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Novel paper-based cholesterol biosensor using graphene/polyvinylpyrrolidone/polyaniline nanocomposite

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ABSTRACT

A novel nanocomposite of graphene (G), polyvinylpyrrolidone (PVP) and polyaniline (PANI) has been successfully prepared and used for the modification of paper-based biosensors via electrospraying. The droplet-like nanostructures of G/PVP/PANI-modified electrodes are obtained with an average size of 160 ± 1.02 nm. Interestingly, the presence of small amount of PVP (2 mg mL^{-1}) in the nanocomposites can substantially improve the dispersibility of G and increase the electrochemical conductivity of electrodes, leading to enhanced sensitivity of the biosensor. The well-defined cyclic voltammogram of standard ferri/ferrocyanide is achieved on a G/PVP/PANI-modified electrode with a 3-fold increase in the current signal compared to an unmodified electrode. This modified electrode also exhibits excellent electrocatalytic activity towards the oxidation of hydrogen peroxide (H_2O_2). Furthermore, cholesterol oxidase (ChOx) is attached to G/PVP/PANI-modified electrode for the amperometric determination of cholesterol. Under optimum conditions, a linear range of 50 μM to 10 mM is achieved and the limit of detection is found to be 1 μM for cholesterol. Finally, the proposed system can be applied for the determination of cholesterol in a complex biological fluid (*i.e.* human serum).

1. Introduction

The development of accurate, sensitive and low-cost biosensor is crucial for early stage screening of disease biomarkers. Recently, cellulose filter paper has become an attractive material for sensor applications due to its large surface area and low cost (Apilux et al., 2010; Dungchai et al., 2011; Songjaroen et al., 2011). Compared to other traditional substrates (*i.e.,* glass, ceramic and polymer), paper-based biosensors offers several advantages, such as low cost, high abundance, biocompatibility and disposability. Furthermore paper-based analysis only requires a small amount of samples and reagents, which make it suitable for biosensor applications (Dungchai et al., 2009). Among the detection techniques, electrochemical detection has attracted much attention due to its ease of use, portable field-based size, high specificity and rapid analysis. Moreover, both qualitative and quantitative information can be obtained simultaneously. Paper-based electrochemical biosensors have been applied to various applications, including clinical diagnosis (Dungchai et al., 2009, 2011), environmental monitoring (Apilux et al., 2010; Nie et al., 2010), and food quality control (Hossain et al., 2009). Paper-based biosensors can be fabricated by several methods, such as photolithography (Apilux et al., 2010; Dungchai et al., 2009), wax screen-printing (Dungchai et al., 2011), wax-dipping (Songjaroen et al., 2011), and wax-printing (Lu et al., 2010; Mentele et al., 2012). In this study, wax-printing is selected to create the disposable paper-based biosensors. Nowadays, an important limitation of paper-based electrochemical biosensors for the detection of low abundant biomarkers is the limited sensitivity; therefore, modification of paper based biosensor with the ultrahigh surface area material, such as metallic nanoparticles and carbon based nanostructures, is still greatly required to improve the sensor sensitivity.

In recent years, graphene (G) has received tremendous attention due to its remarkable physical, chemical, mechanical, and electrical properties. G is a single layer of carbon atoms closely

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packed into a two-dimensional honeycomb arrangement. For fabrication of G-based electronic material, it can provide excellent electrical conductivity and electron mobility. In addition, the small band gap of G is desirable for conducting electrons from target molecules in electrochemical biosensors. The ultrahigh surface area of G is also very useful for loading of bioreceptors (*i.e.,* enzyme, antibody) on its surface. It has been reported that G-based chemical sensors possess very high sensitivity because of the low electronic noise from thermal effect (Geim and Novoselov, 2007; Liu et al., 2012b; Potts et al., 2011). Compared to other carbon allotropes (*e.g.* carbon nanotubes: CNTs), G can be obtained easily by using a chemical conversion of inexpensive graphite (Geim and Novoselov, 2007).

Due to the planar sp^2-carbon of G, it has high tendency to agglomerate together via van der Waals attraction. In terms of applications, preparation of a well-dispersed G solution has become a crucial step. Recently, it has been reported that poly (vinylpyrrolidone) (PVP) can be used to stabilize the high concentration of G in a wide range of organic solvents (Wajid et al., 2012). G/PVP has been used to modify the electrodes for biosensor applications (Liu et al., 2012b; Mano and Heller, 2005). For electrode modification, the nanocomposites between G and conducting polymers have attracted more attention than the pure form of G because the composites are more compatible for electrode fabrication and biofunctionalization (Arya et al., 2011; Huang et al., 2011; Liu et al., 2012a; Qiu et al., 2012). Moreover, using conducting polymers as a matrix for G dispersion can further enhance the sensitivity of electrochemical biosensors. Various conducting polymers, including polyaniline (PANI) (Arya et al., 2011; Huang et al., 2011; Liu et al., 2012b; Qiu et al., 2012), polypyrrole (PPy) (Li et al., 2012; Lu et al., 2012), and poly(3,4-ethylenedioxythiophene) (PEDOT) (Jiang et al., 2013; Karuwan et al., 2012) have been used in biosensors. Among the conducting polymers, PANI is a promising material due to its excellent electrochemical properties, ease of synthesis and functionalization, high environmental stability, and low toxicity. Previously, it has been reported that the conducting form of PANI can be simply prepared by doping PANI with an acid, such as camphorsulfonic acid (CSA) in chloroform (Shin and Kameoka, 2012). Additionally, a number of amino groups ($-NH_2$) of PANI can be readily functionalized with biomolecules (Arya et al., 2011; Qiu et al., 2012), which make it very attractive for biosensor applications. Thus, the development of G/PVP/PANI nanocomposite modified paper-based biosensor is focused in this study.

