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Quantitative gold nanorods based photothermal biosensor for glucose using a thermometer as readout

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ABSTRACT

To meet the increasing need for point-of-care testing (POCT), simple and portable readout strategies would be highly desirable. Thermometer with high accuracy and straightforward readout is an ideal tool for the development of new POCT methods. The exploration of new thermometer-based detection methods is of great significance. In this study, a simple biosensor for glucose based on the photothermal effect of gold nanorods using a simple thermometer as readout has been developed. In the presence of glucose oxidase, glucose can react with the dissolved oxygen to produce H_2O_2. With the help of Fe^{2+}, H_2O_2 can etch gold nanorods (AuNRs) to different aspect ratios. The decrease of the aspect ratio of AuNRs leads to the blue-shift of the localized surface plasmon resonance peak, resulting in a decrease of photothermal effect in the near-infrared regions and the temperature of the system decreased. The change of the temperature has a linear relationship with the logarithm of glucose concentration in the range of 1.0–10.0 mM with a detection limit of 0.8 mM. The proposed method possesses a bias offset of −0.03 mM for glucose detection compared to the hospital method. Since many enzymatic reactions can produce H_2O_2, the principle can be modified to detect different targets by simply change of the enzyme used.

1. Introduction

Signal readout play an important role in biochemical sensing. Conventional signal readout usually relies on sophisticated instruments and trained laboratory personnel, which limited the practical applications. Recent advances in point-of-care testing (POCT) has emphasized the need for affordable and portable signal readout methods. Thermometer, one of the most commonly used devices in daily life, has been used to detect the temperature with high accuracy and reliability and can be found in every home. The advantages such as small size, low cost, and convenient operation, make it an ideal signal readout device [1–7]. Recently, by correlating the temperature change with the target concentration, many biosensors using thermometers as a readout device have been reported using different heated sources, such the photothermal effect of iron oxide nanoparticles [8,9], NIR sensitive dyes [10], and aggregated gold nanoparticles (AuNPs) [11,12]. All these studies indicated that a thermometer can work as an accurate readout device for POCT. But constant effort is still necessary to be paid to find efficient thermal conversion materials.

Gold nanorods (AuNRs) with tailorable longitudinal localized surface plasmon resonance (LSPR) band and large extinction coefficients have high photothermal effects especially at NIR region [13–16]. The changing of aspect ratio of AuNRs will result in the shift in the LSPR band and the change of the photothermal conversion efficiency [17–19]. Although AuNRs-based colorimetric biosensors have been successfully developed for qualitative or semi-quantitative testing by naked eyes, the quantitative detection still relies on large equipment and skilled operators. Comparatively less application based on the change of the photothermal conversion efficiency caused by the changing of aspect ratio of AuNRs had been studied [20–22]. Therefore, the development of AuNRs-based quantitative methods with simplified readout is full of opportunities and challenges.

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In this study, combined with the high specificity of the enzymatic reaction and efficiency of etching AuNRs through the reaction of Fe^{2+} ions with H_2O_2 (the Fenton reaction) [23], a quantitative AuNRs-based photothermal biosensor has been developed using a thermometer as readout. Glucose, one of the most common and important physiological indicators, has been chosen as a model target. At present of glucose oxidase (GOx), H_2O_2 can be produced under enzymatic reaction, which can be followed by the production of hydroxyl radical (•OH) with the help of Fe^{2+} to produce a series of different aspect ratios AuNRs. The difference in the photothermal conversion efficiency of the AuNPs can be monitored by a simple thermometer easily without the need of expensive instruments and skilled operators. Since the aspect ratios of AuNRs has relationship with glucose concentration, a simple method for glucose detection can be developed using a thermometer as readout.

2. Experimental section

2.1. Materials

Hexadecyl trimethyl ammonium bromide (CTAB) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Ascorbic acid was obtained from Energy Chemical in Shanghai. Chloroauric acid ($HAuCl_4{\cdot}4H_2O$, 99.9%), sodium borohydride ($NaBH_4$), silver nitrate ($AgNO_3$), hydrochloric acid (HCl), and uric acid (UA) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai). Glucose oxidase (GOx), glucose, and hydrogen peroxide (H_2O_2) were purchased from Sangon Biotech (Shanghai) Co., Ltd. All other reagents were of analytical grade and used as received. All solutions were prepared with ultrapure water (18.2 MΩ Cm) from the Direct-Q3 UV system (Millipore).

