Analytica Chimica Acta 811 (2014) 76–80

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

A gold@silica core–shell nanoparticle-based surface-enhanced Raman scattering biosensor for label-free glucose detection

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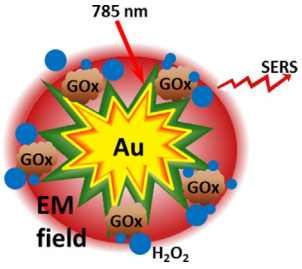
HIGHLIGHTS

- We successfully synthesized Au nanstar@SiO_2 NPs conjugated with the GOx enzyme.
- We quantitatively measured glucose level by examining SERS peak of H_2O_2.
- SERS sensing assay showed good anti-interference capability against UA and AA.
- This sensor can be used in the real biological matrix such as saliva.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 27 September 2013
Received in revised form 2 December 2013
Accepted 4 December 2013
Available online 3 January 2014

Keywords:
Surface-enhanced Raman scattering (SERS)
Biosensor
Glucose

ABSTRACT

The gold nanostar@silica core–shell nanoparticles conjugated with glucose oxidase (GOx) enzyme molecules have been developed as the surface-enhanced Raman scattering (SERS) biosensor for label-free detection of glucose. The surface-immobilized GOx enzyme catalyzes the oxidation of glucose, producing hydrogen peroxide. Under laser excitation, the produced H_2O_2 molecules near the Au nanostar@silica nanoparticles generate a strong SERS signal, which is used to measure the glucose concentration. The SERS signal of nanostar@silica~GOx nanoparticle-based sensing assay shows the dynamic response to the glucose concentration range from 25 μM to 25 mM in the aqueous solution with the limit of detection of 16 μM. The sensing assay does not show any interference when glucose co-exists with both ascorbic acid and uric acid. The sensor can be applied to a saliva sample.

1. Introduction

Diabetes is a globally widespread metabolic disease [1], which elevates the blood glucose level to 80–120 mg/100 mL (4.4–6.7 mM for the normal level) [2]. It affects more than 366 million people worldwide with a predicted number to reach half a billion by 2030 [3]. Diabetes causes a few of complications such as vision loss, heart disease and kidney failure [4]. Monitoring of glucose level in blood or other human fluids help manage the disease and avoid the associated medical risks [5]. Various types of glucose-monitoring devices have been developed using electrochemical, colorimetric conductimetry, optical and fluorescence methods [6]. Electrochemical methods have attracted intense attention because of their high sensitivity, reasonable selectivity, low cost and low limit of detection (LOD) [7]. By versatile modification of the electrodes with polymeric membrane [8–16], fullerene-C_{60} [17,18], carbon nanotubes [19–21] or graphene materials [22–24], selectivity and biocompatibility can be improved. However, the presence of hydrophilic antioxidants including ascorbic and uric acid may

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http://dx.doi.org/10.1016/j.aca.2013.12.009

interfere with the electrochemical signal for human fluid samples such as blood plasma [25]. Hydrogen peroxide, which is the product of glucose catalyzed by glucose oxidase (GOx), may inactivate the electrode as well [26]. These effects diminish the sensitivity and reliability of electrochemical glucose sensors [27]. On the other hand, most existing methods acquire blood samples by phleboto mization finger-prick, which is inconvenient because of the skin irritation and necessity of specific tools [28]. Body fluids such as sweat, urine, tears and saliva are considered as alternative samples for detection [29–31]. However, it is challenging to measure glucose in these fluids due to the very low level of glucose in these samples compared to blood. Therefore, there is an incentive to develop an alternative glucose biosensor with improved sensing performance in terms of LOD, detection range and reliability.

Optical biosensors could be one of the alternative options because they have several advantages including low cost, relatively high molecular specificity and less-invasive [32,33]. However, optical sensors have drawbacks such as high LOD, false positive signals, and vulnerability to interferences particularly in the mid-infrared wavelengths [34]. Raman sensors have good resistance to interferences in an aqueous solution, but Raman sensors have low sensitivity due to inefficient Raman scattering when it is used for measuring trace amounts of analyte. Fortunately, this drawback can be overcome with the use of nanostructures that are capable of generating surface-enhanced Raman scattering (SERS) effects [35,36]. Nanostructure-based SERS sensors are attractive optical transduction technique because of (i) simple sample preparation, (ii) elimination of background signal from water that usually is associated with infrared spectroscopy, and (iii) high sensitivity resulting from plasmonic nanostructures. Owing to these advantages, SERS is a promising technique for biomedical applications [37,38].

