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Electrochemical sensor for selective detection of norepinephrine using graphene sheets-gold nanoparticle complex modified electrode

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Abstract−Neural diseases, like Alzheimer's (AD) and Parkinson's (PD) are widely expanding portions of neurodegenerative diseases, are related to norepinephrine (NE) concentration with proportional correlation. However, quantification of NE is quite difficult because NE coexists with ascorbic acid (AA) and uric acid (UA), which interferes with detecting NE in biological fluid. We fabricated a multi-modified electrode with reduced graphene oxide sheets (GS) and gold nanoparticles (GNPs) for highly selective and sensitive detection of NE. Thus, GS-GNPs modified electrode could enhance the sensitivity for detection of NE, as well as highly sensitive manner with AA. Compared with recent studies, our newly developed sensor appears to have not only a wide detection range (0.2-10 μM), but also superior detection limit (200 nM) in presence of 2000 times higher concentration of AA.

Keywords: Reduced Graphene Oxide Sheets (GS), Gold Nanoparticles (GNPs), Norepinephrine, Electrochemical Sensor

INTRODUCTION

Diverse neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's (PD) have been widely expanding worldwide, ultimately potentially damaging the overall quality of life. According to previous studies, neural disease is the third leading cause of death in South Korea. This means neurodegenerative disorders inflict a great social cost, along with a great loss [1]. In addition, neural cells hardly regenerate when once damaged or degenerated. Therefore, there has been a growing need to develop biosensors for the early detection and prevention of neurodegenerative diseases as well as for efficient therapeutic methods. Several researchers have been studying patient cerebrospinal fluid or blood plasma to measure neural disease biomarker in order to diagnose disease progress for early diagnosis [2,3].

Detection of norepinephrine, which is a monoamine neurotransmitter and marker of PD, has been developed with various chemical sensors such as capillary electrophoresis [4] and high performance liquid chromatography [5]. However, these analytical methods have limitations, such as long required time and expensiveness of equipment. Electrochemistry is one alternative as a detection technique in neural biomarker analysis [6]. Electrochemical sensors have merits in analysis techniques, as it is possible to detect in a rapid, simple and economical manner in real-time. Also, general sensitivity of electrochemical detection is relatively low, around micromolar concentration, compared to other methods. Therefore, NE has enough possibility of being electrochemical analyte.

In electrochemical detection, various materials have been used by their inherent detection voltage range, analyte selectivity, and detection sensitivity. Even though electrochemical detection sensitivity is relatively high, some biomarkers are present in body fluids at very low concentration. Accordingly, researchers have been modifying the electrode surface to enhance its sensitivity. Using gold nanoparticles (GNPs) is one of the most commonly used surface modification method because of its inertness, great conductivity, spacious surface area, and biocompatibility [7,8]. Nano-size GNPs form rough 3-dimension porous structure on flat electrode surface. This structural difference could be induced to improve sensor sensitivity [9].

Another widely-used electrode surface modification material is graphene with high surface area, qualified electrical conductivity, and exceptional mechanical properties [10-13]. Particularly, reduced graphene oxide sheets (GS) have same electrical properties and better solubility than graphene. Reduced graphene oxide structure is a densely packed honeycomb lattice; 2-dimensional single layer is one atom thick of sp2-bonded carbon atoms [14]. It has high surface area, great electrocatalytic activity, and extraordinary electron charge mobility. Noticeable property of reduced graphene oxide (rGO) is adsorption with benzene ring. Because the honeycomb structure of rGO between benzene ring makes a strong π-π interaction [15], benzoic chemicals have great selectivity with GS modified electrochemical sensor.

This study purposes fabrication of an electrochemical sensor to detect the NE for early diagnosis of neurodegenerative disease. Common level of NE is extremely low (around 2 nM) in biological solution compared to ascorbic acid (AA) level (100 to 400 μM) [16]. Moreover, AA has similar electrochemical oxidation potential compared to NE. Thus, AA can interfere in the detection of NE in electrochemical analysis at bare electrode due to the AA oxida-

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tion peak greatly overlapping NE oxidation peak [17]. We modified electrode with rGO sheets (GS) to separate overlapped AA and NE peak owing to π-π interaction between graphene and benzene ring of NE. To increase the sensor detection limit, we synthesized various sizes of gold nanoparticles on GS modified electrode surface to get high surface area and geometric effects. The result shows GS-GNPs modified electrode has great sensitivity and selectivity due to increased electrocatalytic property.

