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Simultaneous and sensitive detection of acetaminophen and valacyclovir based on two dimensional graphene nanosheets

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ABSTRACT

A highly sensitive electrochemical sensor, based on two-dimensional (2D) graphene nanosheets, for the simultaneous detection of acetaminophen and valacyclovir has been developed. This sensor was fabricated through the concurrent electrochemical reduction and deposition of graphene oxide (GO) onto a glassy carbon electrode (GCE) using cyclic voltammetry (CV). Both CV and differential pulse voltammetry (DPV) were employed to study the electrocatalytic properties of the electrochemically reduced graphene oxide (rGO) modified GCE to elucidate the oxidation behaviour of acetaminophen and valacyclovir. The electrodeposition cycle and concentration of GO toward the optimal performance of the prepared sensor were also investigated. This developed sensor exhibited excellent activity for the simultaneous electrochemical oxidation of acetaminophen and valacyclovir. A very low detection limit of 1.34 nM for the exclusive detection of valacyclovir, as well as a promisingly lower detection limit of 4.65 nM for acetaminophen was achieved through the simultaneous detection of acetaminophen and valacyclovir in a mixture. The developed electrochemical sensor was also tested for reproducibility, stability and potential interference capability, which showed an excellent performance. It was further verified utilizing commercially available pharmaceutical tablets in human serum with very high recovery rates, demonstrating a great potential application for pharmaceutical quality control, as well as bioavailability testing and drug monitoring in hospital laboratories.

1. Introduction

Acetaminophen (paracetamol, *N*-acetyl-p-aminophenol) is an extensively used analgesic anti-pyretic drug. It is primarily metabolized in liver and is safe when used at prescribed dosages; despite that all non-prescribed high dosages may cause hepatotoxicity [1,2]. Valacylovir (L-valine 2-[(2-amino-1, 6-dihydro-6-oxo-9h-purin-9-yl) methoxyl]ethyl ester) is a prodrug of the antiviral drug acyclovir, which is used for the treatment of the herpes simplex viruses and the varicella zoster virus. This compound is converted rapidly and extensively to acyclovir (the active antiviral component of valacyclovir) and L-valine, most likely in the liver and the intestine, via hydrolysis, subsequent to oral administration. Acyclovir, a prototype antiviral drug, is a DNA polymerase inhibitor with variable oral bioavailability. By contrast, valacyclovir has an oral bioavailability that is three to five times higher than that of acyclovir [3,4]. Acyclovir and its prodrug valacyclovir comprise guanine analogue antiviral drugs. They are activated by the phosphorylation of virus-specific thymidine kinase. Acyclovir uptake has been shown to be enhanced in herpes virus-infected cells, with a 10 to 30 fold greater affinity for infected cells than uninfected cells [5].

Several traditional methods, for instance, spectrophotometry [6,7], LC-MS [8,3] and HPLC [4,9], have been generally utilized for the determination of valacyclovir compounds. Spectrophotometric and chromatographic methods involve time consuming analytical processes for the determination of these drugs. In recent years, electroanalytical techniques have garnered considerable attention for the detection of drugs and environmental pollutants due to their high sensitivity, excellent selectivity, low cost, rapid response, as well as expedited and straightforward operation [10,11]. A review of the literature has revealed very few publications that describe electrochemical studies on valacyclovir, and a practically nil number of publications as relates to the simultaneous detection of acetaminophen and valacyclovir compounds. Thus, there is an urgent need to explore the quantitative determination of these drugs through electrochemical techniques.

Nanomaterials with high surface areas and electrocatalytic activity have strong potential to be employed in sensor/biosensor fabrication to facilitate electrochemical measurements [12,13]. Graphene comprises a one-atom thick planar sheet of sp^2-bonded carbon atoms, which is attracting tremendous attention in terms of fundamental research and potential applications [14,15]. This nanomaterial holds

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tremendous promise for prospective applications in many technological fields, such as nanoelectronics [16,17] and sensors [18,19]. Graphene and its nanocomposite materials have been utilized for the electrochemical detection of various drug molecules. Cuprous oxide nanoparticles-graphene, graphene/poly(brilliant cresyl blue) nanocomposite, and single walled carbon nanotube/reduced graphene oxide nanohybrids have been utilized for the determination of acetaminophen, epinephrine, valacyclovir [20–22]. Graphene sheet provide an extremely sensitive and remarkable electrical conductor for adsorbed molecules to enable excellent electrocatalytic performance [23–25]. Chemical methods are an efficient approach for the bulk production of graphene-based sheets at low cost [26,27]. However, the excessive use of reducing agents in these techniques raises the risk of contamination in the resulting materials. Additionally, oxygenated species that cannot be fully removed via chemical treatment may degrade the electronic properties of the product to further limit applications [28].

Electrochemical techniques comprise an effective green method for the modification of electronic states through the adjustment of an external power source, to alter the Fermi energy level of surfaces of electrode materials. During electrochemical reduction, the aggregation of GO takes place due to increased π-π interactions between graphene layers [29]. It has been indicated through FTIR spectra that a variety of oxygen-containing functional groups are completely removed from the graphene oxide planes of electrochemically reduced graphene nanosheets. Chemically reduced graphene is not conducive to preserving its typical electronic properties due to residual defects [30].