In the present study, a simple and effective SERS glucose biosensor based on gold nanostar@SiO_2 core–shell nanoparticles is developed. In this sensor, the Au nanostar@SiO_2 nanoparticles are functionalized with glucose oxidase enzyme (GOx), which catalyzes the oxidation of glucose, producing hydrogen peroxide (H_2O_2) and gluconic acid. H_2O_2 molecules, which are generated near the Au nanostars, are detected with a portable Raman microscope. Since the SERS signal takes the finger-print Raman peak of H_2O_2, and is not affected by the amount of water in the sample, this method can be used as a non-invasive diagnostic method for glucose detection in body fluids such as sweat, urine, tears and saliva other than blood [31]. Herein this SERS biosensor has been demonstrated to detect the glucose in saliva.

In the present work, the gold nanostar@SiO_2 core–shell nanoparticles are used as the SERS substrate because this nanostructure brings significant benefits to SERS sensing of glucose [39]. First, the Au nanostar is capable of enhancing the SERS signal remarkably. Second, the silica shell makes the nanoparticle water-soluble in an aqueous solution. Third, the silica shell isolates the Au nanostar from the produced H_2O_2 during collecting the SERS signal to avoid the problem that nano-sized gold particles have strong peroxidase activity.

2. Experimental

2.1. Materials

Chloroauric acid trihydrate ($HAuCl_4{\cdot}3H_2O$), trisodium citrate dehydrate $Na_3C_6H_5O_7{\cdot}2H_2O$ (99.0%), and sodium hydroxide (NaOH) were purchased from Alfa Aesar. N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), ascorbic acid (AA), uric acid, D-(+)glucose, sodium hydrobromide ($NaBH_4$), poly (vinylpyrrolidone) with an average molar mass of 10,000 g mol^{-1} (PVP-10), N,N-dimethylformamide (DMF), glucose oxidase, enzyme (GOx), sodium silicate stock solution (containing ~26.5% SiO_2 and ~10% Na_2O) and hydrogen peroxide (35 wt.% H_2O_2 in water) were purchased from Sigma–Aldrich (MO, USA). 3-Triethoxysilylpropyl succinic anhydride (TEPSA) and 3-mercaptopropyltrimethoxysilane (MPTMS) were from Gelest Inc. (PA, USA). Deionized (DI) water was produced with a Milli-Q Millipore system (18.2 MΩ cm^{-2}, Millipore Corp, USA). All solvents were obtained from commercial sources and used without further purification.

2.2. Apparatus

The nanoparticles were observed with a JEM 2100F transmission electron microscope (TEM, JEOL Ltd., Japan) at an acceleration voltage of 200 kV. UV–vis absorption spectra were measured in 200–1200 nm with a LAMDA 750 UV/vis/NIR spectrometer (MA, USA). X-ray photoelectron spectroscopy (XPS) was performed with a PHI 5000 Versa Probe system (MN, USA). The C 1s peak of aliphatic carbon at 284.8 eV was used as reference to calibrate XPS spectra. Raman spectra were measured with a handheld Raman spectrometer (DeltaNu Inc., WY, USA) at an excitation wavelength of 785 nm with integration time of 5 s.

