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Short Communication

Ultrasound-radiated synthesis of PAMAM-Au nanocomposites and its application on glucose biosensor

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ABSTRACT

Hybrid nanocomposites of carboxyl-terminated generation 4 (*G* 4) poly(amidoamine) dendrimers (PAMAM) with gold nanoparticles (AuNPs) encapsulated inside them were synthesized under ultrasound irradiation. The obtained nanocomposites were used to fabricate highly sensitive amperometric glucose biosensor which exhibited a high and reproducible sensitivity of 2.9 mA/mM/cm^2, response time less than 5 s, linear dynamic range from 0.1 to 15.8 μM, correlation coefficient of $R^2 = 0.9988$, and limit of detection (LOD), based on S/N ratio (S/N = 3) of 0.05 μM. A value of 2.7 mM for the apparent Michaelis–Menten constant K_M^{app} was obtained. The high sensitivity, wider linear range, good reproducibility and stability make this biosensor a promising candidate for portable amperometric glucose biosensor.

1. Introduction

Dendrimers belong to a new class of synthetic macromolecules characterized by a regularly branched treelike structure [1]. Also, dendrimers with high generation numbers ($G \geqslant 4$) usually possess a nearly spherical shape [2] and they can encapsulate metal complexes and other guest molecules. Accordingly, dendrimers have attracted increasing interests in applying dendrimers to the related areas such as chemical and biomedical sensors [3,4] microelectronic and biomimetic systems [5], adhesion, coating, and membrane chemistry [6], and nanotechnology [7].

Metal nanoparticles are interesting because of their inherent size-dependent optical, electrical, catalytic, and magnetic properties [8–13]. Noble metal nanoparticles, especially gold nanoparticles (AuNPs), have received considerable attention [14–17]. Recently, metal nanoparticles have been synthesized in the presence of dendrimers in both aqueous and nonaqueous systems [18–21]. Among various dendrimers, poly(amidoamine) (PAMAM) dendrimers are the most frequently studied. Encapsulation of AuNPs in the interior of the dendrimer (PAMAM-Au) can increase the electrical conductivity of PAMAM and the size of AuNPs can be controlled by using the dendrimer core as a nanotemplate.

Glucose detection is of practical importance in the food and fermentation analysis, in the textile industry, in environmental monitoring, and in medical diagnosis, etc. [22–24]. In this work, PAMAM-Au nanocomposites were synthesized rapidly under ultrasound irradiation and glucose oxidase (GOD) was then covalently immobilized to carboxylic acid terminated groups of PAMAM dendrimers. We report the first attempt to develop an amperometric glucose biosensor based on the immobilization of the multiwall carbon nanotubes (MWCNTs), PAMAM-Au, GOD and Nafion onto the surface of glassy carbon electrode (GCE). This Nafion/GOD/PAMAM-Au/MWCNTs/GCE biosensor exhibited a high and reproducible sensitivity of 2.9 mA/mM/cm^2, response time less than 5 s, linear dynamic range from 0.1 to 15.8 μM with very low detection limit of 0.05 μM.

2. Experimental section

2.1. Reagents and apparatus

A fourth generation ($G = 4$) poly(amidoamine) dendrimer with surface terminated succinamic acid groups [PAMAM (NHCOCH$_2$CH$_2$COOH)$_{64}$], glucose oxidase type VII (136,000 units/g, EC 1.1.3.4. from Aspergillus niger), glucose and Nafion were purchased from Sigma. Amperometric detections were carried out on CHI 1030 electrochemical workstation (CH Instruments, USA)

