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Sensitive Detection of Acetaminophen with Graphene-Based Electrochemical Sensor

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ABSTRACT

Here we report on a high-performance electrochemical sensor for the sensitive detection of acetaminophen based on graphene, which was simultaneously electrochemically reduced and deposited onto a glassy carbon electrode (GCE). The electrocatalytic properties of the electrochemically reduced graphene (ERG) toward the oxidation of acetaminophen were analyzed via cyclic voltammetry (CV), differential pulse voltammetry (DPV) and chronoamperometry. For comparison, various ERG/GCEs were prepared with different electrodeposition cycles to optimize the amount of the ERG. Our experimental results showed that the optimized ERG/GCE possessed robust activity in the electrochemical oxidation of acetaminophen, leading to the development of highly sensitive electrochemical sensor for its detection. An extremely low detection limit of 2.13 nM and a wide linear detection range of from 5.0 nM to 800 μM were achieved via the combination of the amperometric technique and DPV. The developed electrochemical sensor was further employed for the determination of acetaminophen in human serum, with excellent recovery, ranging from 96.08% to103.2%. The fabricated electrochemical sensor also demonstrated high selectivity, stability and reproducibility. The wide linear detection range obtained in this study for the detection of acetaminophen showed strong potential as a promising sensing technique for pharmaceuticals, in terms of quality control and in clinical laboratories for acetaminophen as relates to the determination of hepatotoxicity.

1. Introduction

Acetaminophen, having the chemical name N-acetyl-p-aminophenol (APAP), is a widely utilized analgesic pain reliever and fever reducer [1,2]. It is considered to be safe when administered at recommended dosages; however, it can cause hepatotoxicity at higher doses [3]. Following oral ingestion, acetaminophen is rapidly absorbed and metabolized, primarily in the liver, to achieve peak plasma levels within 1 h. Plasma concentrations during therapy typically range from 2 to 20 mg/L, whereas levels of 30–300 mg/L are often observed in overdosed patients [4,5]. The principle routes of elimination are glucuronidation and sulfation, although oxidation may also occur [6].

Glucuronidation is the primary route of elimination in human adults, accounting for about 45–55% of an acetaminophen dose [6,7]. This step may be catalyzed by a series of uridine diphosphate glucuronyl transferase (UGT) enzymes such as UGT1A1, UGT1A6, UGT1A9, and UGT2B15 in the liver [7–10]. Approximately 30–35% of acetaminophen metabolism occurs via sulfation. In the adult human liver this is catalyzed by SULT1A1, SULT1A3, SULT1A4, SULT1E1 and SULT2A1 [11–13]. The bioactivation of acetaminophen in the formation of N-acetyl-p-benzoquinone imine (NAPQI) is carried out via the CYP450 family of enzymes [6], which are toxic metabolites that are responsible for hepatotoxicity. The toxic metabolite, NAPQI, is initially detoxified by glutathione conjugation within the liver, and subsequently by acetylation in the kidneys, followed by N-acetyl transferase catalyzation, and final excretion in the urine [8]. In cases of overdose the accumulation of NAPQI is high, which causes adverse side effects.

The development of a simple, rapid, sensitive and accurate analytical technique for the determination of acetaminophen in pharmaceuticals and in clinical preparations is indeed warranted. Various methods, such as titrametry [14,15], spectrophotometry [16,17], HPLC [18,19], chemililuminescence [20], have been developed for the determination of acetaminophen in pharmaceutical tablets and biological fluids. However, titrametric, spectrophotometric and chemiluminescence methods involve tedious extraction processes prior to detection. Additionally, liquid chromatography is time consuming, which makes it unsuitable for the analysis of acetaminophen in practice [21]. On the other hand,

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electrochemistry provides powerful analytical techniques with advantages of instrumental simplicity, moderate cost and portability [22]. Since most electroanalytic techniques are selective and capable of highly sensitive and rapid measurements over a wide linear range, which require no sample preparation, and given the fact that acetaminophen is electroactive; electrochemical techniques may be considered as viable and improved alternatives for the determination of acetaminophen over other methods [23].

Since nanomaterials exhibit unique mechanical, electrical, electronic, optical, magnetic, surface and biological properties, which are not found in conventional bulk materials, they have a great potential utility in analytical chemistry for sensor modification [24,25]. Graphene has recently attracted tremendous interest due to its exceptional thermal, mechanical, and electronic properties [26], thus one of its many promising applications lies in the development of electrochemical sensors [27,28]. Single carbon atom thick graphene sheets provide extremely high surface areas with readily available access to surface resident atom populations for electron transport, which impart a high sensitivity to adsorbed molecules [29]. Due to the unique properties of graphene, when it is utilized for the modification of bare electrodes, it has great potential for distinguishing a diverse range of organic compounds. To date, the deposition of graphene films on electrodes has typically been achieved via drop-casting solution-based graphene, which is derived from the chemical reduction of graphene oxide (GO) sheets [30]. However, these methodologies have intrinsic limitations such as a lack of control over film thickness and most importantly, toxic chemicals are involved. Most recently, the electrochemical reduction of GO to graphene has garnered considerable attention due to its rapid and green nature [31–35]. The excellent conductivity, high surface area, and oxygen-related defects of ERG films make them a sensitive promoter of electrochemical sensing processes [36–38].

