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Flow-injection amperometric glucose biosensors based on graphene/Nafion hybrid electrodes

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ABSTRACT

In this research, we demonstrated the fabrication of flow-injection amperometric glucose biosensors based on RGO/Nafion hybrids. The nanohybridization of the reduced graphene oxide (RGO) by Nafion provided the fast electron transfer (ET) for the sensitive amperometric biosensor platforms. The ET rate (k_s) and the charge transfer resistance (R_{CT}) of GOx-RGO/Nafion hybrids were evaluated to verify the accelerated ET. Moreover, hybrid biosensors revealed a quasi-reversible and surface controlled process, as confirmed by the low peak-to-peak (ΔE_p) and linear relations between I_p and scan rate (ν). Hybrid biosensors showed the fast response time of ~3 s, the sensitivity of 3.8 μA mM^{-1} cm^{-2}, the limit of detection of 170 μM, and the linear detection range of 2–20 mM for the flow-injection amperometric detection of glucose. Furthermore, interference effect of oxidizable species such as ascorbic acid (AA) and uric acid (UA) on the performance of hybrid biosensors was prevented at the operating potential of −0.20 V even under the flow injection mode. Therefore, the fast, sensitive, and stable amperometric responses of hybrid biosensors in the flow injection system make it highly suitable for automatically monitoring glucose.

1. Introduction

Graphenes, composed of a two-dimensional single-atom-thick carbon lattice, have attracted strong fundamental and technological interest due to their remarkable physicochemical properties [1]. Drawing from the excellent electrical conductivity, large surface area, chemical and mechanical stability, and biocompatibility [2], graphenes have been significantly investigated for the electrical, optical, and mechanical sensing of various biomolecules [3]. Current research into electrochemical graphene biosensors has been particularly driven by several advantages such as higher sensing performance, portability, cost effectiveness, and capability of detecting turbid sample over other biosensors [3]. Despite intensive studies, a major challenge of graphene-based materials for practical biosensor applications is still posed by the difficulty to assemble into sensing platforms due to the poor processibility [4].

The nanohybridization by polymers is regarded as an effective technology for obtaining the stable colloidal dispersion while simultaneously preserving the inherent properties of carbon nanomaterials [5]. Recently, several groups discovered that the perfluorosulfonated polymer Nafion, which is composed of mainly hydrophobic backbone (–CF_2 groups) and hydrophilic chains (–SO_3^- groups), can easily functionalize carbon nanotubes based on supramolecular assembly by adding Nafion into alcoholic solutions of carbon nanotubes [6,7]. In an analogous manner, Nafion could improve the processibility of graphene due to the intrinsic chemical structure consisting of a hydrophobic backbone and hydrophilic side chains. Along with functioning an electrochemical binder for electrochemical applications, Nafion is capable of effectively immobilizing a variety of enzymes to facilitate biocatalytic electrochemical reactions for the biosensor applications [8,9]. In this regard, various electrochemical graphene/Nafion biosensors have been investigated to detect hydrogen peroxide, nicotinamide adenine dinucleotide (NAD) and its reduced form (NADH), dopamine, glucose, DNA, and cell [10–15]. However, the electrochemical detection of glucose in a flow-injection mode has not been unexplored yet.

Herein, we first report the fabrication of flow-injection biosensors using reduced graphene oxide (RGO)/Nafion hybrid electrodes and their applications into amperometric detection of glucose. RGO/Nafion hybrid electrodes offered the fast response time of ~3 s, the sensitivity of 3.8 μA mM^{-1} cm^{-2}, the limit of detection of 170 μM, and the linear detection range of 2–20 mM through the flow-injection amperometric detection of glucose.

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2. Experimental

2.1. Materials

Graphite powder (<20 μM), hydrazine solution (35 wt% in water), and Nafion (perfluorinated resin solution, 5 wt% in lower aliphatic alcohol and water mixture) were purchased from Sigma–Aldrich. Glucose oxidase (GOx) (EC 1.1.3.4, lyophilized powder, 100–250 units/mg from *Aspergillus niger*) and β-D(+) glucose (97%) were obtained from Sigma. 1-Propanol was obtained from Junsei. Poly(dimethoxy)silane (PDMS) was obtained from Dow Corning.

2.2. Synthesis of RGO/Nafion hybrid

RGO/Nafion hybrids were prepared following our previous work [16]. Graphene oxides (GOs) were prepared from the graphite powder by the Hummers method [17]. For the preparation of RGO/Nafion hybrids, the exfoliated GOs were dispersed in the solution of DI water and 1-propanol at volume ratio of 50:50. 2 wt% of Nafion, which was optimal value for electrochemical applications, was mixed with 0.5 mg/mL of GOs in 20 mL of DI water/1-propanol bisolvents. The homogeneous solution of GO and Nafion was prepared by sonication for 60 min. A highly homogeneous black dispersion was obtained after the reduction of GO into RGO by the addition of 100 μL of hydrazine solution at 85 °C for 24 h. The resulting mixture was washed with DI water and ethanol several times, and the RGO/Nafion hybrids were purified by dialysis for 1 week to remove the excess hydrazine and remaining impurities. Finally, the powder was filtered and dried under vacuum at room temperature and stored.

