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Technical Notes

Fe_3O_4 Magnetic Nanoparticles as Peroxidase Mimetics and Their Applications in H_2O_2 and Glucose Detection

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Artificial enzyme mimetics are a current research interest because natural enzymes bear some serious disadvantages, such as their catalytic activity can be easily inhibited and they can be digested by proteases. A very recently study reported by Yan et al. has proven that Fe_3O_4 magnetic nanoparticles (MNPs) exhibit an intrinsic enzyme mimetic activity similar to that found in natural peroxidases, though MNPs are usually thought to be biological and chemical inert (Gao, L. Z.; Zhuang, J.; Nie, L.; Zhang, J. B.; Zhang, Y.; Gu, N.; Wang, T. H.; Feng, J.; Yang, D. L.; Perrett, S.; Yan, X. Y. *Nat. Nanotechnol.* 2007, *2*, 577−583). In the present work, we just make use of the novel properties of Fe_3O_4 MNPs as peroxidase mimetics reported by Yan et al. to detect H_2O_2. The Fe_3O_4 MNPs were prepared via a coprecipitation method. The as-prepared Fe_3O_4 MNPs were then used to catalyze the oxidation of a peroxidase substrate 2,2′-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS) by H_2O_2 to the oxidized colored product (see eq 1) which provides a colorimetric detection of H_2O_2. As low as 3×10^{-6} mol/L H_2O_2 could be detected with a linear range from 5×10^{-6} to 1×10^{-4} mol/L via our method. More importantly, a sensitive and selective method for glucose detection was developed using glucose oxidase (GOx) and the as-prepared Fe_3O_4 MNPs. The detection platforms for H_2O_2 and glucose developed in the present work not only further confirmed that the Fe_3O_4 MNPs possess intrinsic peroxidase-like activity but also showed great potential applications in varieties of simple, robust, and easy-to-make analytical approaches in the future.

Natural enzymes have been extensively studied for more than 200 years and play central roles in biochemistry.[1] Besides their fundamental importance, natural enzymes also have significant practical applications in medicine, chemical industry, food processing, and agriculture. Natural enzymes as biological catalysts possess remarkable advantages such as high substrate specificities and high efficiency under mild conditions. All natural enzymes are proteins except for a small group of catalytic RNA molecules (i.e., ribozymes). Thus, natural enzymes bear some serious disadvantages such as (a) they can be easily denatured by environmental changes since their catalytic activity depends on the integrity of their native protein conformation; (b) they can be digested by proteases; (c) the preparation and purification are usually time-consuming and expensive.[1] Therefore, a lot of effort has been made to extend the natural enzymes to enzyme mimetics.[2−4]

Through in vitro selection, non-natural ribozymes and deoxyribozymes (catalytic DNA molecules) have been developed.[5−8] Many other enzyme mimetics have also been developed,[9−17] such as cytochrome P450 mimetics,[18−20] serine proteases mimetics,[21] dioxygenase mimetics,[22,23] phosphodiesterase mimetics,[24]

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ligase mimetics,[25] nuclease mimetics,[26] and methanogenesis mimetics.[27] Among these, a lot of research has been focused on peroxidase mimetics including hemin,[20,28,29] hemeatin,[30] hemoglobin,[31] cyclodextrin,[32] and porphyrin,[33,34] which have been used for H_2O_2 and ascorbic acid detection.[30,31,35]

Because of both their fundamental and technological interest and importance, nanomaterials, especially functional nanomaterials, have received considerable attention in recent years.[36−39] Because of their large surface-to-volume ratio, nanomaterials are attractive to use as high-efficiency catalysts and have been extensively studied during the past decade.[39,40] Both homogeneous and heterogeneous catalysis can be realized using transition metal nanomaterials.[39] Magnetic nanomaterials (such as Fe_3O_4) have been extensively studied and used in magnetic resonance imaging, drug delivery, biological separation, and even in biological catalysis.[41−43] As magnetic nanomaterials themselves are usually thought to be chemically and biologically inert, they are typically coated with metal catalysts, enzymes, or antibodies to increase their functionality. When magnetic nanomaterials are used as biological catalysts, dual-functional nanoparticles are usually designed, where magnetic nanoparticles cores are coated with enzymes or metal catalysts. For example, biological catalytic and magnetic separation immunoassay systems have been fabricated using horseradish peroxidase (HRP)-entrapped magnetite-containing spherical silica nanoparticles.[43] Unexpectedly, Yan and coworkers have found that Fe_3O_4 magnetic nanoparticles (MNPs) in fact exhibit an intrinsic enzyme mimetic activity similar to that found in natural peroxidases very recently.[44]

