Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 234 (2020) 118233

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Highly sensitive and simple colorimetric assay of hydrogen peroxide and glucose in human serum via the smart synergistic catalytic mechanism

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ARTICLE INFO

Article history:
Received 9 January 2020
Received in revised form 3 March 2020
Accepted 3 March 2020
Available online xxxx

Keywords:
Colorimetric analysis
Synergistic catalytic mechanism
Glucose
Human serum

ABSTRACT

Due to the own defects of natural enzymes, artificial simulated enzymes are always concerned. Here, the fabricated graphene oxide (GO)/AuNPs nanocomposite exhibits strong synergistic catalysis of peroxidase-mimicking enzymes in combination with the novel property of GO catalytic interface and AuNPs-mediated electron transfer. It can efficiently catalyze the oxidation of enzyme substrate TMB by hydrogen peroxide to form blue TMB oxide. Based on this, the rapid and highly sensitive colorimetric detection of hydrogen peroxide was achieved. Because of the wonderfully synergistic coupling catalysis from GO/AuNPs nanocomposites, the developed artificial enzyme has ultra-strong catalytic activity. For the detection of hydrogen peroxide, the detection limit of this colorimetric analysis is as low as 4.2×10^{-8} M, which is about 1–2 orders of magnitude lower than that of the assays using other single nanoparticles as nanozymes. And it shows high sensitivity. The catalytic oxidation of the prepared nanocomposites to TMB can be completed in minutes, and the response is extremely fast. Combined with the reaction of glucose and glucose oxidase, the colorimetric analysis also realizes the rapid and highly sensitive detection of glucose in human serum. The research results infer that the smart synergy is an effective way to improve the catalytic activity of mimic enzyme. Together with its simplicity in preparation, the GO/AuNPs nanocomposite has excellent development potential in biomedical detection and biosensor design.

1. Introduction

In the process of life evolution, natural enzymes are highly superior biocatalysts and play an extremely important role in the field of biochemistry [1]. However, natural enzymes have their own defects such as difficulty in purification, easy denaturation and inactivation, being expensive and so on [2,3]. Therefore, artificial simulated enzymes as substitutes for natural enzymes have attracted great attention. With the development of nanometer science, the special nanometer interface effect of nanometer materials shows strong catalytic performance in chemical reactions [4–7]. Various nanomaterials as artificial enzymes emerge in this situation. Including Fe_3O_4 magnetic nanoparticles [8] and positively-charged gold nanoparticles (AuNPs) [9], it was also found that other nanomaterials like MoS_2 nanosheets and RhNPs possess intrinsic peroxidase-like activity, and the colorimetric detection of hydrogen peroxide, an oxidant used for the oxidation of peroxidase substrate 3, 3, 5, 5-tetramethylbenzidine (TMB), were developed [10–12]. The artificial enzyme based on the nanomaterials has good catalytic performance. In order to obtain high catalysis, the experimental conditions of synthesis need to be strictly controlled.

Although nanoscience has greatly promoted the development of artificial mimic enzyme, the catalytic performance of nanozymes still remains to be further improved. Intensive efforts have been made to improve the catalytic activity of nanozymes. In the catalytic reactions which nanomaterials participate in, the surfaces conditions of nanomaterials may be related to the electron-mediated energy transfer during catalytic reactions, and thus affect the catalytic performance of nanomaterials [13,14]. The efforts for improving the catalytic activity of nanozymes are mainly through such measures that other species were adsorbed or modified on the surface of nanomaterials to change the catalytic interface properties and enhance the electron and energy transfer on the surface of nanomaterials, in order to improve the catalytic activity of mimicking enzymes [9,15–17]. The peroxidase-like activity of Fe_3O_4 nanoparticles was improved by coating with different ligands [15]. The oxidase-like activity of nanoceria was markedly increased since the adsorbed fluoride can reduce surface energy of nanoceria and facilitate the electron transfer [16]. In addition, the previous studies have found that nanoparticles can enhance their catalytic performance for liquid-phase luminescence reactions through synergistic effects [18–23]. One of the effects that can produce the synergistic

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https://doi.org/10.1016/j.saa.2020.118233
1386-1425/

action is the local concentration effect that is the greater the local concentration of the reactant involved in the reaction system, the stronger the catalytic performance [19,24]. Inspired by this, that is to say, the synergism may also be an effective way to improve the catalytic activity of nanozymes. Graphene can adsorb reactants in reaction systems to generate local concentration effects using its π-rich conjugation surface [25–27], which will lay the theoretical foundation for the improving the catalytic activity of nanozymes through the synergistic action based on graphene nanocomposites. But unfortunately, the research which involved enhancing nanozymes' catalytic activity by the synergistic action of graphene is very few. Liu et al. developed the graphene-based catalytic interface for colorimetric biosensing [28].