2.3. Fabrication of fluidic biosensor device

Biosensing platform was fabricated by integration of fluidic channel substrate with RGO/Nafion hybrid films. RGO/Nafion hybrid films were prepared by filtering homogeneous solutions in DI water/1-propanol (volume ratio of 50:50) bisolvents through an anodisc membrane filter (47 mm in diameter, 0.2 μm pore size, Whatman). And then, as obtained thin film was transferred to a glass substrate pattered with gold electrode through dissolving anodisc membrane using 3 M of NaOH. In order to immobilize the GOx enzymes, the RGO/Nafion films were neutralized by immersing in a water/ethanol mixture (15:85 wt%) up to pH 5.5. After drying the RGO/Nafion films, 1 μL of as-prepared GOx solution (1 mg/mL) in a mixture of water/ethanol (15:85 wt%) was dropped on the surface of the RGO/Nafion film, dried under room temperature for 12 h, and washed with phosphate buffered saline (PBS, pH 7.4) to remove excess enzyme molecules. As-prepared GOx-immobilized RGO/Nafion (GOx-RGO/Nafion) hybrid films were dried at room temperature under vacuum and used as biosensor platforms for glucose detection. Fluidic channel substrates were fabricated using the soft-lithography and replica molding methods. PDMS prepolymers were poured onto a negative master and cured. The PDMS replica was peeled off from the master and then bonded with GOx-immobilized RGO/Nafion hybrid film substrates through plasma treatment to yield fluidic biosensor device. The width and length of the flow channel were 1 mm and 4 cm, respectively. The top of device was equipped with four holes directly above the microfluidic channel, inlet, outlet, Ag/AgCl as reference electrode, and Pt as counter electrode. The device was integrated with a syringe pump (KD scientific, KDS 100) and a sample injector (Rhoedyne, model 7725i) with 20 μL sample loop. GOx-RGO film based biosensing device as a control sample was prepared following the same protocols as GOx-RGO/Nafion hybrid based biosensing device.

2.4. Instrumental methods

Transmission electron microscope (TEM) images were collected on a JEM-3010 HR TEM (300 kV). Scanning TEM (STEM) images were obtained with a probe focused to 0.2 nm and camera length of 20 cm. The scanning electron microscope (SEM) micrographs were obtained using a field emission scanning electron microscope (FEI Sirion model) equipped with an in-house Schottky emitter in high stability. Cyclic voltammogram (CV) scans were recorded using a CHI 760D electrochemical workstation (CH Instruments) with a conventional three-electrode electrochemical cell using an RGO/Nafion hybrid film working electrode, a Ag/AgCl reference electrode, and a Pt wire counter electrode. Amperometric detection was carried out with flow injection biosensor devices. All flow injection analyses were performed at a flow rate of 1 mL min^{-1}. All potentials were collected versus Ag/AgCl reference electrode and performed at room temperature.

3. Results and discussion

The micro- and macroscopic structures of RGO/Nafion hybrids were presented by SEM and TEM images as shown in Fig. 1. Single layers of RGOs were compactly coated by polymer and exfoliated completely by the entanglement of Nafion. The smooth surface of the RGO/Nafion hybrid, which was also observed by SEM image, was beneficial for the fabrication of sensor platforms owing to the efficient immobilization of enzyme. Moreover, the existence and uniform distribution of Nafion on the surface of the isolated RGOs was confirmed by observing carbon and fluorine signals of STEM image. A sample quality of hybrids arises from the favorable interactions between RGO and Nafion, as previously reported [16]. Furthermore, the hybridization of RGOs by Nafion generated the modification of electronic structures, resulting in the fast electron transfer (ET).

Fig. 2 presents the CV curves and Nyquist plots of RGOs and RGO/Nafion hybrids. Compared to RGOs, RGO/Nafion hybrids showed higher current responses and lower peak-to-peak potential separations (ΔE_p) for the redox process of $[Fe(CN)_6]^{3-/4-}$. The higher currents of RGO/Nafion hybrids compared to that of RGOs were due to the large accessible surface area and capacitance. Taking the strong relation of ΔE_p with the ET rate into consideration, the lower ΔE_p of RGO/Nafion hybrids compared to that of RGOs indicates the accelerated ET for a single-electron electrochemical reaction [18]. The electroactive surface area (A) of the RGO/Nafion and RGO electrodes were calculated by the Randles–Sevčik method, described in equation [19]:

$$i_p = (2.69 \times 10^5) n^{3/2} A D_O^{1/2} C_O \nu^{1/2} \quad (1)$$

where i_p is the peak current, n is the number of charges, A is the electroactive surface area, D_O is the diffusion coefficient of the transferred species, C_O is the concentration, and ν is the scan rate. The estimated A value of RGO/Nafion electrode was 0.11 cm^2, which is higher than that of RGO electrode (0.09 cm^2). After the immobilization of GOx, the peak currents of RGO/Nafion hybrids decreased as the redox reactions were inhibited by the resistance effect induced by biomolecule adsorption. In order to demonstrate the facilitated ET, the charge transfer resistance (R_{CT}) was calculated from Nyquist plots. The R_{CT} of GOx-RGOs and GOx-RGO/Nafion hybrids were 242 Ω and 172 Ω, respectively. The low R_{CT} of GOx-RGO/Nafion hybrids were attributed to the facilitated ET of redox agents at the interface of electrode/electrolyte [20].

Fig. 3A shows the chronoamperograms of the oxidation of $Fe(CN)_6{}^{4-}$ ions on the RGO/Nafion hybrid and RGO electrodes.