2.2. Instruments

Microplate Spectrophotometer (Multiskan GO) for the measurement of the UV–vis spectra was purchased from Thermo Fisher Scientific (China). TEM images were acquired by Transmission Electron Microscope operated at an acceleration voltage of 200 kV (JEOLJEM-2100F, Electron optics laboratory Co., Ltd, Japan). The digital camera (EOS 600D) used for taking AuNRs solution photographs was purchased from Canon (China). The digital thermometer used for the measurement of temperature in this study was CIE 305P (Taiflex Scientific CO., LTD). The photothermal testing system was irradiated by a NIR laser pointer (750 nm, 1.5 W).

2.3. Etching of AuNRs and photothermal detection

AuNRs were synthesized by the seed-mediated growth method according to early reported method [24]. The Au seeds were synthesized firstly. Under stirring, $NaBH_4$ solution (0.6 mL, 10 mM) was added in a mixed solution of $HAuCl_4$ (5 mL, 5 mM) and CTAB (5 mL, 200 mM). After 2 min stirring, the solution was set for 30 min to obtain the Au seeds. To prepare the growth solution, ascorbic acid solution (5 mL 10 mM) was added into a mixed solution consisted of CTAB (90 mL, 110 mM), AgNO3 (0.6 mL, 10 mM) and HAuCl4 (5 mL, 10 mM). Finally, the prepared Au seeds (200 μL) were added into the growth solution under vigorous stirring for 30 s and set overnight to obtain AuNRs.

Before the process of AuNRs etching, HCl and FeSO4 solution were prepared. The HCl (2 M) used in this study was prepared by diluting the HCl (12 M) with ultrapure water, and the FeSO4 solution was prepared by dissolving 91 mg FeSO4 in 30 mL ultrapure water. The AuNRs solution was obtained from Section 2.2 without further processing. In the process of AuNRs etching, the typical operation is as follows: AuNRs solution (90 μL) was mixed with HCl (27 μL, 2 M) and FeSO4 (9 μL, 20 mM), followed by the addition of different concentrations of H2O2 (54 μL, 0, 0.5, 1.0, 2.0, or 4.2 mM). After reaction for 50 min, the mixed AuNRs solution was irradiated by NIR laser for 6 min and the temperature of the solution was monitored by a digital thermometer. The room temperature is controlled at 23.0 °C by an air conditioner.

2.4. Analysis of glucose in human serum samples

The human serum samples were donated by the volunteers of the Affiliated hospital of Putian University (Putian, China). The blood samples were first centrifuged at 13500 rpm for 10 min. The supernatant, which is the serum, was collected. The serum samples were stored at −20 °C before use. Then, GOx (54 μL, 50 μg mL^{-1}) was added to samples for the enzymatic reaction. Subsequently, the above mixture was added to the 90 μL AuNRs solution and mixed with HCl (27 μL, 2 M) and $FeSO_4$ (9 μL, 20 mM) to etch AuNRs. The temperature of the system was measured according to the protocol as mentioned in section 2.3. The results provided by the hospital were measured by Power Processor using a Glucose Kit. The principle of this Glucose kit is based on a hexokinase/glucose-6-phosphate dehydrogenase procedure. All experiments were performed under the Guidelines approved by the Affiliated hospital of Putian University and approved by the ethical committee of Affiliated hospital of Putian University. Informed consent was obtained from the human participants of this study.

3. Results and discussion

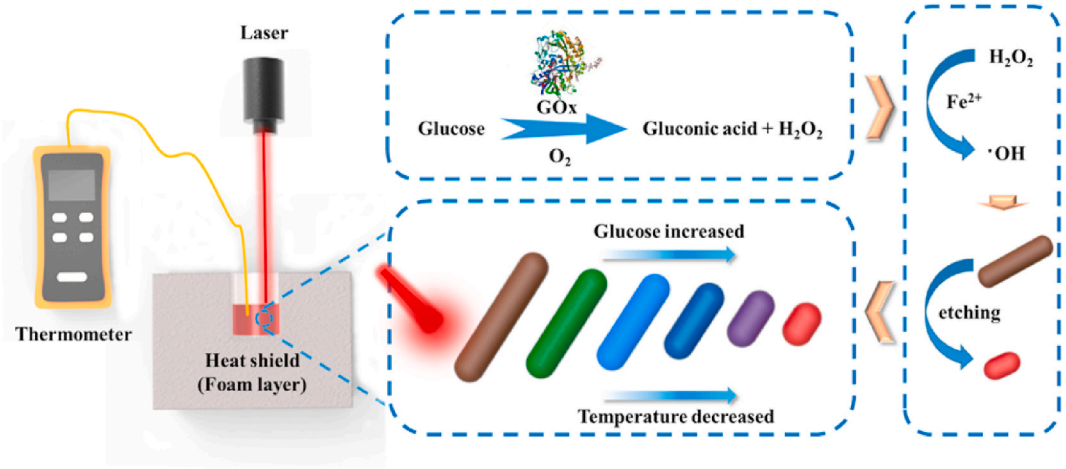
3.1. Principle of the biosensor for glucose using a thermometer as a readout