In this work, we make use of the novel properties of the graphene-based catalytic interface to detect hydrogen peroxide. The novelty of the proposed assay is that the combination of graphene adsorption and catalysis of AuNPs can greatly improve the activity of nanozymes, thus enabling the sensitive detection of hydrogen peroxide and glucose. In view of the operation simplicity from the homogeneous liquid phase in this assay, the assay makes it possible to online detect hydrogen peroxide in environmental media and monitor human blood glucose levels. Graphene oxide (GO)/AuNPs nanocomposite was prepared by hydrothermal synthesis. GO/AuNPs nanocomposite exhibits a synergistic catalysis that can mimic the function of peroxidase and catalyze the oxidation of peroxidase substrate TMB by hydrogen peroxide, causing the solution to change from the originally colorless color to blue (the color of oxidized product). The strong activity of GO/AuNPs nanocomposite as peroxidase mimetics was shown in Fig. 1. The catalytic performance of GO/AuNPs presented by the blue color depends on the concentration of hydrogen peroxide. Based on this, a highly sensitive colorimetric detection of hydrogen peroxide was achieved. Due to the synergistic catalysis at the interface between GO and AuNPs, the GO/AuNPs nanozymes have stronger catalytic activity than previous peroxidase mimics using single nanoparticles like Fe_3O_4 nanoparticles and positively-charged AuNPs. The detection limit for the colorimetric detection of hydrogen peroxide using GO/AuNPs nanozymes was 4.2×10^{-8} M, which is about 1–2 orders of magnitude lower than that of other simulated enzymes [8–11]. The results fully confirm that the synergism from GO and AuNPs is an ideal means to enhance the catalytic activity of nanozymes. In addition, combining the catalytic reaction of glucose with glucose oxidase, the proposed colorimetric analysis can be used to indirectly detect glucose by measuring hydrogen peroxide produced by glucose oxidation. Obviously, not only the smart synergetic coupling catalysis but also the environmentally friendly simple preparation gives the GO/AuNPs nanocomposite great application prospects in the fields of biomedical sensing and environmental science.

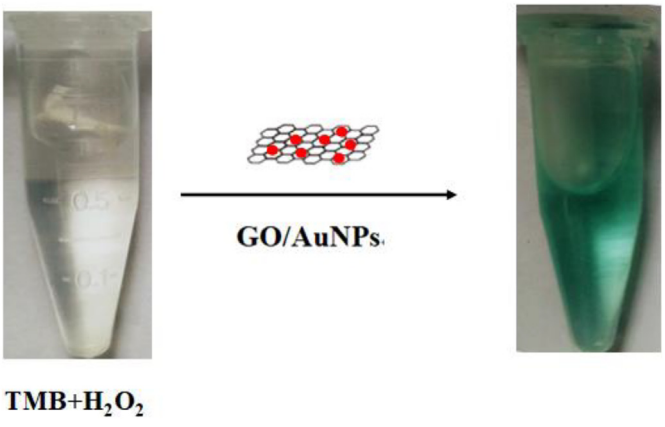


Fig. 1. Strong activity of GO/AuNPs nanocomposite as peroxidase mimetics. Experimental conditions: TMB, 5 mM; H_2O_2, 1 mM; GO/AuNPs, 20 μg/mL. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

2. Experimental section

2.1. Chemicals and materials

Graphite powder (≤300 mesh) was obtained from Tianjin Chemical Reagents Company. Chloroauric acid ($HAuCl_4$) was purchased from Sigma (St. Louis, MO, USA). 3, 3, 5, 5-tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2, 30%) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The working H_2O_2 solutions need to be prepared fresh daily from 30% (w/w) H_2O_2. Glucose oxidase (GOD), glucose, fructose, lactose and maltose were purchased from Shanghai Sangon Biotech Co., Ltd. (Shanghai, China). All other reagents and solvents in this research were purchased in their highest available purity and used without further purification. Millipore Milli-Q water (18 MΩ cm^{-1}) was used in all experiments.

2.2. Instrumentation

A Hitachi U-3900H UV–Visible Spectrophotometer (Tokyo, Japan) was used to scan UV–Visible absorption spectra of the reaction product of TMB at room temperature in the wavelength range from 450 to 800 nm. All the photographs were taken by Olympus C-370 digital camera. The transmission electron microscopy (TEM) of GO/AuNPs nanocomposite was characterized on a JEM-2100 TEM (Japan Electronics Co., Ltd) at room temperature. An HH-1electric-heated thermostatic water bath (Beijing Kewen Instrumental Factory, Beijing, China) was used to control the system temperature at 0.1 °C intervals. A model PB-10 digital ion analyzer (Sartorius Scientific instruments Co., Ltd., China, Beijing) was used for adjusting the pH value of the solution.

2.3. Preparation of GO/AuNPs nanocomposite

Graphene oxide (GO) needs to be prepared first. GO was prepared by an improved Hummers chemical method according to the published protocol [28,29]. The typical experiments are as follows. The dried graphite powder (1.2 g) was evenly dispersed into 25 mL concentrated sulfuric acid and stirred at room temperature for 12 h. Then 3 g $KMnO_4$ was slowly added to the mixture under vigorous stirring in the ice-water bath. The mixture was continuously sonicated for 8 h to obtain a dark-green solution. Next, 45 mL of ultrapure water was added to the resulting dark-green solution and heated to keep boiling for 10 min. After that, 140 mL ultrapure water and 10 mL H_2O_2 (30%) were added successively to terminate the reaction and the resulting bright yellow solution was an aqueous solution of GO. After centrifugation, the product was washed with 5% HCl and ultrapure water for three times. Finally, the solid GO was obtained by vacuum drying. GO/AuNPs nanocomposite was prepared by a simple environment-friendly hydrothermal synthesis [29]. Briefly, 10 mL of 0.5 mg/mL GO aqueous solution, 4 mL of 10 mM $HAuCl_4$ solution and 4 mL of 0.1 M NaOH solution were firstly mixed and diluted to 100 mL. After the mixture was sonicated for 8 h at room temperature, it was transferred to a Teflon-lined autoclave for hydrothermal reaction at 180 °C for 12 h. The as-prepared GO/AuNPs nanocomposites were cooled naturally to room temperature and were stored in the refrigerator (4 °C) and ready for use.