In this work, we make use of the novel properties of Fe_3O_4 MNPs as peroxidase mimetics developed by Yan et al. to detect H_2O_2.[44] The Fe_3O_4 MNPs were prepared via a coprecipitation method. The as-prepared Fe_3O_4 MNPs were then used to catalyze the oxidation of a peroxidase substrate 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) by H_2O_2 to the oxidized colored product (see eq 1) which provides a colorimetric detection of H_2O_2.

$$H_2O_2 + \text{ABTS} \xrightarrow{Fe_3O_4} 2H_2O + \text{oxidized ABTS} \quad (1)$$

$$O_2 + \text{glucose} \xrightarrow{\text{GOx}} H_2O_2 + \text{gluconic acid} \quad (2)$$

Detection of glucose has been paid attention to more and more in biomedical fields and plays an increasingly important role in the improvement of life quality.[45] Combination of the catalytic reaction of glucose with glucose oxidase (GOx) (eq 2) and the Fe_3O_4 MNPs catalytic reaction (eq 1), a colorimetric method for glucose detection was also developed in this work. The developed method exhibited sensitive and selective response toward glucose detection.

EXPERIMENTAL SECTION

Chemicals and Materials. Ferric chloride, ferrous chloride, ammonium hydroxide (25−28 wt %), and 30% H_2O_2 were purchased from Beijing Chemical Reagent Company (Beijing, China). 2,2′-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS), β-D-glucose, maltose, α-lactose, D-fructose, and glucose oxidase (from *Aspergillus niger*, GOx) were purchased from Sigma-Aldrich (Milwaukee, WI). Other reagents and chemicals were at least analytical reagent grade. The water used throughout all experiments was purified by a Milli-Q system (Millipore, Bedford, MA).

Instrumentation. Absorption spectra were recorded on a Cary 500 scan UV−vis−NIR spectrophotometer (Varian, Harbor City, CA). Total Fe concentrations of the Fe_3O_4 MNPs colloidal solutions used were determined using an iCAP 6000 inductively coupled plasma optical emission spectroscopy spectrometer (ICP-OES) (Thermo).

Preparation of Fe_3O_4 MNPs. The Fe_3O_4 MNPs were prepared via a previously reported coprecipitation method.[46] First, 50 mL of 1 M ferric chloride aqueous solution and 10 mL of 2 M ferrous chloride aqueous solution in 2 M HCl were mixed and deoxygenated by purging with nitrogen gas for at least 10 min. Second, the mixed solution of ferrous and ferric salts were then added dropwise into 500 mL of 0.7 M oxygen-free ammonia solution under vigorous stirring for 30 min at room temperature in a nitrogen atmosphere. The formed black Fe_3O_4 colloidal particles were separated by centrifugation and further washed with

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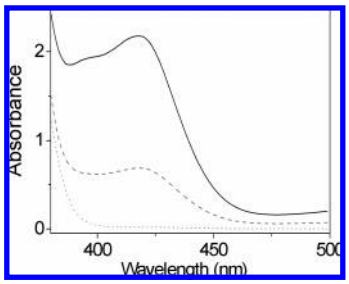

Figure 1. Typical absorption curves of ABTS reaction solutions catalytically oxidized by the as-prepared Fe_3O_4 MNPs in the presence of H_2O_2 incubated at 45 °C in pH 4.0 buffer (—, 100 mM H_2O_2 with Fe_3O_4; – – –, 100 mM H_2O_2 without Fe_3O_4; ···, 0 mM H_2O_2 with Fe_3O_4).

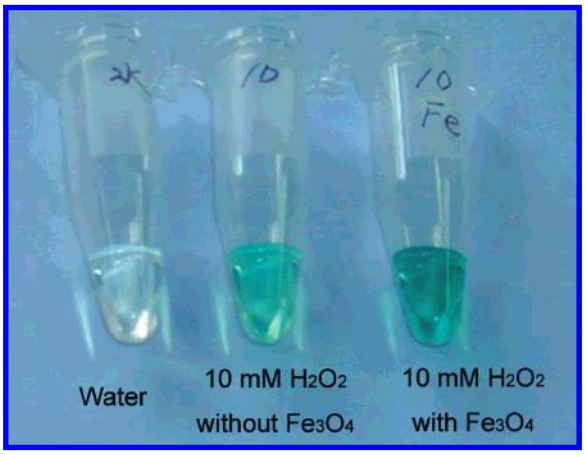

Figure 2. Typical photographs of 24 μL of 60 mM ABTS reaction solutions catalytically oxidized by the as-prepared Fe_3O_4 MNPs in the presence of H_2O_2 incubated at 45 °C in 185 μL of 0.2 M pH 4.0 acetate buffer (from left to right: 0 mM H_2O_2 with Fe_3O_4, 10 mM H_2O_2 without Fe_3O_4, 10 mM H_2O_2 with Fe_3O_4.).