Scheme 1 shown the principle of the proposed biosensor. The AuNRs convert excitation energy into heat upon NIR-laser irradiation, producing a temperature increase of the solution. Fe^{2+} can catalyze the formation of produce hydroxyl radical (•OH) by H_2O_2 in the acidic environment, and then etched AuNRs. It makes the aspect ratio of the AuNRs decrease and results in the blue-shift of the LSPR peak of the AuNRs. The photothermal effect of AuNRs in the NIR regions reduced because of the reduction of absorption in this wavelength and the degree of the temperature enhancement of the system decreased, which can be monitored by a simple thermometer easily. The change of the temperature of the system has some relationship with the amount of hydrogen peroxide (H_2O_2). Since glucose oxidase (GOx) can catalyze the production of H_2O_2 at present of glucose and oxygen, some relationship can be established between the amount of glucose and the temperature of the system. Based on this, a simple photothermal biosensor for glucose can be developed using a thermometer as readout.

3.2. Characterization of the AuNRs etching

Firstly, the TEM images and UV–vis spectra of AuNRs were used to characterize the effect of etching on the AuNRs. As shown in Fig. 1A, the prepared AuNRs is about 70 nm in length and 14 nm in width. After the addition of H_2O_2 and Fe^{2+}, the length of AuNRs becomes short while the diameter of AuNRs didn't change (shown in Fig. 1B). During this process, the LSPR band of the AuNRs was blue-shifted, and obvious color changes can be observed by the naked eyes easily (insert in Fig. 1A and B). These results indicate that the aspect ratio of AuNRs can be significantly changed after the addition of H_2O_2 in presence of Fe^{2+}.

Fig. 1C shows the changing of temperature of the prepared AuNRs solution under laser irradiation. The temperature of the solution increased with the increasing of the irradiation time firstly. After 6 min, the temperature of the system reach a plateau, and the maximum temperature is 44.7 °C. Given that longer irradiation time do not cause significant temperature changes, the time of irradiation was set at 6 min in following studies. Fig. 1D shows the temperature change curve of AuNRs after etching under laser irradiation. The phenomenon is the same with the original one, but the maximum temperature of the solution can only reach 27.9 °C. UV–vis spectra of the system before and after etching had been tested and shown in the insets of Fig. 1C and D. The results showed that the AuNRs has two LSPR peaks. As shown in the



Scheme 1. Schematic illustration of glucose biosensor based on the photothermal effect of the AuNRs using thermometer as readout.