EXPERIMENTAL METHOD

1. Materials

Gold chloride trihydrate ($HAuCl_4$), Norepinephrine ($C_4H_{11}NO_2$), L-Ascorbic acid ($C_6H_8O_6$), Phosphate buffered saline (PBS, pH 7, 10 mM), Polyvinlypyrrolidone K-90 (PVP, MW=360,000), Sodium dodecyl sulfate (SDS, $C_{12}H_{25}NaO_4S$) were purchased from Sigma-Aldrich (USA). Isopropyl alcohol (IPA, C_3H_8O), Ethyl alcohol (EtOH, C_2H_5OH), Sulfuric Acid (H_2SO_4), Hydrogen peroxide (H_2O_2) were purchased from Daejung (Korea). Platinum wire counter electrode (CHI 115), Ag/AgCl reference electrode (CHI 111) were purchased from CH Instruments (USA). Polyethylene glycol (MW=200, Yakuri pure chemicals, Japan), N-N-Dimethylformamide (DMF), $HCON(CH_3)_2$, Junsei, Japan), high surface area reduced Graphene Oxide (rGO, Graphene supermarket, USA) were purchased. Deionized (DI) water was obtained from a Millipore water system.

2. Modifying Electrode with GS and GNPs

Bare gold electrode was cleaned with Piranha solution (H_2SO_4 : H_2O_2=7 : 3, v/v) and rinsed by EtOH and DI water. rGO (powder, 5g/L dissolved in DMF) sonicated in sonicator for 2 hr. 0.1 ml of rGO solution was dropped to the bare gold surface and spin coated at 1,000 rpm, for 1 min with spin coater (Jaesung engineering). Finally, modified GS-coated electrode was fabricated and its surface was identified with FE-SEM (Supra 55VP). Two milliliters of 10 mM $HAuCl_4$ solution was mixed with 8 ml of 4% of PEG. Cleaned bare gold electrode was dipped in 10 ml of 2 mM $HAuCl_4$-PEG solution and run by electrochemical multi-potential step technique. The GNPs of working electrode surface were electrochemically synthesized at −1.3 V for 30 s.

3. Electrical Measurements of Norepinephrine by using GS-GNPs Electrode

GS-GNPs complexes were made through experiment 2 (Fig. 1). Final sensor model for detecting NE consists of 3-electrode as working electrode as a GS-GNPs complexes modified gold substrate, reference electrode as Ag/AgCl, and platinum wire is counter electrode. Electrochemical characteristic of NE was analyzed by electrochemical detection technique (cyclic voltammetry (CV) and differential pulse voltammetry (DPV)), which was performed by potentiostat (CHI-660, CH Instruments, USA).

RESULTS AND DISCUSSION

1. Fabrication of the GS-GNP Modified Electrode to Enhance the Conductivity

As mentioned, GS-GNP modified electrode was developed by spin coating and electrodeposition method (Fig. 1). This functionalization could enhance the performance of the sensor, enabling it to detect specificity of NE by spin coated GS and high sensitivity by electrodeposited gold nanoparticle on the indium tin oxide (ITO) electrode. When gold nanoparticle was immobilized on the electrode, poly ethylene glycol (PEG) was utilized as a surfactant to generate gold nanoparticle on the electrode. Fig. 2(a) shows that the GS and gold nanoparticles were well-functionalized on the electrode

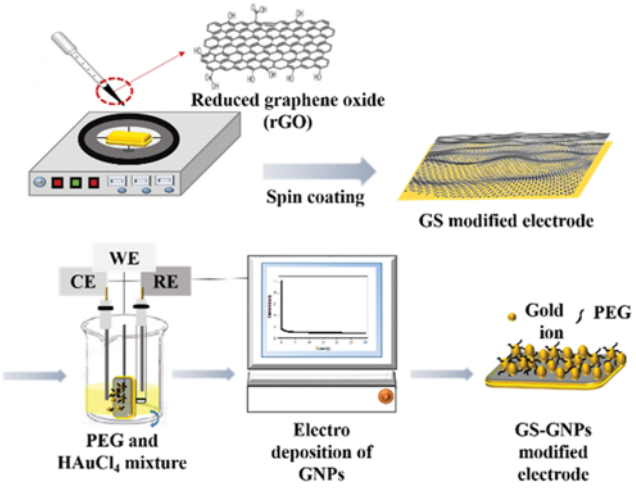

Fig. 1. Schematic diagram of the fabrication GS-GNP modified working electrode. rGO is coated on the ITO using spin coater and the mixture with PEG and Au ion is deposited by electrodeposition.

