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Preparation of chitosan grafted graphite composite for sensitive detection of dopamine in biological samples

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ABSTRACT

The accurate detection of dopamine (DA) levels in biological samples such as human serum and urine are essential indicators in medical diagnostics. In this work, we describe the preparation of chitosan (CS) biopolymer grafted graphite (GR) composite for the sensitive and lower potential detection of DA in its sub micromolar levels. The composite modified electrode has been used for the detection of DA in biological samples such as human serum and urine. The GR-CS composite modified electrode shows an enhanced oxidation peak current response and low oxidation potential for the detection of DA than that of electrodes modified with bare, GR and CS discretely. Under optimum conditions, the fabricated GR-CS composite modified electrode shows the DPV response of DA in the linear response ranging from 0.03 to 20.06 μM. The detection limit and sensitivity of the sensor were estimated as 0.0045 μM and 6.06 μA μM^{-1} cm^{-2}, respectively.

1. Introduction

Over the past few decades, the development of biosensors and chemical sensors for the detection of neurotransmitters has received a great interest due to the vital role in the metabolic system of mammals (Jackowska & Krysinski, 2013; Pradhan et al., 2014). In particular, dopamine (DA) is a well-known inhibitory neurotransmitter and plays an important role in the central nervous system of the human (Nagatsu & Ichinose, 1999). In general, the DA level in the cerebrospinal fluid (CSF) is in the range between 0.5–25 nM (Suominen et al., 2013). Furthermore, the malfunctions of DA in CSF lead to many diseases such as Parkinsonism, schizophrenia, hypertension and pheochromocytoma (Ge, Tan, Xie, Ma, & Yao, 2009). Therefore, the levels of DA in blood or urine are essential indicators in medical diagnostics for the diseases. To date, different analytical techniques have been utilized for the reliable determination of DA in biological fluids, such as liquid chromatography (Meng et al., 2000), capillary electrophoresis (Wey & Thormann, 2001), liquid chromatography coupled with UV detection (Ary & Rona, 2001), calorimetry (Secor & Glass, 2004), native fluorescence detection (Zhang, Li, Gao, Sun, & Chang, 2000) and electrochemical methods (Cabrita, Abrantes, & Viana, 2005; Raj, Okajima, & Ohsaka, 2003). However, the electrochemical methods are simple, rapid, cost-effective and efficient method for determination of DA than that of available traditional methods (Pandikumar et al., 2014).

In electrochemical DA sensors, the unmodified electrodes such as glassy carbon, graphite (GR) and screen printed carbon electrodes are not suitable for detection of DA, due to their poor selectivity, reproducibility, sensitivity and high overpotentials (Chen & Cha, 1999; Ghanbari & Hajheidari, 2015). Therefore, the carbon materials, metal oxide, metal alloy nanoparticles, redox and biopolymers modified electrodes have been widely used for the sensitive and selective detection of DA in lower overpotential (Li et al., 2015; Mao et al., 2015; Pandikumar et al., 2014; Vasantha & Chen, 2006; Wang, Li, Jia, & Xu, 2006; Yan et al., 2015). On the other hand, chitosan (CS) is known non-toxic, highly biodegradable, naturally abundant linear carbohydrate biopolymer and widely used in the construction of electrochemical sensors and biosensors. Its various potential applications include, tissue engineering, artificial skin, burn treatment, wound healing, drug delivery, (Mertins &

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Dimova, 2013, 29; Rinaudo, 2006; Vusa, Manju, Aneesh, Berchmans, & Palaniappan, 2016). Recently, the CS functionalized graphene oxide and CS grafted graphene and carbon nanotubes have been used for the sensing of DA among its various applications (Demirkol & Timur, 2011; Liu, Lian, Yin, & Sun, 2012; Niu et al., 2012; Shan et al., 2010; Wu et al., 2007). However, most of the reported DA sensors are based on CS with pristine graphene and or with carbon nanotubes, in which the composites are prepared by the direct sonication of graphene or carbon nanotubes in CS solution (Liu et al., 2012, 2014; Niu et al., 2012). More recently, we have reported DA sensor using the cyclodextrin grafted GR composite, and the resulting electrode has showed comparable performance over carbon nanomaterials modified electrodes for sensing of DA (Palanisamy et al., 2016). The motivation of the present work is to fabricate a simple, sensitive and reliable DA sensor using the CS grafted GR (GR-CS) composite modified electrode. The CS-GR composite can be easily prepared by sonication of GR and CS in acetic acid for 1 h at room temperature. Fewer reports have already reported for the preparation of CS grafted expanded GR (Jagiello, Judek, Zdrojek, Aksienionek, & Lipinska, 2014; Demitri et al., 2015). However, for the first time we report a potential application of the GR-CS composite for the electrochemical sensing of DA.