The as-prepared GO/AuNPs nanocomposites were characterized with transmission electron microscopy (TEM) and UV–Visible absorption spectra. The TEM images (Fig. S1) show that in the as-prepared GO/AuNPs nanocomposite, AuNPs were uniformly dispersed and deposited on the graphene surface with the size of about 31 nm. The result of UV–Visible absorption spectra (Fig. S2) also showed that the absorption spectra of GO/AuNPs nanocomposite were significantly different from those of GO and AuNPs, indicating the generation of nanocomposites.

2.4. H_2O_2 detection using GO/AuNPs nanocomposite as peroxidase mimic

A typical colorimetric assay for H_2O_2 detection using the GO/AuNPs nanocomposite as peroxidase mimic is realized as follows. Simply, 300 μL of 5 mM TMB, 70 μL of 20 μg/mL GO/AuNPs and 200 μL of H_2O_2 with different concentrations were mixed with 400 μL of 0.5 M acetate buffer (pH 4.0). The mixed solution was incubated in 50 °C water bath for 8 min and cooled to the room temperature. Then, 250 μL of the reacted solutions were decanted into a slit quartz cuvette of 350 μL volume to be scanned the absorption spectrum at room temperature. The concentration of H_2O_2 was quantified by the absorbance at the wavelength of 655 nm.

2.5. Glucose detection using GO/AuNPs nanocomposite and GOD

The procedure for glucose measurement is as follows. 20 μL of 10.0 mg/mL GOD and 200 μL of glucose with different concentrations in 10 mM phosphate buffer solution (pH 7.4) were incubated at 37 °C water bath for 15 min. Then the above glucose reaction solution were added into the mixed solution of 300 μL of 5 mM TMB, 70 μL of 20 μg/mL GO/AuNPs and 300 μL of 0.5 M acetate buffer (pH 4.0) and incubated in 50 °C water bath for 8 min. The resulting solution was cooled to the room temperature and the adsorption spectroscopy was measured. For the control experiment, the procedure was the same except for replacing glucose with maltose, lactose and fructose, respectively.

3. Results and discussion

3.1. Strong activity of GO/AuNPs nanocomposite as peroxidase mimetics

The GO/AuNPs nanocomposite was prepared by hydrothermal synthesis and was characterized by TEM and UV–Visible absorption spectra. TEM results (see the Supporting Information, Fig. S1) showed that spherical nanoparticles were uniformly dispersed and embedded on the surface of GO and the particle size of nanoparticles was about 31 nm. The results of UV–Visible absorption spectra were shown in Supporting Information, Fig. S2. AuNPs have the characteristic absorption peak at the wavelength of about 520 nm, which is the result of plasma resonance on the surface of AuNPs [30,31]. GO has no apparent absorption peak in the visible region. The GO/AuNPs nanocomposite also has an obvious absorption peak at the corresponding wavelength (about 520 nm), but the absorption intensity is less than that of AuNPs. It indicates that in GO/AuNPs nanocomposite, the nanoparticles of gold have achieved stable growth on the surface of GO. The nanoparticles of gold are scattered on the surface of graphene, so that the absorption spectrum of the GO/AuNPs nanocomposite changes completely different from that of GO and AuNPs. Therefore, GO/AuNPs nanocomposite is a new composite nanomaterial which is different from GO and AuNPs.

The catalytic properties of the GO/AuNPs nanocomposite for the peroxidase substrate TMB oxidation by H_2O_2 were investigated as peroxides mimic enzymes. The results are shown in Fig. 2A. The GO/AuNPs nanocomposite can catalyze the oxidation of TMB by hydrogen peroxide, and the solution appears blue (Fig. 2Ac), which is the color of the oxidation product of TMB. In the absence of GO/AuNPs nanocomposites, the solution's blue color is much lighter and almost colorless (Fig. 2Ab). This demonstrated that TMB was almost not oxidized, and there are few oxidation products of TMB in blue color. The UV–Visible absorption spectra (Fig. 2B) also revealed that the solution with GO/AuNPs nanocomposite has much stronger absorption at the wavelength of 655 nm than the solution without GO/AuNPs nanocomposite. In order to further confirm the peroxidase-mimicking enzyme properties of GO/AuNPs nanocomposites, GO and AuNPs were prepared separately by hydrothermal synthesis for control experiments, and their catalytic effect on the oxidation of TMB by H_2O_2 was surveyed. The results (Fig. 3b, c) found that the solutions in GO alone and AuNPs alone was pale blue color and almost colorless. For the system where GO and AuNPs are mechanically mixed together, the solution is also very pale in color (Fig. 3d). It declared that GO alone, AuNPs alone, or the mechanical mixtures of GO and AuNPs cannot catalyze the oxidation of TMB by hydrogen peroxide. The solution color of the only system with GO/AuNPs nanocomposites is prominent blue (Fig. 3e), indicating that only this new nanocomposite can catalyze the oxidation of TMB. The catalytic properties of the peroxide mimic enzyme are derived from the nanocomposites themselves. The prepared GO/AuNPs nanocomposites do indeed mimic the enzyme properties of peroxides effectively.