water three times. The Fe_3O_4 MNPs were then redispersed in water and stored at room temperature for use (referred to as the Fe_3O_4 MNPs stock solution with a total Fe concentration of 3.737 ± 0.007 mg/mL).

H_2O_2 Detection Using Fe_3O_4 MNPs as Peroxidase Mimetics. To investigate the peroxidase-like activity of the as-prepared Fe_3O_4 MNPs, the catalytic oxidation of the peroxidase substrate ABTS in the presence of H_2O_2 was tested. In a typical experiment (referred to as Test I), (a) 24 μL of 60 mM ABTS, 10 μL of the Fe_3O_4 MNPs stock solution, and 24 μL of 100 mM H_2O_2 were added into 185 μL of 0.2 M acetate buffer (pH = 4.0); (b) the mixed solution was incubated in a 45 °C water bath for 10 min; (c) the Fe_3O_4 MNPs were then removed from the reaction solution by an external magnetic field, (d) 100 μL of the resulting reaction solution without Fe_3O_4 MNPs was added to 900 μL of water, mixed, and used for adsorption spectroscopy measurement.

To examine the influence of reaction buffer pH on the Fe_3O_4 MNPs activity, 0.2 M acetate buffer solutions from pH 2.0 to 12.0 were investigated, under conditions identical to those used for Test I.

To examine the influence of incubation temperature on the Fe_3O_4 MNPs activity, catalytic reactions incubated in different temperature water baths from 20 to 60 °C were investigated, under conditions identical to those used for Test I. As control experiments, 10 μL of water was used instead of 10 μL of Fe_3O_4 MNPs.

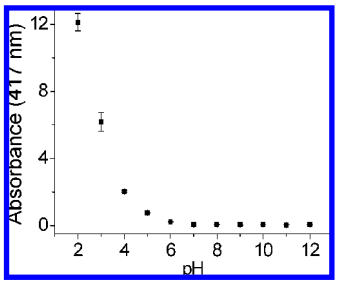

Figure 3. A pH dependent response curve for H_2O_2 detection using the as-prepared Fe_3O_4 MNPs incubated at 45 °C. The error bars represent the standard deviation of three measurements.

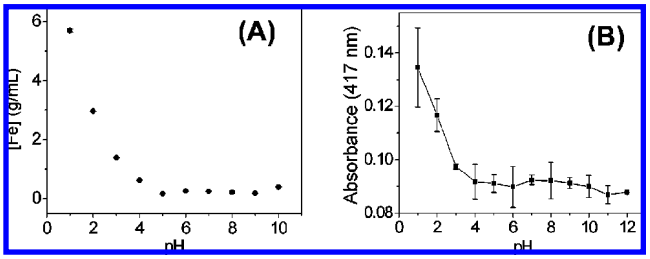

Figure 4. (A) The pH dependence of the Fe leaching from the as-prepared Fe_3O_4 MNPs and (B) the response curve for H_2O_2 detection using the Fe leaching from the as-prepared Fe_3O_4 MNPs incubated at 45 °C in different pH solutions.

Leaching of Iron Ions. To investigate the leaching of iron ions from the Fe_3O_4 MNPs in the reaction buffer solution, the Fe_3O_4 MNPs were incubated in the standard reaction buffer solutions with different pH values for 10 min, then the Fe_3O_4 MNPs were removed by an external magnetic field. The catalytic activity of the leaching solutions was tested under the same conditions for Test I.

The iron contents of the leaching solutions were analyzed using ICP-OES.

Glucose Detection Using GOx and Fe_3O_4 MNPs. Glucose detection was realized as follows: (referred to as Test II) (a) 20 μL of 20 mg/mL GOx and 200 μL of glucose of different concentrations in 10 mM phosphate buffered saline (PBS, pH 7.0) were incubated at 37 °C for 30 min; (b) 24 μL of 60 mM ABTS, 10 μL of the Fe_3O_4 MNPs stock solution, and 800 μL of 0.2 M acetate buffer (pH = 4.0) were added into the above 220 μL glucose reaction solution; (c) the mixed solution was incubated in a 45 °C water bath for 10 min, and the Fe_3O_4 MNPs were then removed from the reaction solution by an external magnetic field, (d) 900 μL of the final reaction solution was used to perform the adsorption spectroscopy measurement.