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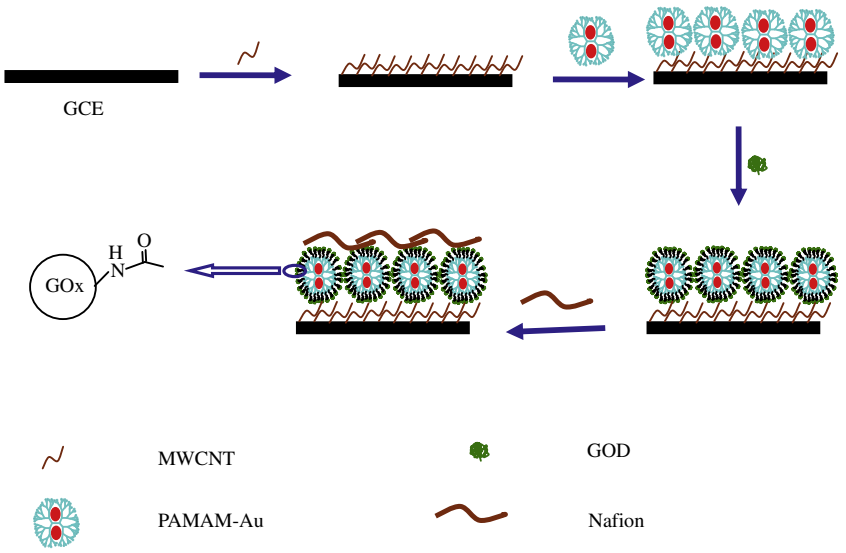

Fig. 1. Fabrication of Nafion/GOD/PAMAM-Au/MWCNTs electrode.

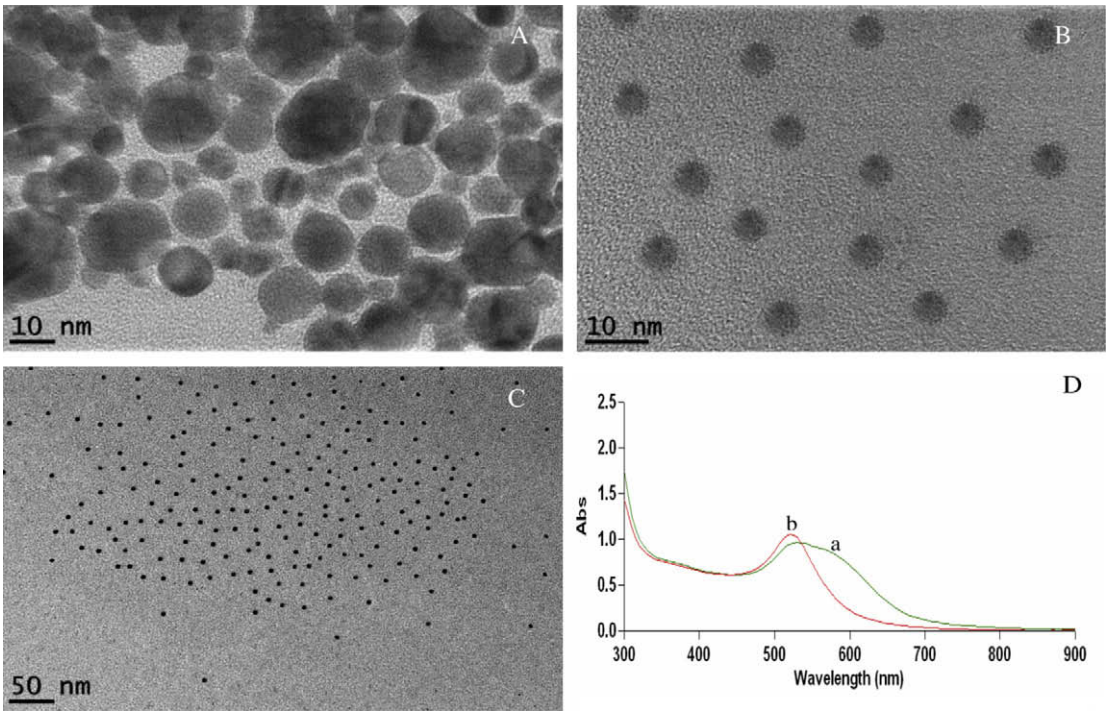

Fig. 2. TEM images of the PAMAM-Au nanocomposites (A) synthesized under stirring; high (B) and low magnification (C) PAMAM-Au nanocomposites synthesized under irradiation of ultrasound; (D) UV spectra of (a) PAMAM-Au nanocomposites synthesized under stirring and (b) PAMAM-Au nanocomposites synthesized under irradiation of ultrasound.

with a three-electrode system, a Nafion/GOD/PAMAM-Au/MWCNTs modified electrode as working electrode, a Ag/AgCl as reference electrode and a platinum electrode as counter electrode. All experiments were performed at ambient temperature (25 ± 1 °C).