In the oxidation reaction with hydrogen peroxide which nanoparticles participate in, hydrogen peroxide can be adsorbed on the surface of nanoparticles [13,32]. The O—O bond of hydrogen peroxide is opened to form HO• free radicals, which can be adsorbed at the nanometer interface through particle mediated electron transfer, thus promoting the oxidation reaction [6,33,34]. For the reaction of TMB oxidation by hydrogen peroxide catalyzed by GO/AuNPs nanocomposites, at the extremely close nano-interface between GO and AuNPs, the adsorption of GO to hydrogen peroxide (from π-rich conjugation surface of GO) and electron transfer mediated by AuNPs have a synergistic coupling effect, thus greatly improving its catalytic oxidation activity. However, the mechanical mixture of GO and AuNPs could not produce such synergistic action, mainly because the bonding between GO and AuNPs in the mechanical mixture was not close enough to perform synergistic catalysis. It can be inferred that the strong property of the peroxide mimic enzyme exhibited by GO/AuNPs nanocomposites is mainly due to the synergistic catalytic effect at the nanometer interface, and the intimate binding in the nanocomposite is an important factor to produce the synergistic catalytic effect.

3.2. Highly sensitive colorimetric detection of hydrogen peroxide

The TMB oxidation with hydrogen peroxide catalyzed by the GO/AuNPs nanocomposite as peroxidase mimic is influenced by the pH value and reaction temperature of the system. Acetic acid buffer solution was used as the reaction medium for the experiment. The results for the influence of pH value of the system found that the TMB oxidation reaction catalyzed by GO/AuNPs nanocomposite is slow and the catalytic activity is weak under neutral and alkaline systems. The catalytic activity of GO/AuNPs nanocomposite for TMB oxidation was the strongest in the acid buffer medium (pH = 4). In acidic systems, the oxygen-containing groups such as epoxy and carboxyl groups on the surface of GO exist in their hydrogen-containing form, which promotes the solubility of GO and increases its amount in solution. Thus, in GO/AuNPs nanocomposite, AuNPs are embedded and dispersed more stably on the surface of GO with more amount in the solution system, so the interface between AuNPs and GO is more conducive to synergistic catalysis. The influence of temperature on the catalytic activity of GO/AuNPs nanocomposite as peroxidase mimic was investigated by measuring the absorbance of the TMB–H_2O_2 system in the absence and presence of GO/AuNPs nanocomposite. As shown in the Supporting Information, Fig. S3a, the ΔA, where ΔA = A (the absorbance in the presence of GO/AuNPs nanocomposite, 655 nm) − A (the absorbance in the absence of GO/AuNPs nanocomposite, 655 nm), reached the maximum at 50 °C. The results found that the catalytic activity was the strongest when the reaction temperature was 50 °C. So, the temperature of the catalytic oxidation reaction was selected as 50 °C. In addition, the reaction time required for TMB oxidation catalyzed by GO/AuNPs nanocomposite was also investigated. The results (see Supporting Information, Fig. S3b) showed that the absorbance at the wavelength of 655 nm increased gradually with the extension of reaction time, and tended to be constant after 8 min. It indicated that the catalytic oxidation of TMB by GO/AuNPs nanocomposite was completed after 8 min. Thus, the TMB oxidation reaction time is determined to be 8 min.

In fact, the catalytic oxidation efficiency of the GO/AuNPs nanocomposite for the enzyme substrate TMB depends on the amount of hydrogen peroxide. The correlation between the absorption spectrum of the

