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Short communication

Graphene oxide functionalized with silver@silica–polyethylene glycol hybrid nanoparticles for direct electrochemical detection of quercetin

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ABSTRACT

A direct electrochemical detection of quercetin based on functionalized graphene oxide modified on gold-printed circuit board chip was demonstrated in this study. Functionalized graphene oxide materials are prepared by the covalent reaction of graphene oxide with silver@silica–polyethylene glycol nanoparticles (~12.35 nm). Functionalized graphene oxide electrode shows a well-defined voltammetric response in phosphate buffered saline and catalyzes the oxidation of quercetin to quinone without the need of an enzyme. Significantly, the functionalized graphene oxide modified electrode exhibited a higher sensitivity than pristine gold-printed circuit board and graphene oxide electrodes, a wide concentration range of 7.5 to 1040 nM and detection limit of 3.57 nM. Developed biosensor platform is selective toward quercetin in the presence of an interferent molecule.

1. Introduction

Quercetin is one of the abundant flavonoid molecules with a wide range of biological roles such as anti-oxidant (Zhou et al., 2001), anti-inflammatory, anti-allergic (Guardia et al., 2001; Rogerio et al., 2010), anti-cancer (Murakami et al., 2008) and anti-bacterial activity (Bravo and Anacona, 2001). Due to its polyhydroxy functional groups in the aromatic rings, quercetin (3,3′,4′,5,7-pentahydroxyflavone) is demonstrated to form a complex reaction with metal ions, influencing the transportation, reactivity, bioavailability and toxicity of metal ions (Dolatabadi, 2011). Transition metal complexes with quercetin are studied to have improved sequence-selective DNA binding and photochemical properties (Tan et al., 2009; Dolatabadi, 2011). Therefore, determination of quercetin with high sensitivity is of vital importance from therapeutics point-of-view. Recently, quercetin has been determined by spectrophotometry (Nikolovska-Coleska et al., 1996), high performance liquid chromatography (Wang et al., 2005), capillary electrophoresis (Prasongsidh and Skurray, 1998) and spectrofluorimetry methods (Liu et al., 2012). Later, electrochemical quercetin biosensors especially supported by nanomaterials were studied to have predominant advantages than conventional methods such as relatively rapid analysis time, simplicity in operation and low cost for fabrication of devices. For instance, multi-walled carbon nanotube (MWCNT) electrode (He et al., 2005), carbon nanotubes and nafion (Xu and Kim, 2006), monosuccinyl beta-cyclodextrin doped MWCNT (Jin et al., 2009) and Ag nanoparticles in ionic liquid and laccase immobilized on β-cyclodextrin modified with epichlorohydrin were developed for the determination of quercetin (Franzoi et al., 2010). Recently, successful determination of quercetin was demonstrated by using p-aminothiophenol functionalized graphene oxide/gold nanoparticles (Yola et al., 2013) and molecularly imprinted polymer with graphene oxide (Sun et al., 2013).

Modification of conventional electrodes with different nanostructures such as zero dimensional nanoparticles, one-dimensional nanotubes and two-dimensional graphene-materials has attracted a great deal of interest due to their heterogeneous composition and high surface-to-volume ratio with interesting electrochemical properties (Amal Raj et al., 2011; Lavanya et al., 2012; Palanisamy et al., 2013). Stability of hybrid nanomaterials modified at the electrode surface and binding affinity of analyte biomolecules on the electrode-material interface is highly depends on the physicochemical composition of hybrid nanomaterials. Recently, our group has constructed a new class of, three-dimensional nanostructure, functionalized graphene oxide (FGO) containing metalloid polymer hybrid (MPH) nanoparticles, which possess significant electrochemical properties (Veerapandian et al., 2012a). It has been explained that the FGO utilized for biosensing platform i.e., MPHs chemically conjugated (via the amidation process) on graphene oxide (GO), which is distinct from conventional composite film that are physically mixed or sequentially dropped on the electrode surface.

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The strong affinity between chemically bonded hybrid particles and GO is expected to induce enhanced electron transfer kinetics and long term stability at the electrode interface. Furthermore, the MPHs are composed of three different chemical compositions, such as metal (silver, Ag), non-metal (silica, SiO_2) and polymer (polyethylene glycol, PEG), together as single nanocomposite particles.

In this communication, we demonstrate the FGO modified gold-printed circuit board (Au-PCB) electrode as a new class of biosensor platform for quercetin determination with a wide range of detection and high sensitivity. From this present study, our results showed that FGO modified electrode is significantly responsive toward the electrocatalysis of quercetin and required no additional supporting matrix on the electrode surface-interface. This approach is attractive not only for new system but also for convenient in fabrication and operation of quercetin biosensing platform.