2.3. Procedures

2.3.1. Synthesis of gold nanostar@SiO_2 core–shell nanoparticles

Gold nanostars (NS) were synthesized according to the protocol reported previously [39]. Briefly, the gold seed solution was prepared by diluting 1 mL of aqueous solution of $HAuCl_4{\cdot}3H_2O$ (1 wt.%) with 90 mL of water. A 2 mL of 38.8 mM trisodium citrate was added and mixed with the gold solution. Subsequently, 1 mL of freshly prepared $NaBH_4$ solution (0.075 wt.% in 38.8 mM trisodium citrate solution) was added. The reaction mixture was kept at room temperature with constant stirring overnight. Then 50 mL of generated solution was mixed with 10 mM polyvinylpyrrolidone (PVP) at room temperature under constant stirring for 24 h to prepare the PVP-coated gold seed colloidal solution. To prepare the gold nanostars, 82 μL of 50 mM $HAuCl_4$ was added into 15 mL of 10 mM PVP in DMF solution followed by rapid addition of 43 μL of the PVP-coated gold seed solution (4 mM) under constant stirring at room temperature for 13 h. Finally, the nanostars were separated with centrifugation at 12,000 rpm for 30 min and cleaned twice with ethanol and deionized (DI) water, respectively. The Au nanostars were re-dispersed in DI water and kept at room temperature until use.

The absorbance of the Au nanostar solution was adjusted to 2.5 at the longer surface plasmon peak position before coating with SiO_2 layer. 50 μL of freshly prepared (3-mercaptopropyl) trimethoxysilane (MPTMS) solution (50 mM) was then dropped into 1 mL of the Au nanostar solution under constant stirring for 30 min to modify the Au nanostar surface with silane. Subsequently, 100 μL of fresh sodium silicate solution (0.54 wt.%, pH > 12) was added under stirring for 10 min. The mixture was kept undisturbed for 24 h before adding 1 mL of anhydrous ethanol to produce a condensed SiO_2 layer. The resulting AuNS@SiO_2 solution was then centrifuged and washed with anhydrous ethanol, and re-dispersed in deionized water for future use.

2.3.2. Immobilization of glucose oxidase enzyme

To immobilize glucose oxidase, carboxyl functionalized AuNS@SiO_2 nanoparticles were prepared by adding 400 μL of 200 mM 3-triethoxysilylpropyl succinic anhydride (TEPSA) into 800 μL of AuNS@SiO_2 aqueous solution and then incubating the

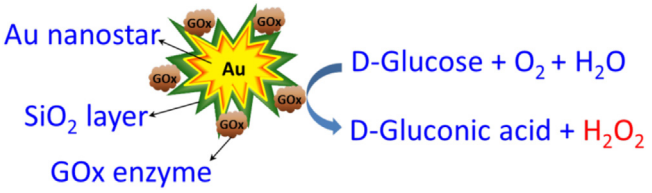

Fig. 1. Scheme illustration of the nanoparticle-based SERS sensor for glucose detection.

solution at room temperature overnight. The —COOH group on AuNS@SiO_2 was then activated by 100 μL of 50 mM NHS with 200 mM EDC. After 2 h of incubation, 20 μL of 25 mg mL^{-1} GOx was added and incubated overnight under stirring at room temperature. The solution was centrifuged at 12,000 rpm to collect the precipitate and washed with phosphate buffered saline (PBS, 10 mM, pH 7.0) solution to obtain the final product of GOx-conjugated AuNS@SiO_2 (AuNS@SiO_2~GOx). The nanoparticles were then dispersed in 800 μL of PBS solution for future use.

2.3.3. Performance test of the sensor

150 μL of AuNS@SiO_2~GOx was mixed with 150 μL of glucose and then incubated at room temperature for 10 min. 75 μL of this mixture was placed on a gold-coated chip for SERS measurement. Raman signals were acquired with the Raman spectrometer and normalized by a blank sample without containing any glucose. Three random sites on the gold chip were selected to obtain the SERS spectra for each sample, and the average was taken.

To test the possible interferences from biologically relevant molecules, 0.62 mM ascorbic acid (AA) and 85 μM uric acid (UA) [40] were added to the analyte solution, respectively. The SERS spectra were obtained following the same protocol that was used above.

To examine the applicability of the method to the real-world samples, saliva sample collected from a fasting volunteer without food intake for 8 h was treated and tested. Before collection, oral cavity of the volunteer was rinsed with 150 mL of water three times, and then 500 μL of saliva was obtained with a small sterilized pasture pipette. The contents were immediately transferred into a sterilized tube and divided into 125 μL of aliquots for further use. A mixture containing 125 μL of AuNS@SiO_2~GOx and 125 μL of 4 mM (or 8 mM) glucose in PBS solution was mixed individually with the saliva sample, and then incubated at room temperature for 10 min. Subsequently, 75 μL of the mixture was placed on a gold-coated chip for SERS measurement.