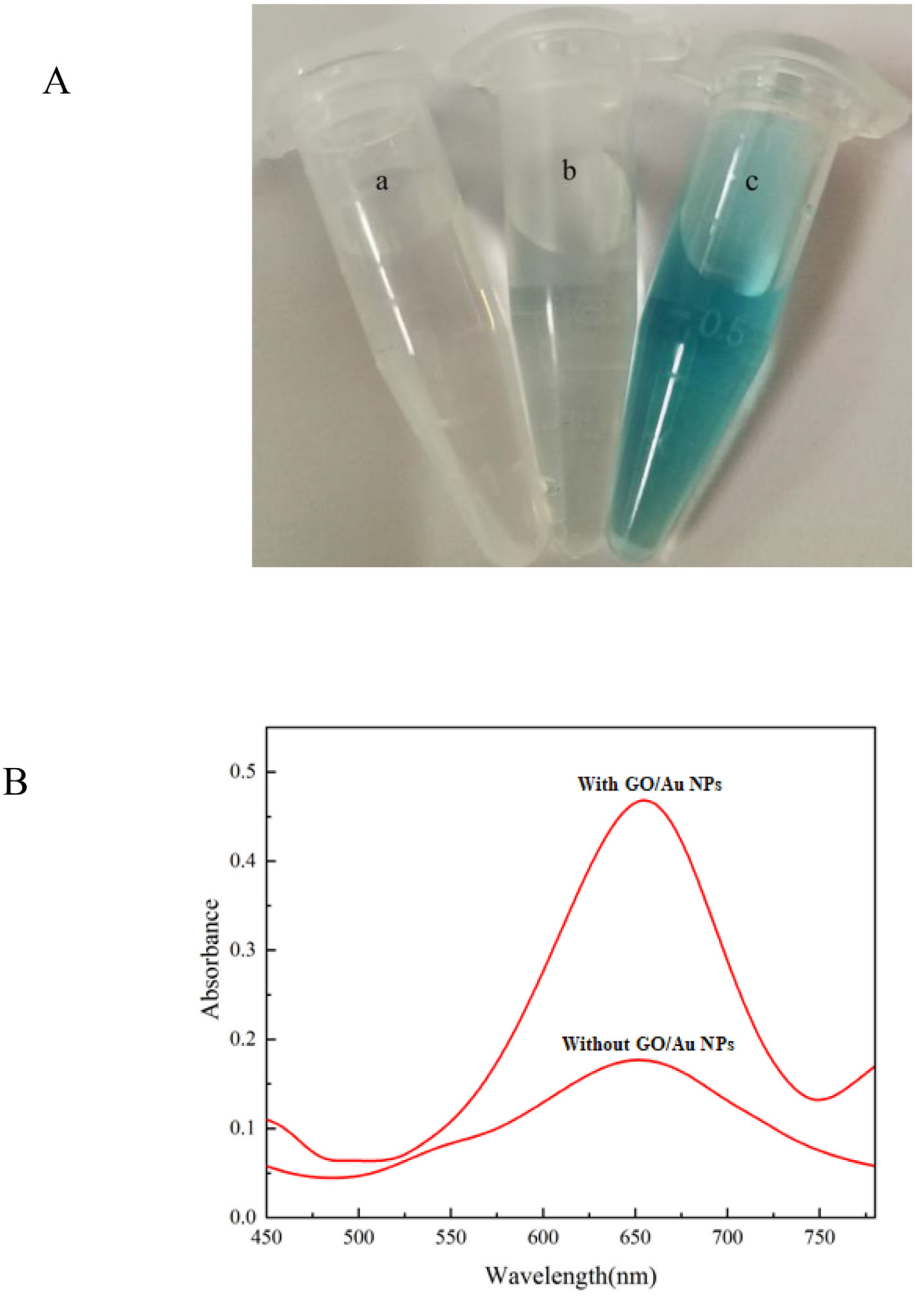


Fig. 2. (A) Typical images for catalytic oxidation of peroxidase substrate TMB: (a) 0 M H_2O_2 with GO/AuNPs, (b) 1 mM H_2O_2 without GO/AuNPs, (c) 1 mM H_2O_2 with GO/AuNPs. (B) UV–Visible absorption spectra of catalytic oxidation solution of TMB by H_2O_2 with and without GO/AuNPs. Experimental conditions: TMB, 5 mM; H_2O_2, 1 mM; GO/AuNPs, 20 μg/mL. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

reaction solution and the amount of hydrogen peroxide in the catalytic oxidation of TMB by GO/AuNPs nanocomposite was investigated. As shown in Fig. 4A, with the increase of hydrogen peroxide concentration, the absorption gradually enhanced at the wavelength of about 655 nm. Under the above optimized experimental conditions, the result found that (Fig. 4B) the concentration of hydrogen peroxide in the range of 3.8×10^{-7}–5.5×10^{-5} M is linearly correlated with the absorbance at 655 nm with the detection limit of 4.2×10^{-8} M. The colorimetric assays using different nanozymes [8–12,35–37] were compared (see Supporting Information, Table S1). For hydrogen peroxide detection, it was about 1–2 orders of magnitude lower than other colorimetric analyses using single nanoparticle like Fe_3O_4 magnetic nanoparticles [8] and positively-charged AuNPs [9] as simulated enzyme. The high sensitivity of the developed colorimetric analysis was attributed to the synergistic catalysis of GO adsorption and AuNPs mediated electron transfer in GO/AuNPs nanocomposites, while single nanoparticles could not produce the synergistic catalysis. This synergistic catalysis significantly increased the catalytic activity of the simulated enzyme and thus increased the sensitivity of colorimetric analysis. Moreover, the specificity for catalytic oxidation of TMB by H_2O_2 using GO/AuNPs nanocomposite as peroxidiase mimic was investigated through different substrate of $K_3Fe(CN)_6$ and dissolved O_2 instead of hydrogen peroxide. Absorption spectra of the reaction solutions from different substrates were shown in Fig. 5. The results found that the absorption from hydrogen peroxide was significantly stronger than that from $K_3Fe(CN)_6$ and dissolved O_2. It indicates that hydrogen peroxide is highly specific to the oxidation of TMB in the catalytic reaction using GO/AuNPs nanocomposites as peroxidiase mimic.