In control experiments, 5 mM maltose, 5 mM lactose, and 5 mM fructose were used instead of glucose, under conditions identical to those used for Test II.

RESULTS AND DISCUSSION

The As-Prepared Fe_3O_4 MNPs as Peroxidase Mimetics and Their Use in H_2O_2 Detection. To investigate the peroxidase-like activity of the as-prepared Fe_3O_4 MNPs, the catalytic oxidation of peroxidase substrate ABTS in the presence of H_2O_2 was tested. As shown in Figures 1 and 2, the as-prepared Fe_3O_4 MNPs indeed exhibited a catalytic behavior toward ABTS oxidation by H_2O_2. The absorption spectra indicated that the presence of the as-

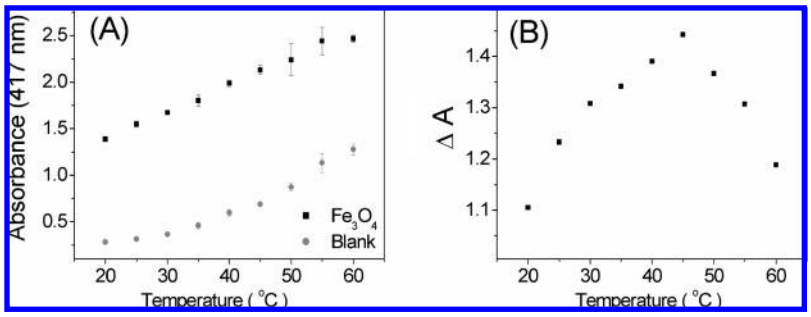

Figure 5. (A) Temperature−response curves for H_2O_2 detection in the absence (●) and presence (■) of the as-prepared Fe_3O_4 MNPs and (B) temperature−ΔA curve for H_2O_2 detection where $\Delta A = A(Fe_3O_4, 417\ nm) - A(blank, 417\ nm)$. The error bars represent the standard deviation of three measurements.

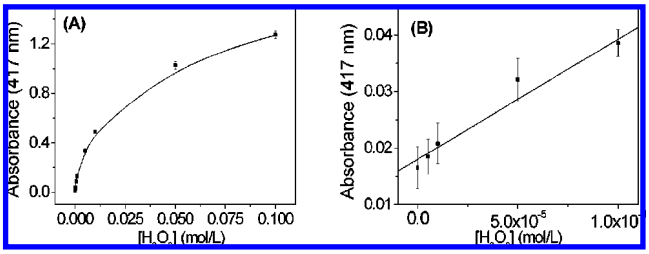

Figure 6. (A) A dose−response curve for H_2O_2 detection using the as-prepared Fe_3O_4 MNPs as artificial enzymes and (B) the linear calibration plot for H_2O_2. The error bars represent the standard deviation of three measurements.

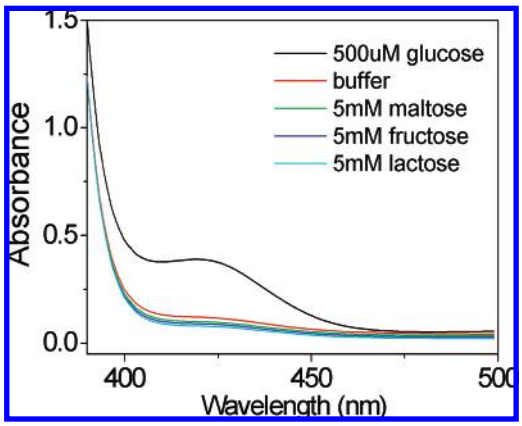

Figure 7. Typical absorption profiles for glucose detection with the colorimetric method developed using GOx and the as-prepared Fe_3O_4 MNPs (black line, 500 μM glucose; red line, buffer; green line, 5 mM maltose; blue line, 5 mM fructose, and cyan line, 5 mM lactose.).

prepared Fe_3O_4 MNPs gave a 320% response when compared with the one in the absence of the as-prepared Fe_3O_4 MNPs (Figure 1). It should be noted that ABTS could be oxidized by H_2O_2 in the absence of any catalysts under our conditions. This phenomenon was different from that in Yan's previous study where the peroxidase substrate 3,3,5,5-tetramethylbenzidine (TMB) could not be readily oxidized by H_2O_2 in the absence of the catalyst Fe_3O_4 MNPs.[44] Also, the difference between our result and Yan's might be assigned to the nature and concentrations of the two peroxidase substrates used (see Figure S1 in Supporting Information). However, the Fe_3O_4 MNPs indeed gave higher response and showed catalytic activity toward ABTS oxidation by H_2O_2 in the current study, which was consistent with Yan's early work[44] (Figures 1 and 2).