In this work, a sensitive and selective DA sensor was developed based on GR-CS composite modified electrode for the first time. The GR-CS composite modified electrode shows an enhanced sensitivity with lower oxidation peak potential for DA than that of pristine GR and CS modified electrodes. In addition, the GR-CS modified electrode shows a superior performance towards the oxidation of DA than graphene-CS composite, due to its strong intercalation of CS on exfoliated GR sheets. The selectivity and stability of the sensor were studied and discussed in detail. The practicability of the sensor also been evaluated in biological samples and discussed.

2. Experimental

2.1. Chemicals

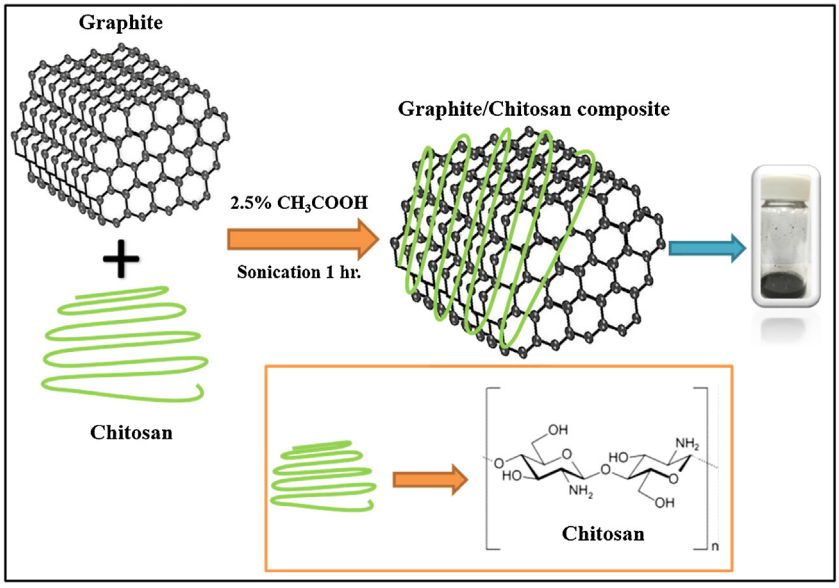
Raw graphite, dopamine and chitosan (from crab shells, minimum 85% deacetylated) were obtained from Sigma. Uric acid, ascorbic acid and acetic acid were purchased from Aldrich. Graphene nanopowder (8 nm flakes, product number UR-GNAPHENE) was purchased from UniRegion Bio-Tech, Taiwan. Human blood serum sample was collected from valley biomedical, Taiwan product & services, Inc. This study was reviewed and approved by the ethics committee of Chang-Gung memorial hospital through the contract no. IRB101-5042A3 (Palanisamy et al., 2016). Human urine sample were collected from the two healthy persons and used for real sample analysis with their permission. The supporting electrolyte 0.05 M phosphate buffer pH 7 (PBS) was prepared by using 0.05 M Na_2HPO_4 and NaH_2PO_4 solutions in doubly distilled water and the pH were adjusted using 0.1 M H_2SO_4 and NaOH. All chemicals used in this study were of analytical grade and the solutions were prepared using double distilled water without any further purification.

2.2. Apparatus

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) measurements were performed by the CHI 750a electrochemical work station. Scanning electron microscopy (SEM) was performed using Hitachi S-3000 H electron microscope. Raman spectra were recorded using a Raman spectrometer (Dong Woo 500i, Korea) equipped with a charge-coupled detector. Fourier transform infrared spectroscopy (FT-IR) was carried out using the Thermo SCIENTIFIC Nicolet iS10 instrument. Conventional three-electrode system was used for the electrochemical experiments, the glassy carbon electrode (GCE) with geometric surface area of 0.079 cm^2 was used as a working electrode, a saturated Ag/AgCl as a reference electrode and a platinum electrode as the auxiliary electrode. All electrochemical measurements were carried out at room temperature in N_2 atmosphere.

2.3. Preparation of GR–CS composite

To prepare the CS-GR composite, first 15 mg of CS was dissolved in 3 mL of 2.5% acetic acid with the aid of ultra-sonication. Then, 10 mg GR (2:3 w/w, optimum) was added to the CS solution and continuously sonicated for 1 h at room temperature. The resulting GR-CS composite was centrifuged and dried in an air oven. The GR-CS composite was re-dispersed in ethanol and used for further electrochemical experiments. The preparation of CS-GR composite is shown in Scheme 1. For controls, GR solution was prepared by dispersing 10 mg of GR in dimethylformamide and CS solution was prepared by dissolving 15 mg of CS in 2.5% acetic acid. To prepare GR-CS modified electrode, about 9 μL (optimum) of GR-CS disper-



Scheme 1. Schematic representation of preparation of GR-CS composite.