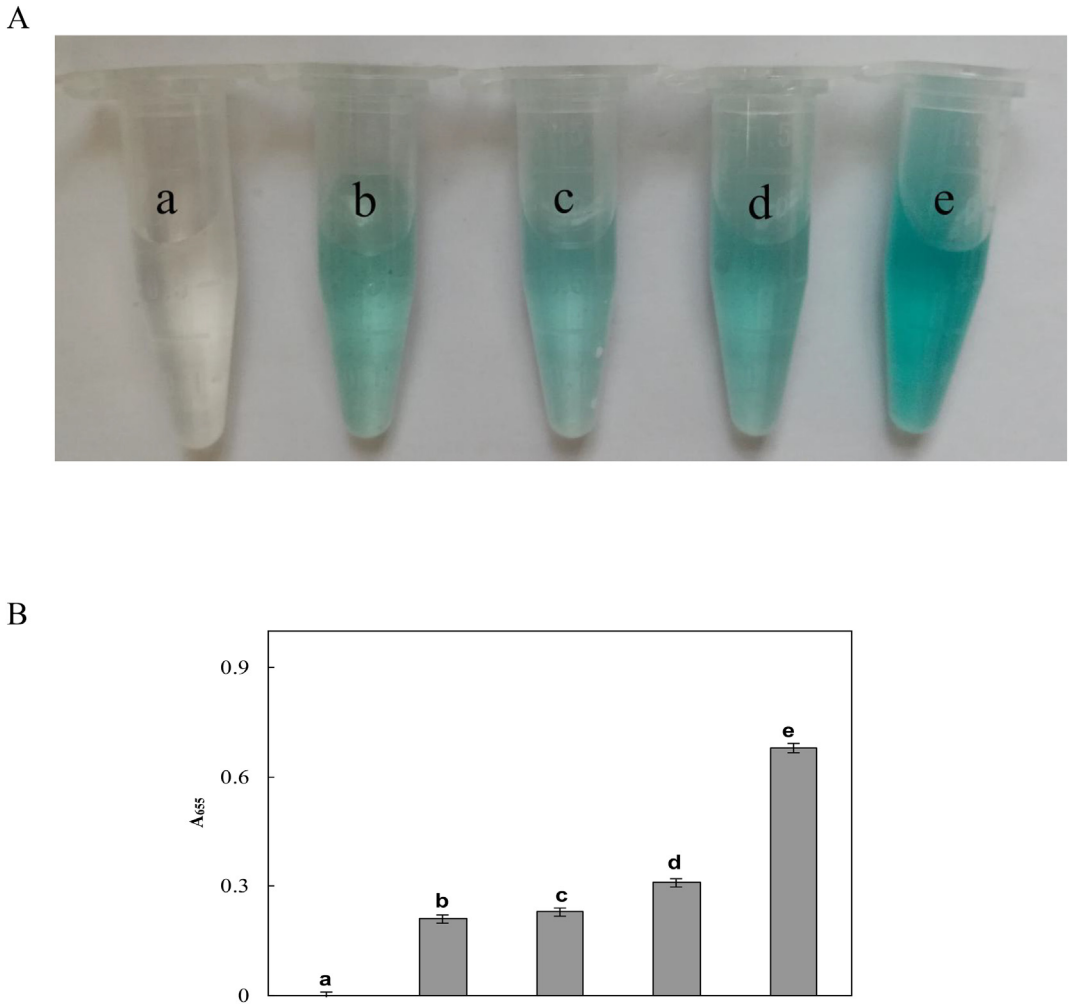


Fig. 3. (A) Images and (B) the corresponding absorption of the oxidation solution of TMB by H_2O_2 under different cases: (a) without nanoparticles, (b) with GO, (c) with AuNPs, (d) with GO+AuNPs mixture, and (e) with GO/AuNPs nanocomposite. Experimental conditions: 5 mM TMB, 1 mM H_2O_2, 0.5 M acetate buffer (pH 4.0). (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

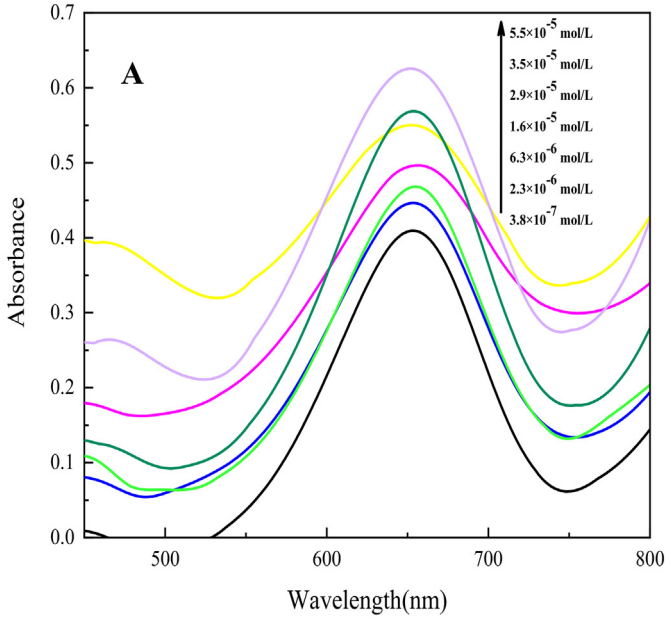
3.3. Highly sensitive colorimetric detection of glucose

As we know, glucose oxidase can oxidize glucose reaction to produce hydrogen peroxide. By detecting the hydrogen peroxide produced from the oxidation reaction of glucose, the colorimetric analysis based on GO/AuNPs nanocomposite used as peroxidase-mimicking enzyme should be able to measure glucose indirectly. The procedure for glucose detection is described in Experimental section. The detection of glucose by the colorimetric analysis using GO/AuNPs nanocomposite for peroxidase catalysis was performed in two processes. (1) Glucose reacts with glucose oxidase to produce hydrogen peroxide. (2) Detection of hydrogen peroxide in glucose oxidation reaction solution by the colorimetric analysis based on GO/AuNPs nanocomposite. The results (Fig. 6A) showed that the concentration of glucose in the range of 5.1×10^{-6}–5.1×10^{-4} M has a good linear relationship with the absorbance at 655 nm, and the detection limit was 6.3×10^{-7} M. The colorimetric assays using different nanozymes for the detection of glucose were compared (see Supporting Information, Table S2). It is also about 1 order of magnitude lower than the colorimetric analysis using single nanoparticle as simulate peroxidase, which further proves the highly efficient catalytic activity of GO/AuNPs nanocomposites due to synergistic effect. Furthermore, fructose, lactose and maltose were used as the control experiments to investigate the selectivity of the method for glucose detection. The results (Fig. 6B) found that under the same test conditions, 3 mM of other sugars showed little response and only 300 μM of glucose solution system appeared to be obvious blue color. The results indicate that the colorimetric analysis has high specificity for glucose detection, which provides the necessary support for the application of this analysis in biological media.

3.4. Analytical application for real samples

In order to evaluate the feasibility of practical application of this colorimetric analysis, the hydrogen peroxide of determined amount and the glucose in the real serum samples were detected respectively by this method, and the recovery experiments of standard addition were carried out. The detection results for hydrogen peroxide are shown in Table 1. For three determined hydrogen peroxide solutions with different concentrations, the detection results from the present method are basically consistent with the known contents. The relative standard deviation (RSD) for three parallel measurements is ≤3.8%, and the recovery rate ranged from 93.3 to 103.3%. The results were satisfactory.