JEM-2100 High Resolution Transmission Electron Microscope (HRTEM) (JEOL Co. Ltd., Japan) was used for characterization of the PAMAM-Au. UV spectrum was obtained at ambient temperature using a Cary 50 Conc UV–Visible Spectrophotometer (Varian, US). Sonication was performed in a KQ-2100DA ultrasonic cleaner with a frequency of 40 kHz and a nominal power 100 W. The reaction flask was placed with maximum energy area in the cleaner and reaction temperature was controlled by water bath.

2.2. Preparation of PAMAM-Au nanocomposites

PAMAM-Au nanocomposites were prepared as follows: 2 mL $KAuCl_4$ solution (1 mM) was added to 2 mL PAMAM (0.07 mM) and 2 mL formic acid (1 mM). This pale yellow solution was under irradiation of ultrasound for 30 min. When the zerovalent Au complex was formed, the color immediately changed from yellow to

red. This reaction occurred over a 30 min time period which was largely shorter than the method previously [25].

2.3. Preparation of Nafion/GOD/PAMAM-Au/MWCNTs modified electrode

Fig. 1 presents a schematic diagram for the fabrication of Nafion/GOD/PAMAM-Au/MWCNTs electrode. Firstly, 0.1 mg/mL functionalized MWCNTs (in DMF) and 0.1 mg/mL GOD solutions were prepared. Then 5.0 μL MWCNTs was dropped onto the surface of pretreated GCE [26]. After drying for 10 min, MWCNTs electrode was dropped by 5.0 μL PAMAM-Au Nanocomposites. Then the PAMAM-Au/MWCNTs electrode was dipped in 0.1 mg/mL GOD solutions for 12 h and then dropped by 3.0 μL 2.5% Nafion (in methanol). At last, the Nafion/GOD/PAMAM-Au/MWCNTs modified electrode was rinsed thoroughly with doubly distilled water and stored in PBS (pH 7.0) at 4 °C.

3. Results and discussion

3.1. TEM images of the PAMAM-Au nanocomposites

The structure of PAMAM/Au nanocomposites can be characterised directly by TEM. As shown in Fig. 2 PAMAM/Au nanocomposites were synthesized in smaller sizes and better dispersion with the irradiation of ultrasound than with stirring. In Fig. 2A, the diameter of AuNPs which were synthesized under stirring was about 5–20 nm variably and not dispersed equally inside the hole of the PAMAM dendrimers. While it synthesized under irradiation of ultrasound was about 4 nm and dispersed equally inside the hole of the PAMAM dendrimers. UV spectroscopy was used to characterize the formation of PAMAM-Au nanocomposites (Fig. 2D). PAMAM-Au in its aqueous solution showed a surface plasma resonance absorption band at about 520 nm, confirming the formation of gold nanoparticles [27]. In Fig. 2D, the absorption peak at 530 nm (Fig. 2D), curve a) for the PAMAM-Au nanocomposites synthesized under stirring had a red shift (ca. 10 nm) and a broader band than synthesized under irradiation of ultrasound. While the red shift observed under stirring may support the enlargement of the particles [28] and the broader band can support the uneven PAMAM-Au nanocomposites size synthesized under stirring.

