product due to their therapeutic effect [13].

To date, a variety of classical analytical techniques such as high performance liquid chromatography (HPLC), liquid chromatography-tandem mass spectrometry (LC-MS/MS), resonance light scattering (RLS), capillary electrophoresis, mass spectrometry (MS), thin layer chromatography (TLC), electrochemiluminescence and electrochemical methods have been adapted for the real time monitoring, separation and detection of palmatine and other natural alkaloids [14–23]. These analytical techniques have some drawbacks such as highly expensive analytical devices, complicated preconcentrations, more time consuming and so on. Therefore, the electrochemical methods have been mostly considered for various potential applications due to its remarkable advantages such as fast response, low cost, handle free and higher sensitivity. Hence, we have used the electrochemical method for the determination of palmatine content in human serum and urine samples. Nevertheless, so far only one report has been available for the electrochemical determination of palmatine.

In past few decays, the carbon nanomaterials have been utilized for different potential applications due to its classical structural and optical properties [24,25]. In particular, the acid treated functionalized multiwalled carbon nanotubes (f-MWCNT) have been received much attention for the development of biocompatible nanomaterials owing to their classical advantages such as high conductivity, large surface area, excellent mechanical strength and low cost [26]. The admirable nature of f-MWCNT has been continuously used in variety of potential applications including supercapacitors, batteries, electrochemiluminescent, transistors and mainly focused in the field of high performance of electrochemical sensors [27,28]. Recently, the CNTs based studies were generally focused the interaction between the CNTs and biological active molecules such as DNA, peptides and proteins for biosensor applications [29–31]. On the other hand, poly-L-lysine (PLL) is an essential biocompatible polyamino acid and possesses more electroactive species containing terminal amine groups on the side chains [32–34]. The biocompatible nature of PLL has been showed several advantages such as good biocompatibility, plentiful amino groups, a flexible molecular backbone, enzyme degradation and excellent solubility in water [35]. Thus, the fabrication of MWCNTs with several kinds of polymers have been reported such as polydopamine, polyaniline and polypyrrole [36–38]. Hence, in this study we have utilized PLL as a biopolymer for the fabrication of a biocompatible f-MWCNT/PLL nanocomposite for the electrochemical detection of palmatine.

In this study, we report a novel palmatine sensor using f-MWCNT/PLL biocompatible nanocomposite. The f-MWCNT was directly attached with hydrophilic nature of PLL as a linker through the amide group. The amine groups of PLL were easily cross linked with the carboxylic group of MWCNT and act as a friendly linker between the f-MWCNT and biologically active molecules. A simple and facile electro-polymerization takes place for the preparation of f-MWCNT/PLL composite. The electrocatalytic activity of f-MWCNT/PLL composite towards the oxidation palmatine was carefully optimized and discussed in details. This proposed composite will opens a large number of opportunities for the preparation of various biosensors and DNA based biosensor.

2. Experimental section

2.1. Materials and methods

L-lysine and MWCNTs (MWCNT, purity > 95%, diameter < 10 nm, length of 5–15 μm) were purchased from Sigma-Aldrich. Palmatine hydrochloride was obtained from Aldrich and the stock solution was prepared using DI water, stored at 4 °C in dark. All other chemicals were used of analytical grade as received without further purification. The supporting electrolyte of sulfuric acid medium was prepared by mixing stock solutions of 0.1 mol L^{-1} sulfuric acid and sodium sulfate. All experiments were done at room temperature using double-distilled water. A standard three-electrode cell was utilized for the electrochemical experiments using a computerized CHI410a electrochemical workstation. The GCE was used as the working electrode, Ag/AgCl electrode (Sat. KCl) and platinum wire (0.5 mm) used as reference and counter electrodes, respectively. All electrochemical experiments were carried out at a room temperature in an inert atmosphere (N_2 atmosphere).

2.2. Preparation of f-MWCNT/PLL composite

The MWCNTs were acid treated for 4 h according to the previous literature [39]. Then, the obtained carboxylic functionalized MWCNT (f-MWCNT) was redispersed in DI water. The mirror like GCE was polished using alumina powder and washed with DI water followed by ethanol via ultra sonication. About 6 μL of f-MWCNT was drop casted on the surface of pre-cleaned GCE and dried at room temperature. The f-MWCNT modified GCE was transferred to a solution containing 10 mM L-lysine containing phosphate buffer solution (pH 6) at a scan rate of 100 mV/s in the potential range of –1.5 to 2.5 V for 10 successive cycles. The prepared composite was rinsed with DI water and dried at room temperature. The obtained composite was denoted as f-MWCNT/PLL modified GCE and the overall procedure was illustrated in Scheme 1.

3. Results and discussion

3.1. Structural analysis

The structural morphologies of the prepared composites were characterized by using FESEM. Fig. 1 depicts the FESEM images of (A) f-MWCNT and (B) f-MWCNT/PLL composite. It can be seen that the nanotubes are interconnected randomly and arranged a mat like structure (Fig. 1A) and there is no damage after the acid treatment. This result confirms that the covalent functionalization was not affecting the structure of the MWCNTs. On the other hand, the f-MWCNT/PLL composite display a uniform sheet like morphology and the nanotubes were arranged a thin layer of nanosheets after the successful polymerization (Fig. 1B). The morphological changes of the composite ensures that the successful polymerization of PLL on the surface of f-MWCNT. As we know that the negatively