Glucose plays an important role in the life system and glucose content levels in the blood are closely related to people's health. Daily monitoring of blood glucose levels is essential for the care of people with diabetes and the prevention of health hazards caused by hyperglycemia or hypoglycemia. The real human serum was used as test samples to investigate the practical application of the proposed analysis. Human



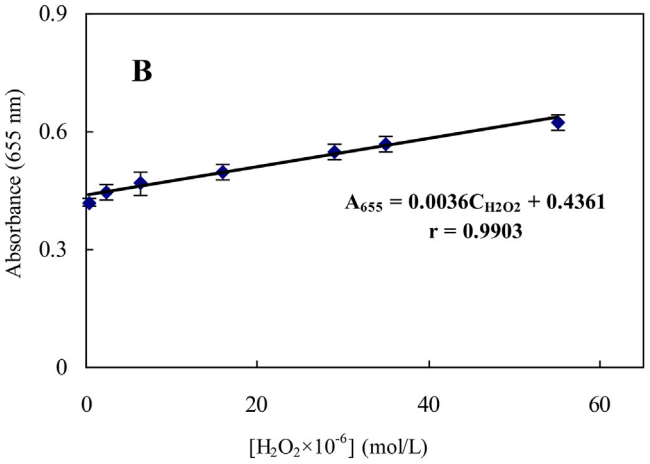


Fig. 4. (A) UV–vis absorption spectra of catalytic oxidation solution of TMB by GO/AuNPs under different concentrations of H_2O_2. (B) The linear calibration curve of the absorbance at 655 nm versus H_2O_2 concentration.

serum samples were taken from Xi'a University of Science and Technology Hospital. As the normal content of human blood glucose is 3.9–6.1 mmol/L [38], before the test, the collected serum samples need to be diluted to meet the requirements of this method after centrifugation. The glucose test results are shown in Table 2. For three different test samples, the detection results from this method are not substantially different from those given by the hospital. The RSD for three parallel measurements of the same test sample was ≤4.6%, and the recovery was from 105.3% to 108.3%. Experimental results demonstrated that the developed analysis is accurate and reliable for the detection of glucose in real serum samples. In light of the simplicity of this colorimetric analysis, it has an excellent application prospect in clinical diagnosis and routine nursing related to blood glucose. Furthermore, the reagents used in this assay are very cheap except for chloroauric acid. The amount of chloroauric acid needed to prepare nanocomposites is very small, and only 4 mL of 10 mM $HAuCl_4$ solution is required for a

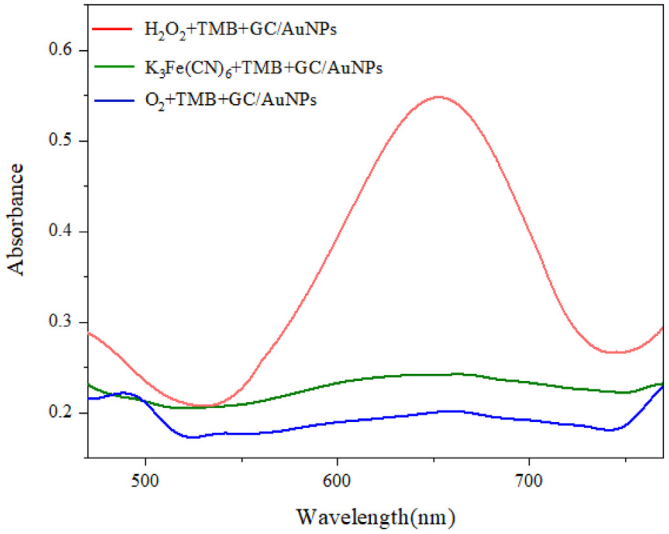


Fig. 5. Absorption spectra of different substrate in the presence of the same amount of TMB and GO/AuNPs nanocomposite.

single synthesis. The GO/AuNPs amount required for the test is also small, with only 70 μL of 20 μg/mL GO/AuNPs needed for a single test. According to the estimation, the cost of a single test should not exceed 0.1 RMB. So, the cost of testing for this assay is very low and suitable for the use of instant care in less developed areas.

4. Conclusions

Herein, we synthesized GO/AuNPs nanocomposites, which demonstrated strong peroxidase-mimicking activity and was able to rapidly and efficiently catalyze the oxidation of enzyme substrate TMB by hydrogen peroxide to produce blue products. Based on this, the colorimetric analysis was established to detect hydrogen peroxide. Combined with the oxidation reaction of glucose oxidase and glucose, the developed colorimetric analysis can also detect glucose in human serum. The advantage of the prepared GO/AuNPs nanocomposites over single nanoparticles in the use of peroxidase simulation is that the synergistic catalysis generated from GO adsorption and AuNPs-mediated electron transfer in GO/AuNPs nanocomposites greatly improves the catalytic activity of the simulated enzymes. In addition to easy synthesis and the superiority of artificial simulated enzyme itself, GO/AuNPs nanocomposite has great potential applications in the fields of biomedicine, biosensors and environmental sciences.

CRediT authorship contribution statement

Yingying Qi: Conceptualization, Methodology, Investigation, Writing - original draft, Validation. **Yiting Chen:** Investigation, Data curation.**Jiahuan He:**Investigation, Visualization, Software.**Xiang Gao:** Supervision, Visualization, Writing - review & editing.

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.