To fabricate G/PVP/PANI nanocomposites on the paper-based biosensor, electrospraying is selected because a 3D droplet-like nanostructure can be created on the modified electrode surface. Compared to thin-film modified electrodes, G/PVP/PANI-nanodroplet-modified electrodes offer a higher specific surface area, which leads to an enhanced electrochemical sensitivity of the biosensors. Increasing the electrode surface area through this method might be very useful for further biofunctionalization, such as enzyme loading.

Biomarkers are biomolecules that can indicate a normal or pathogenic process in a biological system, including the level of exposure to environmental factors, genetic susceptibility, and an indication of response to therapy. One of the most important biomarkers for cardiovascular disease and high blood pressure is cholesterol; therefore, development of a method that can quantitatively determine the cholesterol level is very crucial. The conventional method based on spectrophotometry has been widely used for cholesterol determination (Arya et al., 2007; Dhand et al., 2007); however, this technique requires an expensive instrument and complicated sample preparation. With the advent of nanomaterials, metal nanoparticles and carbon-based nanomaterials have been applied for electrochemical biosensors to improve the electrochemical performance of cholesterol biosensors (Dey and Raj, 2010; Dhand et al., 2007; Eguilaz et al., 2011; Manjunatha et al., 2012).

Herein, a novel nanocomposite of G/PVP/PANI was prepared and used to modify the working electrode of a paper-based biosensor via electrospraying. The droplet-like structures of the G/PVP/PANI-nanocomposite-modified electrode was used for the sensitive determination of H_2O_2 and cholesterol using amperometry. The performance of this sensing system was optimized and then applied to the determination of cholesterol concentration in a complex biological fluid (*e.g.,* human serum).

2. Material and methods

2.1. Chemicals and materials

Graphene (G) nanopowders were purchased from SkySpring Nanomaterials, Inc. (Houston, TX). Cholesterol and 418 U mg^{-1} cholesterol oxidase (ChOx) from *Streptomyces* sp., sodium dodecyl sulfate (SDS), polyoxyethylene octyl phenyl ether (Triton X-100), camphor-10-sulfonic acid (CSA), polyaniline (PANI), polystyrene (Mw ~ 180,000; PS), poly (vinyl pyrrolidone) (Mw = 10,000; PVP) and trichloroacetic acid (TCA) were obtained from Sigma (St. Louis, MO). Dimethylformamide (DMF), Potassium dihydrogen phosphate (KH_2PO_4) and chloroform were purchased from Carlo Erba Reagenti-SDS (Val de Reuil, France). Disodium hydrogen phosphate (Na_2HPO_4), Potassium chloride (KCl), and Sodium chloride (NaCl) were purchased from Merck (Darmstadi, Germany). Carbon ink and silver/silver chloride ink were obtained from Gwent group (Torfaen, United Kingdom). Filter paper grade no. 1 (size, 46 × 57 cm^2) was purchased from Whatman. All chemicals were used as received without further purification. All solutions were prepared by using high-purity water from MilliQ Water System (Millipore, USA, $R \geq 18.2$ MΩ cm^{-1}). Phosphate buffered saline (PBS) was prepared by dissolving 0.144% (w/v) Na_2HPO_3, 0.024% (w/v) KH_2PO_4, 0.02% (w/v) KCl, 0.8% (w/v) NaCl in high-purity water. A stock solution of cholesterol was prepared in 5% (w/v) of Triton X-100 and high-purity water and then stored at 4 °C. A stock solution of ChOx was freshly prepared in PBS (Ruecha et al., 2011). For the determination of the cholesterol in a real biological sample, lyophilized human serum (CONSERA), obtained from Nissui Pharmaceutical, was used (Tokyo, Japan). The serum samples were precipitated using TCA prior to use.

2.2. Apparatus

All electrochemical measurements, including cyclic voltammetry and amperometry, were performed on a CHI 1232A electrochemical analyzer (CH Instruments, Inc., USA). A three electrode system was used and the working electrode was a G/PVP/PANI-modified, screen-printed carbon electrode (4 mm in diameter). An in-house electrospraying system was used for the electrode modification. A JSM-6400 field emission scanning electron microscope (Japan Electron Optics Laboratory Co., Ltd, Japan) with an accelerating voltage of 15 kV and a JEM-2100 transmission electron microscope (Japan Electron Optics Laboratory Co., Ltd, Japan) were used for the electrode characterization.

2.3. Fabrication of paper-based biosensor

In this work, paper-based biosensor was fabricated using wax-printing method according to a previous report (Mentele et al., 2012) with slight modification. First, the patterned paper-based biosensor was designed by Adobe Illustrator and then printed onto filter paper (Whatman no. 1) using a wax printer (Xerox Color Qube 8570, Japan). Next, the printed paper-based biosensor was