3.2. Electrocatalytic oxidation of glucose

The amperometric detection was performed with Nafion/GOD/PAMAM-Au/MWCNTs/GCE biosensor at room temperature and different concentrations of glucose solution after reacted for 3 min in PBS (pH 7.0). Fig. 3A depicts a current–time plot for the biosensor on successive step changes of glucose concentration at the applied potential of 0.6 V. The calibration curve for the biosensor under the optimized experimental conditions is shown in Fig. 3B. The biosensor displayed a linear response to glucose in the concentration range from 0.1 to 15.8 μM (R^2 = 0.9988, n = 5, RSD_{av} = 0.9988%). The linear equation was I (μA) = 0.1126 + 0.2025C (μM), and the sensitivity of the biosensor was 2.9 mA/mM/cm^2, which was higher than values found in the literature previously [29,30]. The high

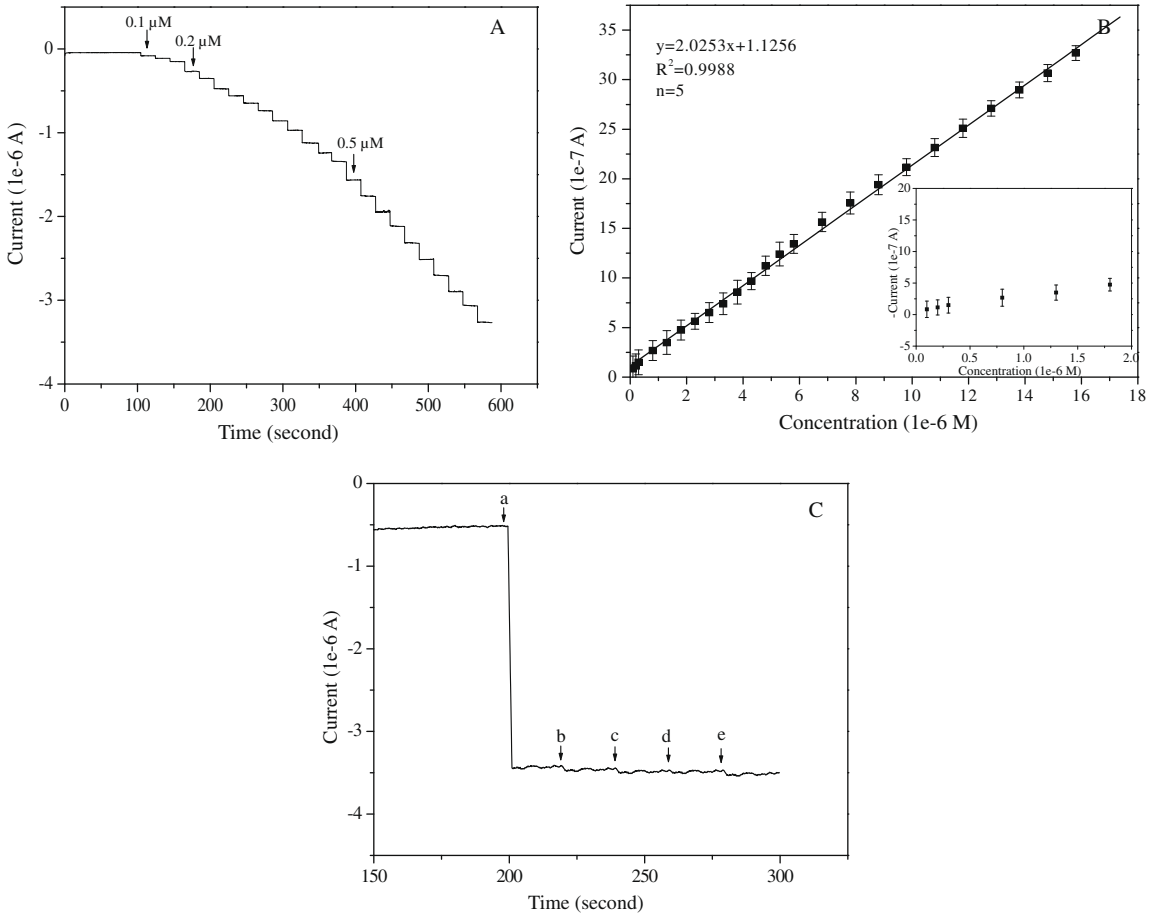

Fig. 3. (A) Current response of the biosensor upon various concentrations of glucose in PBS at 0.6 V (vs. Ag/AgCl). (B) Steady-state calibration curve of the biosensor in pH 7.0, 0.1 M PBS at 0.6 V (vs. Ag/AgCl). (C) Effect of interfering species on biosensor response. (a) 0.1 mM glucose, (b) 0.1 mM uric acid (UA), (c) 0.1 mM L-Cysteine (L-cys), (d) 0.1 mM Glutamate (GA) and (e) 0.1 mM ascorbic acid (AA).

sensitivity can be attributed to the introduction of the Au nanoparticles in the core of PAMAM that can increase the electrical conductivity of PAMAM, the catalytically electroactive surface area and provide the biocompatible microenvironment to maintain the activity of the enzyme. The detection limit of the biosensor was estimated to be 0.5 μM at a signal/noise ratio of 3 with a fast current response time (within 5 s), which was lower than previous method [29,30].

The apparent Michaelis–Menten constant K_M^{app}, a reflection of the enzymatic affinity, can be calculated from the Lineweaver–Burk equation $1/i = K_M^{app}/i_{max}(1/C) + (1/i_{max})$, where i is the current, i_{max} is the maximum current measured under saturated substrate conditions, and C is the glucose concentration. According to the Lineweaver–Burk plot, the K_M^{app} was calculated to be 2.7 mM. The obtained value K_M^{app} was less than the previously reported literature [29]. The high GOD affinity to glucose was assigned to the biocompatible nature, high