Acknowledgements

This work was supported financially by the National Natural Science Foundation of China (No. 21605018), the Natural Science Basic Research Project of Shaanxi Province of China (No. 2018JM5149), and the

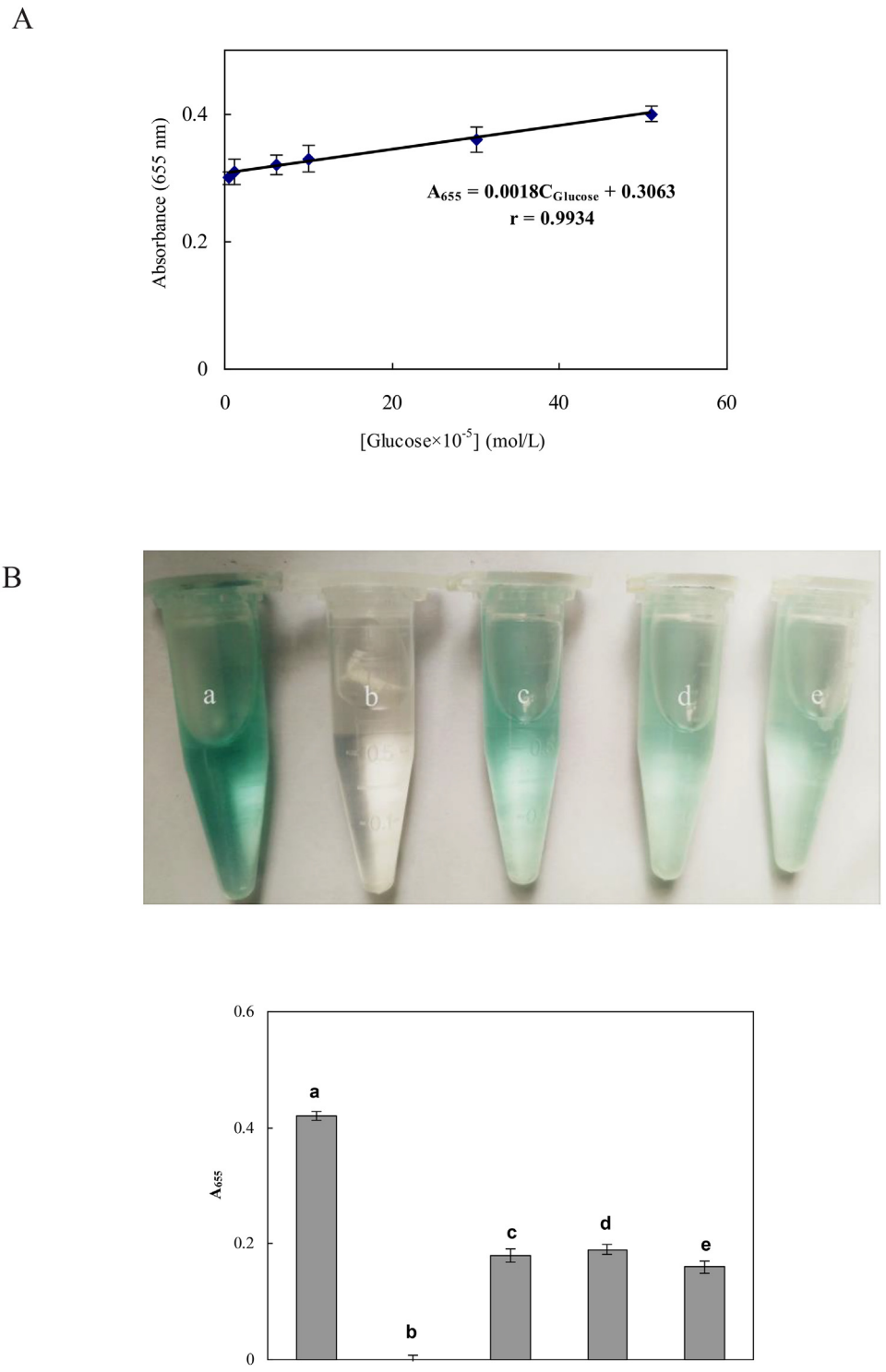


Fig. 6. (A) The linear calibration curve of the absorbance at 655 nm versus glucose concentration. (B) Typical photographs and (C) the corresponding absorption for glucose detection with the present colorimetric method using GOD and GO/AuNPs nanocomposite: (a) 300 μmol/L glucose, (b) buffer, (c) 3 mmol/L maltose, (d) 3 mmol/L lactose and (e) 3 mmol/L fructose.

Table 1
Determination of hydrogen peroxide by the GO/AuNPs colorimetric assay.

Samples	Standard content (mol/L)	Present method (mol/L)	RSD %	Added (mol/L)	Found (mol/L)	Recovery %	RSD %
1	3.0×10^{-6}	2.92×10^{-6}	3.3	1.0×10^{-6}	3.93×10^{-6}	93.3	2.9
2	1.0×10^{-5}	1.04×10^{-5}	2.8	1.0×10^{-6}	2.09×10^{-5}	94.8	2.1
3	3.0×10^{-5}	2.93×10^{-5}	3.8	1.0×10^{-5}	4.03×10^{-5}	103.3	3.0

Table 2
Analytical results of glucose detection in human serum ($n = 3$).

Samples	Local hospital (mM)	Present method (mM)	RSD %	Added (mM)	Found (mM)	Recovery %	RSD %
1	4.20	4.39	4.5	1.0	5.28	108.3	3.9
2	5.12	5.33	4.2	1.0	6.18	106.2	4.3
3	6.33	6.13	4.6	1.0	7.38	105.3	4.1

Foundation Research Project of Shaanxi Provincial Key Laboratory of Geological Support for Coal Green Exploitation (No. MTy2019-05).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2020.118233.

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