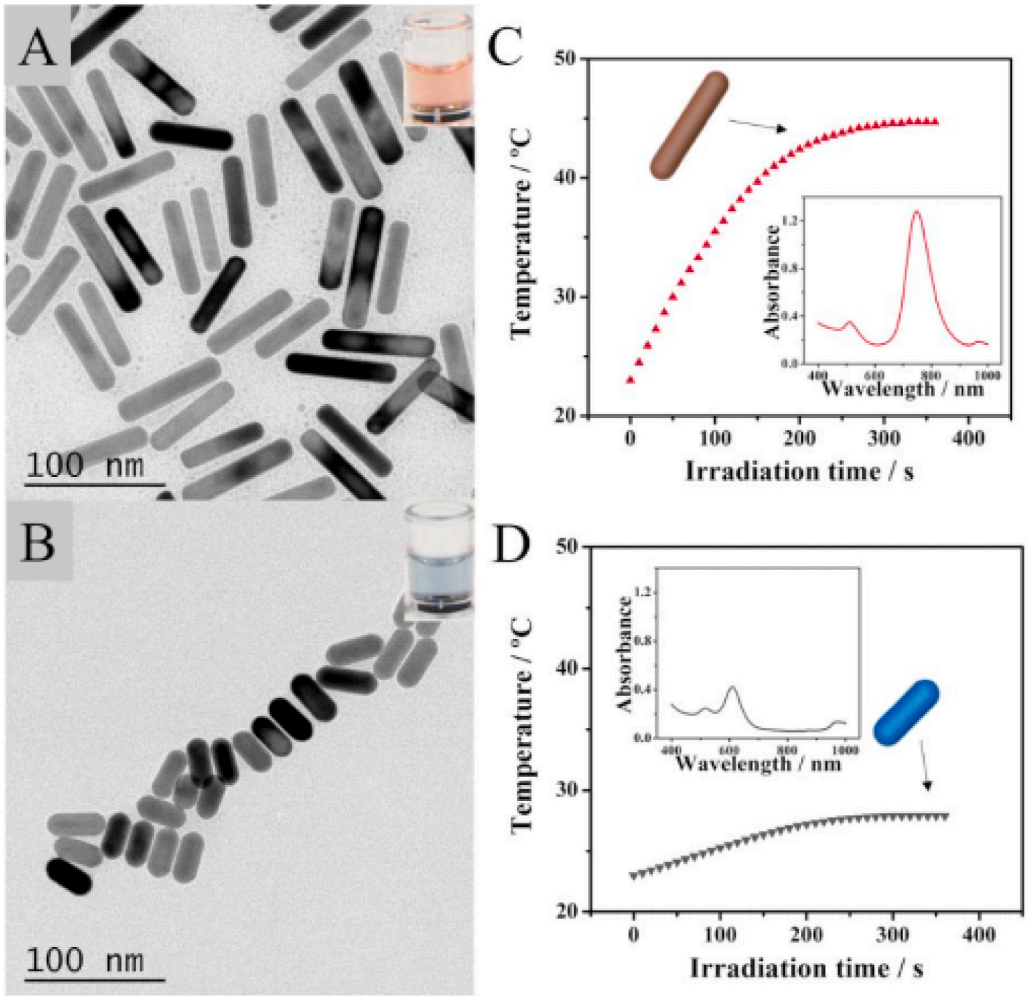


Fig. 1. TEM images of the AuNRs (A) before and (B) after etching (insets are the photographs of the corresponding samples); Temperature curve against the time of system (C) before and (D) after etching under the irradiation (insets are the UV–vis spectra of the corresponding samples).

UV–vis spectra, the peak at 511 nm corresponds to the short-axis LSPR peak of the AuNRs, and the peak at 750 nm corresponds to the long-axis absorption peak of the AuNRs (Fig. 1C). After etching, the short-axis LSPR peak of the AuNRs is nearly kept constant, but the long-axis LSPR peak of the AuNRs has a significant blue-shift (Fig. 1D). This blue-shifted is caused by the etching of AuNRs under the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow \cdot OH + Fe^{3+} + OH^-$), and the aspect ratio decrease. The blue-shift of the plasma absorption peak is responsible for the reduction of the photothermal effect of the AuNRs.

3.3. Feasibility validation

Simple experiments had been performed to verify the feasibility of the proposed method. UV–vis spectra of the AuNRs mixed with 20 mM Fe^{2+} and different concentrations of H_2O_2 were shown in Fig. 2A. As the H_2O_2 concentration increases, the long-axis absorption peak of the AuNRs shifted blue and the absorption value decreases greatly in the NIR, when the short-axis absorption peak of the AuNRs shows no significant changes. The corresponding temperature changing of the different AuNRs solutions under NIR laser irradiation was shown in Fig. 2B. With the enhancing amount of H_2O_2 added, the degree of temperature increasing under laser irradiation decreases significantly, because the photothermal effect of AuNRs reduced at reducing aspect ratio. These results indicated that it is feasible to develop a quantitative analysis method by controlling the H_2O_2 produced. Since the present of glucose oxidase can catalyze glucose to produce H_2O_2 [25,26], it is possible to establish a glucose biosensor using thermometer as readout.