3. Results and discussion

Fig. 1 illustrates the sensing principle of the AuNS@SiO_2~GOx nanoparticle-based SERS sensor. In this sensor, the Au nanostar was chosen as the SERS substrate. Based on our previous study [39], Au nanostar is an excellent SERS substrate because it can achieve higher amplification of SERS signal than both Au nanosphere and nanorod. The AuNS@SiO_2 core–shell particles have better water solubility than bare Au nanoparticles [39], which ensures the stability of the SERS substrate and the repeatability of detection. Fig. 2(a) shows that the gold nanostars were about 70 nm in size; and the silica shell was about 8 nm thick (Fig. 2(b)). The UV–vis spectra in Fig. 2(b) indicate that the plasmonic absorption peak of the Au nanostarred-shifted from 854 nm to 861 nm after coating with the silica shell. In this SERS sensor (Fig. 1), GOx is covalently linked to the AuNS@SiO_2 core–shell particle as the molecular recognition probe for selective detection of glucose. Successful immobilization of GOx on the AuNS@SiO_2 particles was confirmed by the XPS spectra in Figure S1 in Support Information. The GOx enzyme catalyzes

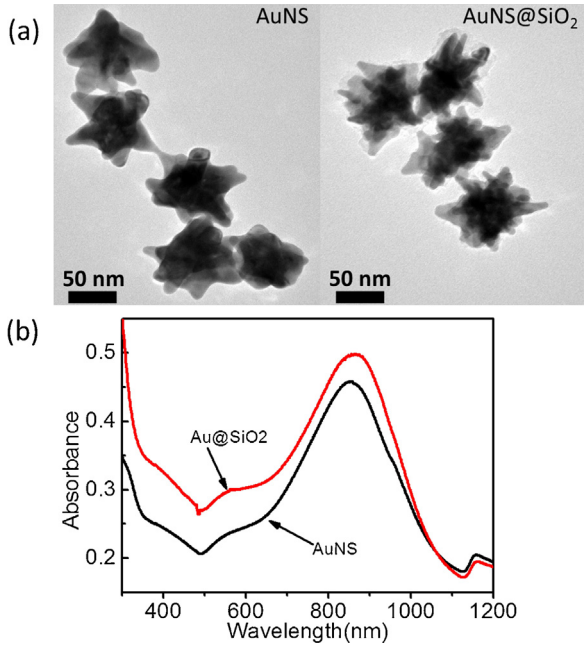

Fig. 2. (a)TEM images and (b) UV–vis absorption spectra obtained from the bare gold nanostars and the Au nanostar@silica core–shell nanoparticles.

the glucose oxidation to produce hydrogen peroxide and gluconic acid as follows:

$$\text{D-glucose} + O_2 \xrightarrow{\text{GOx}} \text{D-gluconic acid} + H_2O_2 \quad (1)$$

The produced H_2O_2 generates the SERS signal under the laser excitation. Although the concentration of produced H_2O_2 is low, the H_2O_2 molecules produced are close to the surface of AuNS@SiO_2 core–shell particles. Hence the H_2O_2 molecules are exposed to strong electromagnetic field created by local surface plasmon resonance (LSPR) of the Au nanostar under laser excitation. As demonstrated in our previous study [39], the local electromagnetic field near the plasmonic Au nanostar can greatly amplify the SERS signal of molecules. It is worth noting that the785 nm laser (excitation source) was in resonance with the LSPR absorption band of the Au nanostar, which benefited to SERS enhancement. Therefore, the SERS signal of H_2O_2 molecules can be detected even at a low concentration.

Based on Eq. (1), the produced H_2O_2 amount is directly proportional to the concentration of glucose. Hence the change in the intensity of the SERS peak of H_2O_2 reflects the variation of glucose concentration. Fig. 3(a) shows the SERS spectra obtained from the sensing assay containing different concentrations of glucose. The peak at 873 cm^{-1} came from the υ(O—O) vibration mode, which was characteristic of H_2O_2. The peak at 1073 cm^{-1} was ascribed to gluconic acid [40]. Both the peaks increased with an increase in the glucose concentration.