The objective of this work was to establish a convenient, cost-effective and highly sensitive method for the determination of acetaminophen in pharmaceutical formulations and human bodily fluids based on the ERG/GCE. In the present study, the electrochemical oxidation of acetaminophen on electrochemically reduced graphene (ERG)-modified glassy carbon electrodes (GCEs) was investigated, leading to the development of a high-performance electrochemical sensor for the analysis of acetaminophen, with an extremely low detection limit and a wide linear detection range. In addition, the electrochemical sensor developed in this study was successfully employed for the detection of acetaminophen in human serum and pharmaceutical samples, demonstrating that the proposed electrochemical sensor has strong potential for practical utility in clinical and quality control laboratories, as well for therapeutic drug monitoring and hepatotoxic serum level determination in hospital laboratories.

2. Experimental

2.1. Apparatus

All electrochemical experiments, including cyclic voltammetry (CV), differential pulse voltammetry (DPV) and amperometry were performed with a CHI 660 electrochemical workstation (CH Instruments Inc. USA) using a conventional three-electrode system that consisted of a platinum coil counter electrode, a Ag/AgCl (3 M KCl) reference electrode, and a working electrode, which was comprised of 3 mm in diameter (modified and unmodified) glassy carbon electrodes (GCEs). A field-emission scanning electron microscope (FE-SEM) (Hitachi SU-70) was utilized for the characterization of the graphene-modified GCE surface. All experiments were performed at room temperature, $20 \pm 2\,^{\circ}C$, and the electrode potentials quoted are versus an Ag/AgCl electrode.

2.2. Chemicals and reagents

Acetaminophen (AP), graphene oxide (GO) dispersed in water (2 mg/mL), and human serum (from human male AB Plasma) were purchased from Sigma-Aldrich. Generic tablets (350 mg each, containing 325 mg acetaminophen) were obtained from the Thunder Bay Regional Health Sciences Center pharmacy. All other reagents were of analytical grade and utilized as supplied. All solutions were prepared with pure water (18.2 MΩ cm), which was generated by a Nanopure® water purification system. All acetaminophen solutions were freshly prepared and used within 24 h.

2.3. Electrode fabrication

Prior to modification, a glassy carbon electrode (GCE) was polished with 0.05 μm alumina powders, subsequently sonicated in pure water, and allowed to dry at room temperature. To produce the ERG, a 2 mg/mL GO solution was added to a 0.067 M pH 7.4 phosphate buffer solution (PBS) via homogenous mixing, to form a 0.3 mg/mL GO colloidal dispersion. The GO suspension in the electrochemical cell was deoxygenated using Ar gas for 15 min. Simultaneous electrochemical reduction and deposition of graphene on the GCE were performed in the GO suspension (0.3 mg/mL) in the electrode potential range between -1.5 and 0.5 V at a sweep rate of 10 mV/s. The resulting ERG/GCE was cleaned with pure water, and then dried at room temperature for 1 h.

2.4. Electrochemical measurements

Electrochemically reduced graphene modified glassy carbon electrodes were used as working electrodes in a three-electrode electrochemical cell. A stock solution of 0.01 M acetaminophen was prepared, where after a calculated amount of stock solution was added to 20 mL of 0.1 M phosphate buffer solution (PBS) at pH 7.4 to obtain the desired concentration of acetaminophen. The experiments were carried out by studying the cyclic voltammetric behavior of the acetaminophen at a potential range of from 0.0 to 0.6 V. The DPV was performed at potential range of from 0.0 V to 0.6 V, with a pulse width of 0.2 s, pulse period of 0.5 s and potential increment of 4 mV. All amperometric measurements were acquired at 0.5 V with continuous magnetic stirring in order to maintain a homogeneous concentration.

2.5. Determination of pharmaceutical samples in human serum

The developed sensor was tested for the determination of acetaminophen using the generic acetaminophen tablets in human serum. Prior to conducting the experiment, the human serum sample had been stored in a freezer. The tablets were weighed, ground into a powder, and then dissolved in 5 mL of human serum samples to obtain a 0.01 M stock solution concentration. The solution was further treated with acetonitrile for protein precipitation and then sonicated for 5 min. The acetaminophen/human serum solution was then centrifuged at 4000 rpm for 15 min in order to remove any protein residues. The supernatant was subsequently collected and diluted to obtain 10–25 μM concentrations of acetaminophen in 0.1 M PBS. The recovery tests were carried out using DPV for the determination of acetaminophen in human serum.

3. Result and Discussion

3.1. Surface and electrochemical characterization of the ERG/GCE

Fig. 1A presents the 1st (blue), 3rd (green) and 5th (red) cycle of the cyclic voltammograms (CVs) during the simultaneous