3.4. Optimization of experimental conditions

To acquire the best performance, the effect of some experimental conditions were optimized. ΔT (the change of temperature enhancement) is used as the evaluation criterion of each condition ($\Delta T = T_0$-T, T_0, and T represent the temperature enhancement when the target object is absent and present). H_2O_2 cannot etch the AuNRs without Fe^{2+}. Therefore, the concentration of Fe^{2+} is crucial. As shown in Fig. 3A, ΔT gradually increases as the concentration of Fe^{2+} increases. When the concentration of Fe^{2+} is greater than 20 mM, the ΔT reaches a plateau. So this concentration had been chosen for the following experiments. The process in which Fe^{2+} catalyzes the formation of •OH by H_2O_2 is in the acidic environment. Then the amount of HCl added had been optimized also. The results showed that the ideal effect can be obtained when the concentration of HCl is 2 M (Fig. 3B). The amount of GOx affects the amount of H_2O_2 produced. Fig. 3C showed that ΔT increased with the enhanced dosage of GOx firstly and when the concentration of GOx is greater than 50 μg mL^{-1}, the ΔT reaches a plateau. So this concentration had been chosen for the following experiments. Besides, the enzymatic reaction time was optimized (Fig. 3D). The results showed that the best performance can be obtained after 50 min. Furthermore, the optimization pH value for the enzyme reaction had been optimized. The results showed that pH 7.4 is the best condition (data not shown). So these conditions had been chosen for the following study.

3.5. Performance of proposed biosensor

Combined with glucose oxidase catalyzing glucose to produce H_2O_2, the proposed principle had been applied to detect glucose. As shown in Fig. 4A, ΔT has a linear relationship with the logarithm of glucose concentration in the range of 1.0–10.0 mM. The equation is:

$$\Delta T = 13.228 \lg C_{glucose} + 2.704 \ (R^2 = 0.981)$$

Where $C_{glucose}$ is the concentration of glucose molecules, and R is the linear correlation coefficient. The detection limit is 0.8 mM (S/N = 3). The etching of AuNRs had been applied to develop multicolor biosensor for glucose early [27]. The insets are the corresponding photographs of the system. When the glucose concentration is low (such as 1 mM), it is difficult for the naked eye to distinguish the color difference, but the obvious temperature change can be detected by the thermometer. This indicated the proposed biosensor has better sensitivity when compared with the multicolor biosensor based on the etching of AuNRs.

Good selectivity is the guarantee for the specific detection of the target. AA, DA, and UA had been chosen as model interferences (all set at 30 mM). The results show that the ΔT caused by the interferences is little (Fig. 4B). But at present of 3 mM glucose, the value of ΔT is great. The specificity of glucose oxidase for glucose can ensure the selectivity of the proposed method. Furthermore, the precision of photothermal assay was studied by monitor 2.0 mM of target for 5 times, the relative standard deviation (RSD) is 4.2%. If five different samples with the same target concentration (2.0 mM) had been tested, the RSD is 4.8%. These results indicated the proposed method had good precision and reproducibility.

3.6. Determination of glucose in human serum

The proposed method had been applied to detect the glucose concentration in ten human serum samples obtained from ten volunteers, respectively. And the obtained results were compared with the results provided by the hospital (Table S1). As shown in Fig. 5A, a strong linear correlation can be found between both methods ($y = 1.35x + 0.72$, $R^2 = 0.994$, where y represents the results provided by the hospital, and x represents the results obtained by the proposed method). Besides, the Bland–Altman plot was used to analyze the agreement between both methods. As shown in Fig. 5B, the photothermal assay possesses a bias offset of −0.03 mM at the limit of agreement from −0.38 to 0.32 mM for glucose detection compared to the hospital method. These results indicate that the photothermal detection method can be applied to quantitative analysis glucose in real samples with satisfying accuracy.