The normal level of glucose in blood and in saliva are 4.4–6.7 mM [2] and 0.1–0.3 mM [41,42], respectively. Typically, a glucose sensor should be able to detect glucose as high as 25 mM [43] and as low as the normal levels. Based on such information, the SERS sensor was optimized by investigating the effects of the GOx loading, the pH of the assay, and the incubation time necessary for reaching the reaction equilibrium. The optimized assay solution contained 0.2 mg mL^{-1} AuNS@SiO_2~GOx in 0.2 M PBS inaqueous solution with pH of 4.8. The optimized incubation time prior to SERS measurement was 10 min after the analyte was added into the assay solution. Detailed information on the optimization studies can be found in the Supporting Information.

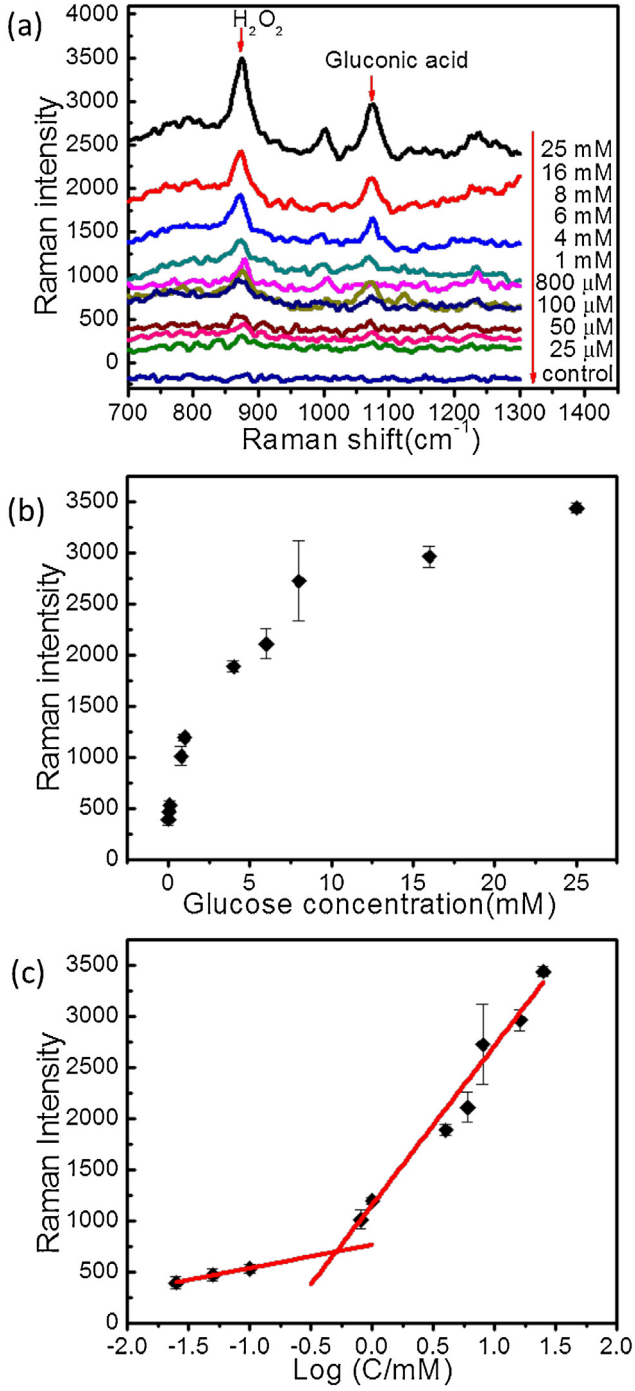

Fig. 3. (a) SERS spectra obtaind from the sensing assay containing different concentrations of glucose; (b) a plot of the resposne of sensor to the glucose concentration; (c) the sensor response versus the logarithmic concentration of glucose. All measured SERS spectra were normalized by subtracting the background in the absence of glucose.