2. Experimental

2.1. Chemicals

Silver nitrate ($AgNO_3$), tetraethoxysilane (TEOS) ($Si(OC_2H_5)_4$), sodium borohydride ($NaBH_4$), ammonium hydroxide (NH_4OH), poly(ethylene glycol) (PEG) (average M_n=10,000), 3-aminopropyltriethoxysilane (3-APTES), expandable graphite powder and quercetin were purchased from Sigma-Aldrich. Sulfuric acid (H_2SO_4), potassium permanganate ($KMnO_4$), hydrogen peroxide (H_2O_2), hydrochloric acid (HCl) and anhydrous ethanol (C_2H_5OH) were obtained from Daejung Chemicals and Metal Ltd., Republic of Korea. The 2 μM quercetin stock solution was prepared in ethanol and utilized for preparation of further solutions. Phosphaste-buffered saline (PBS, Sigma-Aldrich) was used as the common electrolyte solution for all electrochemical measurements, prepared by dissolving 1 tablet of PBS (Sigma-Aldrich) in 200 mL of deionized water, which gives a final concentration of 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4 at 25 °C. All the chemical reagents were of analytical grade and used as received without further purification.

2.2. Synthesis of GO nanosheets

Aqueous colloidal dispersions of GO nanosheets were synthesized by vigorous oxidation of graphite powder following the modified Hummers method (Hirata et al., 2004).

2.3. Synthesis of MPHs

MPHs composed of Ag@SiO_2–PEG with an average particle size distribution of ~12.35 nm were synthesized following an ultrasonochemistry (Veerapandian and Yun, 2010a). Briefly, an aqueous solution of a metal precursor $AgNO_3$ (30 mM) was added to a reaction vessel containing PEG (stabilizing agent) solution and 30 mM of $NaBH_4$ (reducing agent). The reagent mixture was kept under probe ultrasonicator for a period of 15 min, with an optimized conditions such as amplitude (35%), probe temperature (65 °C) and pulse on–off cycle (5–10 s), in order to form the Ag core. Following this, the desired 30 mM TEOS and NH_4OH were simultaneously added. The reaction vessel was again underwent ultrasonication for a period of 30 min to ensure complete reduction and formation of the hybrid structure. The resulting colloidal dispersions containing hybrid nanoparticles were separated by centrifugation. As-separated nanoparticles were washed twice with ethanol and deionized water and were utilized for further experimentation.

2.4. Synthesis of FGO nanosheets

MPHs were first silanized and then covalently reacted with oxygenated functional groups of GO nanosheets, following a procedure detailed elsewhere (Veerapandian et al., 2012a). Briefly, an aqueous dispersion of MPHs (5 mg mL^{-1}) and 3-APTES (30 μL mL^{-1} in C_2H_5OH) was added to a vial containing 3 mL of anhydrous C_2H_5OH. This was kept under magnetic stirring at 800 rpm in room temperature for a period of 10 h. Subsequent to this, an aqueous solution of GO (2.5 mg mL^{-1}) was added and kept under magnetic stirring at 800 rpm for another 10 h to facilitate a covalent reaction between silanized MPHs and GO. After this reaction period, the GO functionalized with MPHs were separated by centrifugation, washed thrice with ethanol and used for construction of the Au-PCB modification.

2.5. Construction of Au-PCB-GO or Au-PCB-FGO electrode for quercetin detection

Prior to surface modification, Au-PCB electrode substrate was sequentially washed with acetone, ethanol and deionized water. Following this, the Au-PCB electrode was exposed to oxygen plasma treatment for ~2 min. Then, typically, 5 μL of aqueous GO or FGO suspension (2 mg mL^{-1}) was drop casted and allowed to evaporate at ambient temperature for 1 h. As-fabricated Au-PCB electrodes were then utilized for further electrochemical measurements.

A custom-designed Au-PCB substrate with an area of ~2 mm in diameter (circle shaped) was used as the working electrode and the two crescent-shaped Au substrates with a length of 4.3 mm and a breadth of 0.8 mm were used as counter and reference electrodes, respectively. Digital photograph for an Au-PCB electrode was shown in Supplementary information (Fig. S3).

2.6. Instrumentation

UV–visible absorbance spectra were measured from a Varian Cary 50 UV–vis spectrometer. Raman spectra were recorded with a LabRam HR800 micro-Raman spectroscope (Horiba Jobin-Yvon, France) using 100× objective lens at room temperature, with a 532 nm Nd:YAG laser beam and 1800 lines per mm grating. High resolution transmission electron microscope (HR-TEM) images of GO and FGO were obtained from Cs-corrector equiped HR-TEM (FEI Titan 80-300) operated at 300 kV. Cyclic voltammograms (CVs) and linear sweep voltammograms (LSVs) were measured using VersaSTAT 3 (Princeton Applied Research) in a three electrode configuration.

3. Results and discussion

3.1. Characterization of functionalized graphene oxide nanostructures

Fundamental surface topography and optical properties of GO and FGO nanosheets were characterized by HR-TEM micrographs and UV–vis absorption spectrum. As observed from Fig. 1(a) FGO nanosheet shows significant surface modification with well-distributed hybrid nanoparticles. Especially a higher number of nanoparticles functionalized on the edge plane, indicating that the existence of carboxyl groups (at the edge plane) has predominantly involved in the covalent reaction (amidation process) with silanized-MPHs. On the other hand, the pristine GO nanosheet (Fig. 1(a), inset) reveals the transparent sheet morphology with wrinkles and corrugated texture. The HR-TEM image of MPHs and its respective size distribution histogram graph were shown in