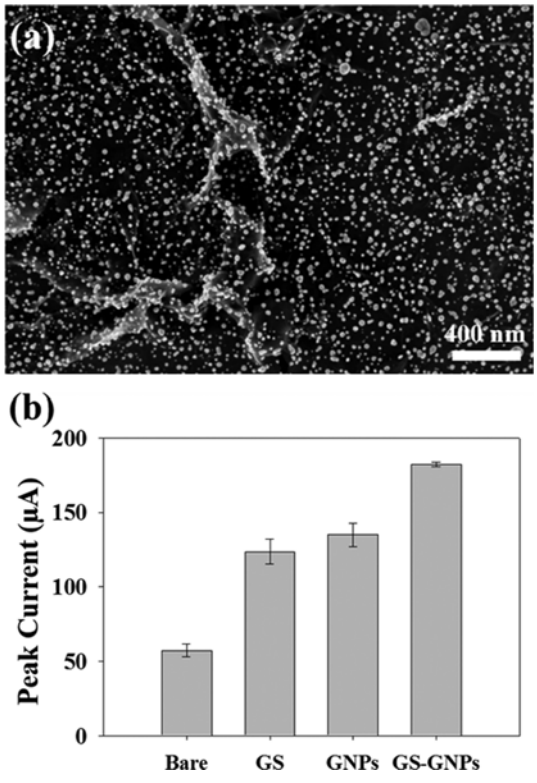

Fig. 2. GS-GNP modified working electrode. (a) SEM image of the electrode. (b) Peak current of the 10 μM NE with bare, GS, GNPs and GS-GNP modified electrodes.

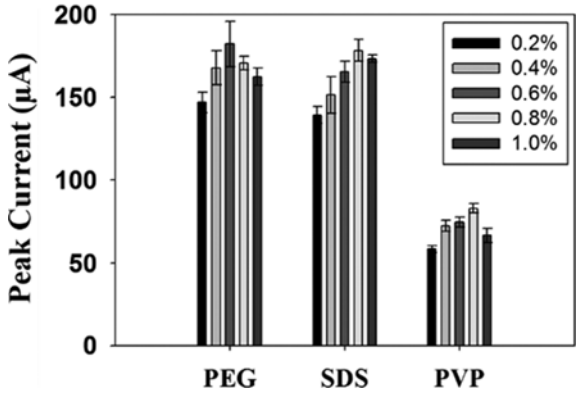

Fig. 3. Comparison of GS-GNP electrodes using various surfactants at 10 μM NE with 5 different concentrations.

through the SEM image. GS-coated surface has winkled structure and the average gold particle size is 21.78 nm (±8.387 nm). In Fig. 2(b), it is compared to electrochemical conductivity of bare/modified electrode with GS/GNPs/GS-GNPs. As a rresult, modifying both GS-GNPs complexes electrode is shown to have highest peak current due to its inherent electrical properties and broadened surface area of the electrode.

2. Optimization of GS-GNPs Structure

Prior to measuring NE concentration, it is necessary to decide the optimal surfactant and its concentration to improve the sensitivity for early detection of NE. We selected three typical surfactants, polyethylene glycol (PEG), sodium dodecyl sulfate (SDS), polyvinylpyrrolidone (PVP), to choose the best one for functionalizing GNP on the GS-coated electrode. To find optimization condition and evaluate electrical performance of GNP-functionalized electrode depending on various concentrations of surfactant, 10 μM of NE was utilized to measure the current peaks and compare their intensities by differential pulse voltammetry, which measures current with high sensitivity. Fig. 3 shows the peak current intensity for comparison of the best condition of surfactant and its concentration between 0.2 to 1.0%. Overall, PVP displays low intensity at any concentration compared to the other surfactant. It was also evaluated through the SEM image, where the GNPs were attached on the electrode surface as a relatively small density (data not shown). Among the PEG and PVP surfactants, using 0.6% of PEG had the largest oxidation peak current value (182.3 (±13.7) μA), smallest particle size (21.782 (±8.387) nm) and highest density (4370 (±226.192)× 10^8 of particles/cm^2). Small particles with high density on the GS electrode can improve its conductivity because surface-to-volume ratio increases with equivalent $HAuCl_4$.