As previously reported, the catalytic activity of the Fe_3O_4 MNPs is dependent on pH and temperature.[44] Thus, the pH and

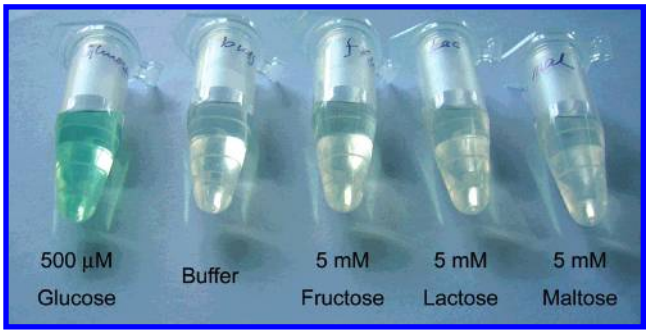

Figure 8. Typical photographs for glucose detection with the colorimetric method developed using GOx and the as-prepared Fe_3O_4 MNPs (from left to right: 500 μM glucose, buffer, 5 mM fructose, 5 mM lactose, and 5 mM maltose.).

temperature dependent-activity of the Fe_3O_4 MNPs was investigated in the current study. The reaction solution pH-dependent response curves are shown in Figure 3. The catalytic oxidation of ABTS with H_2O_2 using the Fe_3O_4 MNPs was much faster in acidic solutions than in neutral or basic solutions. Because (1) the Fenton's reagent (i.e., Fe^{2+}/Fe^{3+} ions in solution) can catalyze the breakdown of H_2O_2 and the iron ions contained in the Fe_3O_4 MNPs might leach into the reaction buffer solution, and (2) Yan et al. suggested that Fe^{2+} ions in the Fe_3O_4 MNPs may play a dominant role in the catalytic peroxidase-like activity of Fe_3O_4 MNPs,[44] the further control experiments were performed to confirm the catalytic activity originated from the Fe_3O_4 MNPs. The amount of iron ions that leached was determined by ICP-OES. As shown in Figure 4A, the iron ions indeed could be leached from the Fe_3O_4 MNPs in an acid reaction solution. When pH $\geq$ 4.0, the amount of iron ions that leached was lower than the concentration required for the Fenton reaction.[44,47] The catalytic activity of the iron ions that leached was further tested. As shown in Figure 4B, when first incubated in pH $\leq$ 3.0 buffer solutions, the catalytic activity could be detected. When first incubated in pH $\geq$ 4.0 buffer solutions, nearly no catalytic activity could be detected. Both the results indicated that the as-prepared Fe_3O_4 MNPs were stable in pH $\geq$ 4.0 buffer solutions. Thus, the 0.2 M pH 4.0 acetate buffer solution was taken as the optimal reaction solution to get a high catalytic activity from the as-prepared Fe_3O_4 MNPs and to ensure that the catalytic activity originated from Fe_3O_4 MNPs themselves and not from the Fe^{2+} ions leached.

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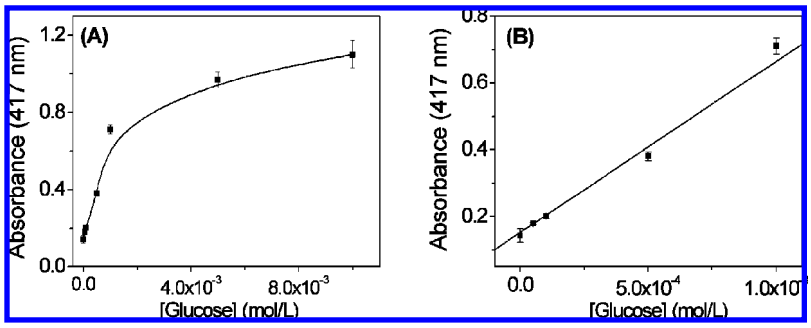

Figure 9. (A) A dose−response curve for glucose detection using GOx and the as-prepared Fe_3O_4 MNPs and (B) the linear calibration plot for glucose. The error bars represent the standard deviation of three measurements.