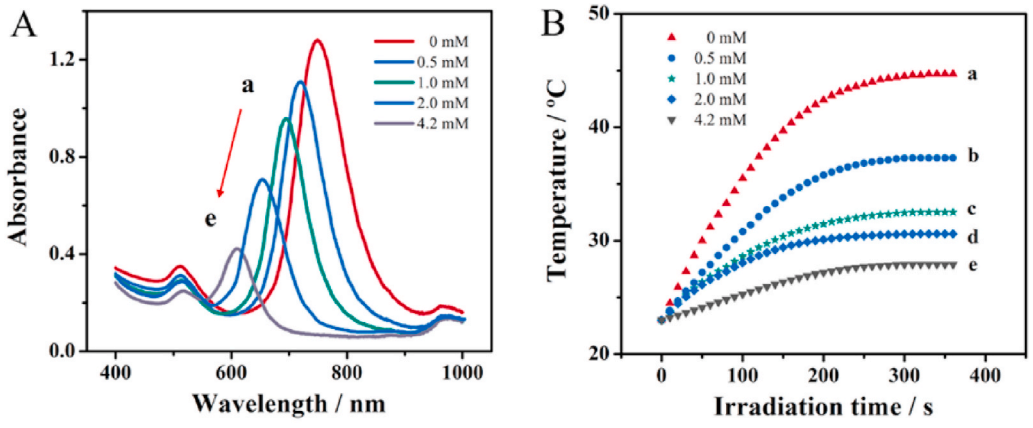


Fig. 2. (A) UV–vis spectra of the AuNRs solutions in the presence of different concentrations of H_2O_2. The concentrations of H_2O_2 were 0, 0.5, 1.0, 2.0, and 4.2 mM, respectively, corresponding to the curve a to e. (B) Temperature comparison of the AuNRs solutions after etching with different concentrations of H_2O_2. The concentration of Fe^{2+} is 20 mM and HCl is 2 M.

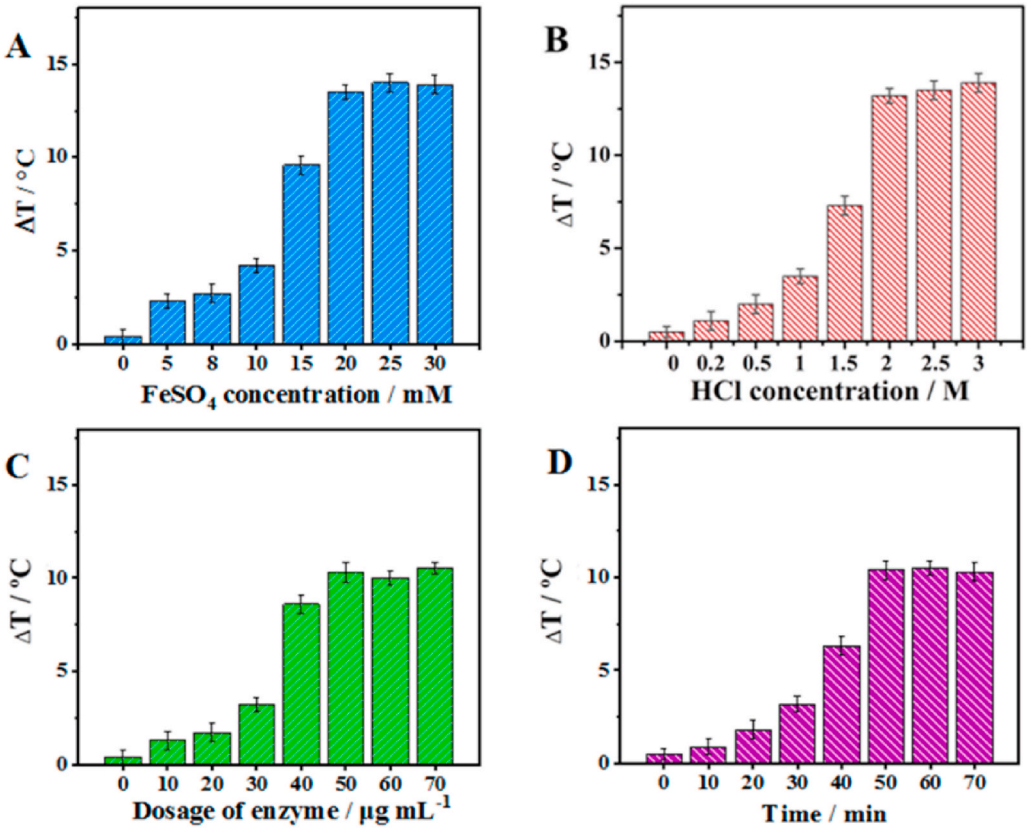


Fig. 3. Effect of concentration of (A) Fe^{2+} and (B) HCl on the ΔT of system. 2.0 mM H_2O_2 was used in the experiments. The optimization of (C) the dosage of glucose oxidase and (D) enzymatic reaction time. 20 mM Fe^{2+}, 2.0 mM H_2O_2, and 10 mM glucose were used in the experiments.