3. Electrochemical Measurements of Norepinephrine by using GS-GNPs Complexes Modified Electrode

To detect the low concentration of the NE, 0.6% of PEG, which is the best condition of the surfactant, was used to make a GS-GNP electrode. In Fig. 4(a), NE was measured by GS-GNP electrode with various concentrations, from 200 nM to 100 μM, based on the DPV technique. To verify its selective detection and emulate actual fluid in the brain, NE was determined in presence of AA 400 μM. The GS-GNPs modified electrode could detect 0.2 μM with high selectivity and sensitivity in presence of AA 400 μM, because integration of carbon-based materials and gold nanoparticles usually shows synergistic effects in electrocatalytic applications. When we compared the performance of the developed GS-GNPs sensor with that of other sensors recently studied, our newly developed sensor has a wide detection range (200 nM to 100 μM) and superior detection limit (0.2 μM) [18-20].

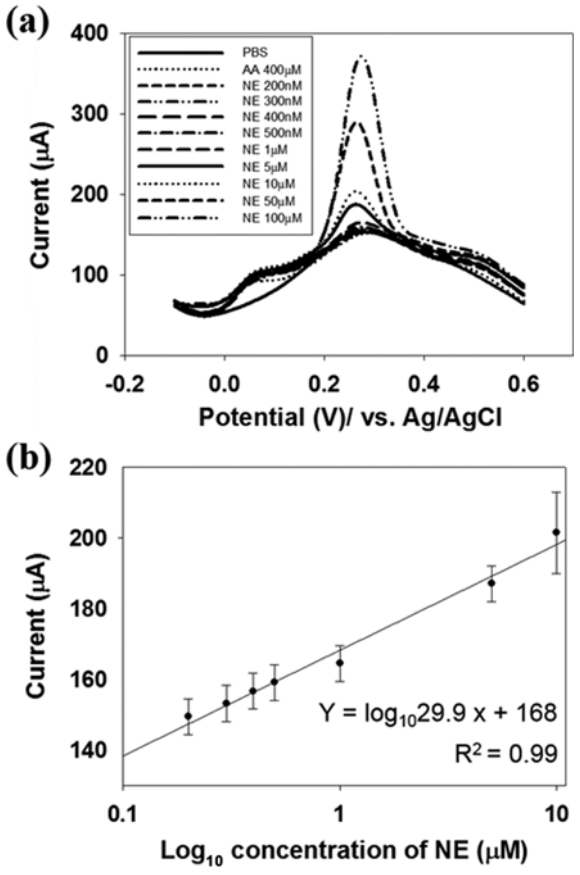

Fig. 4. Detection of varying concentration of NE by using GS-GNP modified electrode. (a) DPVs of varying concentrations of NE (0.2 μM-100 μM) with AA (400 μM) at GS-GNP modified electrode. (b) Linear plot (0.2 μM-10 μM) of anodic current peak as a function of NE concentration.

CONCLUSION

Wehave developed a highly selective and sensitive electrode with reduced graphene oxide sheets and gold nanoparticle. Since graphene and NE are attracted to each other with π-π interaction, the sensor has great selectivity of NE in presence of ascorbic acid compared with bare electrode. To increase detection sensitivity of NE in low concentrations, the electrode was fabricated by simple and rapid technique, which is electrochemical deposition of gold nanoparticles on the GS-coated electrode. GNPs modified electrode has faster electron transfer to the target materials with its 3D structure geometrical effect, and enhances sensor sensitivity. Our GS-GNPs modified electrode was successfully fabricated and detected. Compared with recent studies, our newly developed sensor detected NE not only in a wide detection range (0.2-10 μM), but also enhanced

detection limit (200 nM) in presence of 2000 times higher concentration of ascorbic acid.

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