specific surface area, chemical stability, high conductivity which provide high electron communication features that enhance the direct electron transfer of the PAMAM-Au nanocomposites in the GOD/PAMAM-Au/MWCNTs/GC electrode surface. It was observed by comparing with the previously reported results that the fabricated glucose biosensor based on the PAMAM-Au nanocomposites possessing a higher sensitivity and low detection limit as compared with other reported results [29,30].

3.3. Application to real samples

The applicability of this Nafion/GOD/PAMAM-Au/MWCNTs/GCE glucose biosensor was assessed by the detection of glucose concentration in diluted human serum samples utilizing the standard addition method. The recovery was estimated to be 102 ± 5% for the prepared biosensor. The recovery test demonstrates that the glucose biosensor offers an excellent method for the determination of glucose in diluted human serum and could be used clinically.

3.4. Selectivity and stability

The biosensor also showed a good selectivity for glucose. In an air-saturated and stirred 0.1 M pH 7.0 PBS containing 0.1 mM glucose, the response arising from 0.1 mM uric acid (UA), 0.1 mM L-cysteine (L-cys), 0.1 mM glutamate (GA) and ascorbic acid (AA) was negligible (Fig. 3C).

Additional experiments were carried out to test the reproducibility and stability. No obvious change could be seen from the CV curves after 100 cyclic scans in pH 7.0 PBS at 100 mV/s. The biosensor was stored at 4 °C when not used, and it retained about 90% of its original bioactivity after three months, indicating a good stability.

4. Conclusions

In this paper, a novel method was exploited to encapsulate AuNPs in PAMAM under ultrasound irradiation. An amperometric glucose biosensor Nafion/GOD/PAMAM-Au/MWCNTs/GCE was developed for the rapid determination of glucose. The fabricated biosensor exhibited a high and reproducible sensitivity of 2.9 mA/mM/cm^2, response time less than 5 s, linear dynamic range from 0.1 to 15.1 μM, $R^2 = 0.9988$, and LOD based on S/N ratio (S/N = 3) of 0.05 μM. These results demonstrate that PAMAM-Au nanocomposites which were synthesized under ultrasound irradiation are an attractive material for the fabrication of efficient amperometric biosensors.

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