The calibration curve was established by acquiring the SERS spectra from the individual assay solutions containing various concentrations of glucose, as shown in Fig. 3(b). In this calibration curve, the intensity of the SERS peak at 873 cm^{-1} was plotted as a function of the glucose concentration. The AuNS@SiO_2~GOx glucose sensor exhibited a dynamic range of concentration from 25 μM to 25 mM. When the intensity of SERS peak was plotted as a function of the logarithmic concentration of glucose, the linear regression

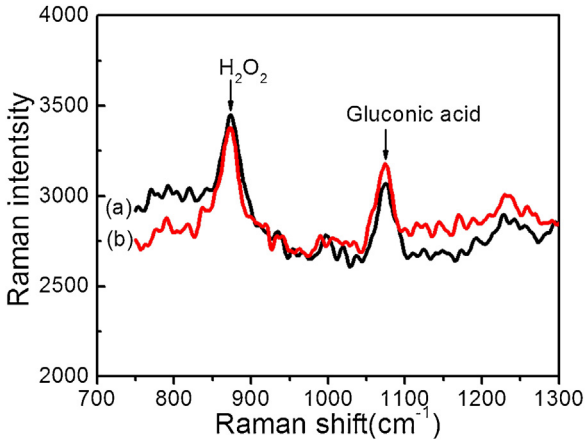

Fig. 4. SERS spectra obtained from the sensing assay containing 25 mM glucose, (a) in the absence of and (b) in the presence of 0.62 mM uric acid and 85 μM ascorbic acid.

curves were generated. At a high level of glucose (0.5–25 mM), the linear curve was fit by

$$y = 1575x + 1109 \quad (2)$$

(R^2 = 0.97), where y represents the SERS signal intensity for H_2O_2 and x represents the logarithmic concentration of glucose, as shown in Fig. 3(c). At a low level of glucose (25 μM to 0.5 mM), the linear curve was fit by

$$y = 230x + 763 \quad (3)$$

(R^2 = 0.985). The limit of detection (LOD) was measured to at 16 μM at a signal-to-noise ratio of 3. This LOD was better than the values reported previously [42,43].

To test the robustness of the AuNS@SiO_2~GOx sensor, the SERS signal was acquired in the presence of uric acid (UA) and ascorbic acid (AA), which are commonly found in human fluids. Fig. 4 shows that the charactersitic H_2O_2 peak was not affected when 0.62 mM UA and 85 μM AA was present in the glucose assay solution. No significant interference from AA and UA was found. The SERS signal for glucosewas obtained from human saliva obtained from healthy adults (Figure S6). The intensity of the Raman peak at 873 cm^{-1} was negligible in the absence of AuNS@SiO_2~GOx nanoparticles. When the AuNS@SiO_2~GOx nanoparticles were present in the saliva sample, the SERS peak at 873 cm^{-1} was observed. When the saliva sample was spiked with 4 mM and 8 mM glucose, the intensity of the SERS peak increased. These results demonstrated that the AuNS@SiO_2~GOx SERS-based glucose sensor can be used without any significant interference to clinical samples. Moreover, compared with other methods (Table S1), the low LOD and the wide range of our glucose sensor make it a promissing non-invasive tool that could monitor the glucose level in saliva of diabetes patients, who need periodic glucose testing to establish the blood glucose levels at 3–10 times per day [5].

4. Conclusions

The Au nanstar@SiO_2 core–shell nanoparticles conjugated with GOx enzyme were successfully synthesized. Using this AuNS@SiO_2~GOx SERS-based glucose sensor, glucose concentration from 0.025 mM to 25 mM was quantitatively detected with a LOD of 16 μM. This was lower than the physiological levels of glucose in blood and saliva (0.1–0.3 mM). Hence, the developed sensor has a potential in detection of sudden drop in the glucose level. Moreover, the glucose sensor showed robust signals even in the presence of uric acid and ascorbic acid. This AuNS@SiO_2~GOx

SERS-based glucose sensor holds promise as a non-invasive tool for monitoring the glucose level in human.

Acknowledgments

The resource and facilities used were partially supported by the NSF (EPS 1003907). The use of WVU Shared Facility was appreciated.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aca.2013.12.009.

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