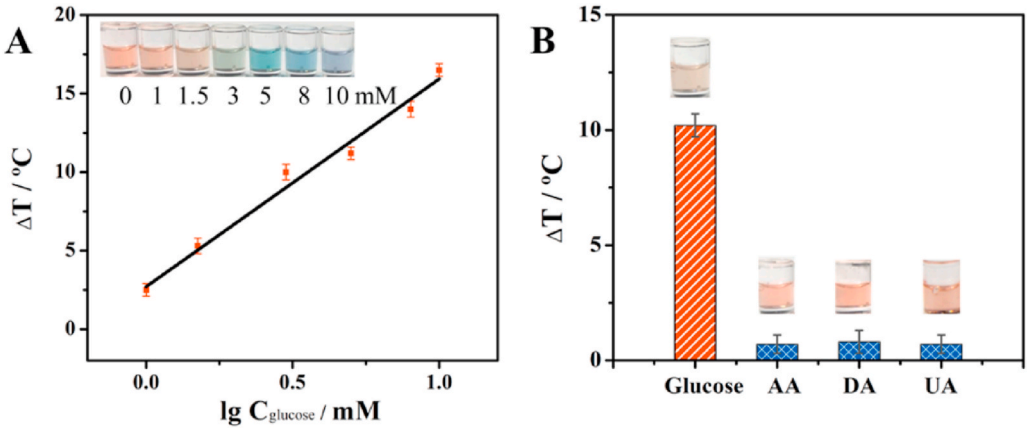


Fig. 4. (A) Calibration curve for quantification of the glucose concentration. (B) The selectivity of the proposed photothermal assay for glucose detection. Insets are the photographs of the corresponding samples.

4. Conclusion

In summary, a quantitative photothermal biosensor based on the photothermal effect of AuNRs was developed for glucose determination. This photothermal biosensor exhibited high sensitivity and selectivity owing to significant temperature changes caused by target induced etching of AuNRs and the highly specific enzymatic reaction. This photothermal biosensor is low-cost and portable and can avoid complex analytical instruments and operational training because the temperature signal can be measured by a commercial thermometer precisely and simply. Besides, the potential application of the proposed method to detect the concentration of glucose in human serum was evaluated. Moreover, the proposed mechanism can be adjusted to detect different targets by changing the enzyme used easily, because many enzymatic reactions can produce H_2O_2 for the Fenton reaction to etch AuNRs.

CRediT author statement

Yingzhou Tao: Conceptualization, Methodology, Investigation, Writing - Original Draft. Fang Luo: Software, Project administration, Writing - Review & Editing. Yisheng Lin: Formal analysis, Validation. Nuo Dong: Visualization. Cheng Li: Formal analysis. Zhenyu Lin: Resources, Review & Editing, Supervision.

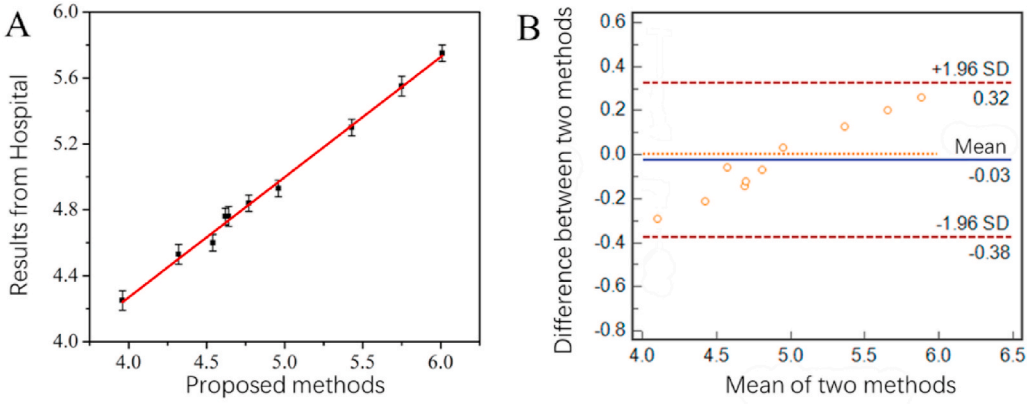


Fig. 5. (A) Correlation analysis of results obtained from the photothermal assay and standard clinical detection in 10 real samples; (B) Bland−Altman analysis for the difference between the photothermal assay and clinical results of 10 real samples.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2021.122364.

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