Market Research and Global Industry Analysts, Inc. has released recent research on the global market for acetaminophen, which is to reach $999.4 million USD by 2020 with an annual 3.8% growth, whereas valacyclovir is to attain $4.8 billion USD by 2017. According to the guidelines made by the Center for Disease Control (CDC) for prevention and treatment of varicella zoster virus, analgesic (acetaminophen) drugs are usually prescribed along with valacyclovir as treatment regime to relieve from the pain. Thus, simultaneous monitoring of these two drugs is highly recommended during the suspicious acetaminophen overdose cases. Herein, we have demonstrated a promising graphene based electrochemical sensing platform for the exclusive detection of valacyclovir, as well as for the simultaneous detection of acetaminophen and valacyclovir. The aim of the present work was to design a graphene sheet modified GCE via a facile electrochemical method for the electrochemical quantification of both emerging drugs toward practical applications in pharmacology and in biological fluids.

2. Experimental

2.1. Apparatus

For all electrochemical analysis, a three-electrode system electrochemical cell comprised of platinum wire as counter electrode, 3 M KCl saturated Ag/AgCl as reference electrode, and a 3 mm glassy carbon electrode (GCE) as working electrode were utilized through CHI 660 electrochemical workstation (CH Instruments Inc., USA). Electrochemical techniques including e.g. cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were employed for the electroanalysis of the drug compounds. The as-prepared rGO/GCE surface during sensor fabrication was characterized through the field-emission scanning electron microscope (FE-SEM) (Hitachi SU-70) equipped with an energy dispersive X-ray (EDX) spectrometer (Oxford AZtec operated at 20 kV). Raman spectra were recorded with a confocal micro-Raman spectrometer system built in-house. In brief, the system consists of a Nikon eclipse E400 upright microscope coupled to a Chromex 250is spectrograph with intermediate coupling lenses and Rayleigh reject filters. The detector was a Santa Barbara Instruments (SBIG) low noise ST-10 cooled CCD camera cooled to −10 °C. An Ar + ion laser with 514.5 nm line was used for excitation and focused to ~2 μm diameter spot at a power of 5 mW. Catalina Scientific Kestrelspec software was used to control the spectrometer and collect the spectra.

2.2. Chemicals and reagents

All the chemicals including valacyclovir, acetaminophen (AP), and 2 mg mL^{-1} graphene oxide (GO) dispersed in water and human serum (from human male AB plasma) were purchased through Sigma-Aldrich. Generic tablets valacyclovir (500 mg) and acetaminophen (325 mg) were purchased from local pharmacy in Thunder Bay. All chemicals were used as received without further purification. Pure water was supplied through Nanopure® water purification system for all solutions preparation. All working solutions were freshly prepared to overcome possible drug hydrolysis problem.

2.3. Sensor fabrication

For the sensor fabrication, GCE was polished using alumina slurry (0.05 μm) followed by sonication in pure water. 0.3 mg mL^{-1} of GO suspension was prepared in 0.1 M (pH 9.0) phosphate buffer solution (PBS) followed by 30 min of sonication. The prepared GO suspension was de-aerated with Ar gas to completely remove oxygen from the solution. Cyclic voltammetric reduction was performed in the GO suspension (0.3 mg mL^{-1}) with applied potential between 0.5 and −1.5 V, using a three-electrode system, with a bare GCE serving as the working electrode [31]. Five reduction cycles were employed in this study to deposit electrochemically reduced graphene oxide (rGO) onto the GCE surface. The sensor was ready to use for electrochemical measurements after rinsing with pure water followed by air dry. DPV was employed for the detection of acetaminophen and valacyclovir, where quite time was 60 s, pulse width was 0.2 s and pulse period was 0.5 s.

2.4. Determination of pharmaceutical tablets in biological fluids (human plasma)

The fabricated sensor was utilized for the detection of generic tablets (acetaminophen and valacyclovir) spiked in human serum. For the working solution, both tablets (acetaminophen and valacyclovir) were crushed and spiked in human serum to obtain a final concentration of 0.01 M as a stock solution. The spiked plasma was then treated with acetonitrile for protein precipitation and sonicated for 6 min. This spiked human serum solution was then centrifuged at 12,200*g* for 15 min in order to remove any residual proteins [32,33]. The supernatant was employed as a sample for the determination of acetaminophen and valacyclovir concentrations through DPV.

3. Result and discussion

3.1. Characterization of electrochemically reduced graphene oxide (rGO) on the GCE

Fig. 1A presents the FE-SEM image of the rGO/GCE surface prepared with 0.3 mg mL^{-1} GO dispersed in 0.1 M PBS (pH 9) by continuous five-cycle electrodeposition scanned in the potential range between 0.5 and −1.5 V. Energy dispersive X-ray spectroscopy (EDS) was employed to study the degree of oxygen removal from GO, subsequent to electrochemical reduction. As shown in Fig. 1B, a dramatic change in the carbon:oxygen (C:O) ratio was found in exfoliated GO and rGO. Through EDS, it was observed that a C:O ratio of 1:3 existed in exfoliated GO on the GCE surface, whereas a 6:1 ratio of total C:O was revealed in the rGO on the GCE surface, which indicated the successful removal of a large quantity of oxygen from the graphene oxide during electrochemical reduction. Aside from the primary carbon and oxygen peaks, additional small peaks were also observed. The prominent Na and P peaks found in the EDS measurement might have been obtained through PBS during sample preparation.