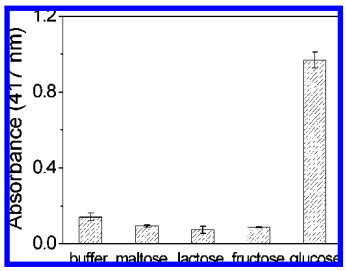

Figure 10. Specificity analysis of the colorimetric method for glucose detection (from left to right: buffer, 5 mM maltose, 5 mM lactose, 5 mM fructose, and 5 mM glucose). The error bars represent the standard deviation of three measurements.

The reaction temperature-dependent response curves were shown in Figure 5. Both in the absence and presence of the Fe_3O_4 MNPs, the signals increased as the reaction temperature increased (Figure 5A). This was different from the previous results where a peak value was obtained at 40 °C.[44] However, when the ΔA, where $\Delta A = A(Fe_3O_4, 417\ nm) - A(blank, 417\ nm)$, was used for plotting, a peak value could be obtained at 45 °C (Figure 5B). Thus, 45 °C was taken as the optimal reaction temperature.

Because the catalytic activity of the Fe_3O_4 MNPs is H_2O_2 concentration dependent (eq 1), the system discussed above could be used to detect H_2O_2. Under the optimal conditions (i.e., 45 °C, 0.2 M pH = 4.0 acetate buffer), the method developed was used for H_2O_2 detection. Figure 6A shows a typical H_2O_2 concentration−response curve where as low as 3×10^{-6} mol/L H_2O_2 could be detected with a linear range from 5×10^{-6} to 1×10^{-4} mol/L (Figure 6B).

Glucose Detection Using GOx and the As-Prepared Fe_3O_4 MNPs. When the catalytic reaction shown in eq 1 is coupled with the glucose catalytic reaction by GOx (eq 2), colorimetric glucose detection could be readily realized. A typical absorption profile for glucose detection using the colorimetric method developed is shown in Figure 7. Because GOx could be denatured in pH 4.0 buffer solution, the glucose detection was performed in two separated steps as mentioned in the experimental section. When the reaction of eq 2 was finished in a pH 7.0 buffer solution, the H_2O_2 produced by the glucose oxidation with GOx was detected using the as-prepared Fe_3O_4 MNPs (eq 1). Therefore, colorimetric detection of glucose could be easily realized using our colorimetric method (Figure 8). Figure 9A shows a typical glucose concentration−response curve where as low as 3×10^{-5} mol/L glucose could be detected with a linear range from 5×10^{-5} to 1×10^{-3} mol/L (Figure 9B).

For testing if the detection of glucose is specific, control experiments were taken using fructose, lactose, and maltose. The selectivity of the colorimetric method is shown in Figure 10 (also see Figure 8). As high as 5 mM control samples were investigated, and no detectable signals were obtained. Thus the colorimetric method developed here showed high selectivity toward glucose detection.

CONCLUSION

In summary, Fe_3O_4 MNPs were prepared and investigated as peroxidase mimetics. The catalytic oxidation of peroxidase substrate ABTS with H_2O_2 using the Fe_3O_4 MNPs was realized. The Fe_3O_4 MNPs as peroxidase mimetics provide a colorimetric assay for H_2O_2. The colorimetric method showed good response toward H_2O_2 detection with a linear range from 5×10^{-6} to 1×10^{-4} mol/L. More importantly, a sensitive and selective analytical platform for glucose detection was fabricated using glucose oxidase (GOx) and the as-prepared Fe_3O_4 MNPs. The analytical platform developed exhibited sensitive and selective detection of glucose with a linear range from 5×10^{-5} to 1×10^{-3} mol/L. Because the Fe_3O_4 MNPs can rival natural enzymes due to their easy preparation, robustness, and stability in rough conditions, the analytical platform for the detection of H_2O_2 and glucose developed further here not only confirmed that the Fe_3O_4 MNPs possess intrinsic peroxidase-like activity but also showed great potential applications in varieties of simple, robust, cost-effective, and easy-to-make biosensors in the future.

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SUPPORTING INFORMATION AVAILABLE

Absorption curves of TMB catalytically oxidized by the as-prepared Fe_3O_4 MNPs and chemical structures of ABTS and TMB. This material is available free of charge via the Internet at http://pubs.acs.org.

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