Peroxidase-like activity of FeVO₄ nanobelts and its analytical application for optical detection of hydrogen peroxide

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Abstract: In this paper, FeVO_4 materials were prepared via a facile hydrothermal method under different pHs and were developed as highly efficient biomimetic catalysts for the first time. Different FeVO_4 materials with different crystal structures, morphologies and surface properties showed distinct peroxidase-like activities. Experimental results showed that the products obtained at $\text{pH} = 4$ ($\text{FeVO}_4\text{-4}$) possessed a belt-like nanostructure with a large BET specific surface area and exhibited the best intrinsic peroxidase-like activity compared to other FeVO_4 materials. $\text{FeVO}_4\text{-4}$ nanobelts (NBs) could efficiently catalyze the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) in the presence of H_2O_2 to generate a blue oxide. Based on the highly efficient catalytic activity of $\text{FeVO}_4\text{-4}$ NBs, a novel system for optical determination of H_2O_2 was successfully established, and the detection limit of H_2O_2 could reach $0.2\ \mu\text{M}$. $\text{FeVO}_4\text{-4}$ NBs also exhibited excellent selectivity, reproducibility, long-term stability, and easy recovery property. Furthermore, a peroxidase mimetic mechanism of $\text{FeVO}_4\text{-4}$ NBs was proposed based on the active species trapping experiments. This work developed a novel, accessible and highly sensitive system for visual detection of H_2O_2 , making $\text{FeVO}_4\text{-4}$ NBs a potential biomimetic catalysts in H_2O_2 detection and biomedical analysis.

Keywords: FeVO_4 nanobelts; Peroxidase-like activity; Optical determination; Hydrogen peroxide

1. Introduction

For living organisms, enzymes are involved in numerous metabolic activities. Due to their strong selectivity for substrate and high efficiency under optimum conditions, natural enzymes have been widely used in various fields of production and processes, such as chemical industry [1, 2], biosensor [1, 2], pharmaceutical processes [1, 2], and food processing [1, 2]. However, since most of the natural enzymes are proteins (a few are catalytic RNA molecules), they can be decomposed easily and lose enzyme activity in extreme external environments, such as under strong acid, strong alkali and high temperature conditions [3]. In addition, the high cost of separation, extraction and purification also greatly limits the use of natural enzymes in production [1-5]. Therefore, researchers have been focusing on developing novel and efficient mimetic enzymes.

Until now, a variety of mimetic enzymes have been developed, and a large portion of these enzyme mimetics are peroxidase-like, such as nanomaterials [6], cyclodextrin [7], Schiff base complex [8], and DNA complexes [9]. Among them, nanomaterials are attracting lots of attention due to their similarities to natural enzymes in the size, shape and surface charge [10, 11]. Moreover, the large specific surface areas, more surface activation centers, and highly efficient catalytic activities of nanomaterials greatly enhance their mimetic performances [1, 4]. Furthermore, nanomaterials mimetic enzymes have some other advantages over natural enzymes, such as simple synthesis process, low cost, good stability and efficient enzymatic activity under extreme environmental conditions, structure and morphology diversity, and satisfying repeatability and recyclability [12, 13]. Therefore, nanomaterials mimetic enzymes are becoming a research focus and have been used in biology, medicine, environment and other fields [12, 13]. Since Gao et al. [14] reported the simulated peroxide enzyme properties of ferroferric oxide nanoparticles in 2007, studies in nanomaterials mimetic enzymes have been widely developed and many novel nanomaterials have been reported in recent years, including graphene oxide [15], V_2O_5 [6], $AgVO_3$ [16], $MnSe$ [4], and Co-Al layered double hydroxides [10].

Although the developed nanomaterials mimetic enzymes have wide and favorable prospects,

their application is limited by the difficulty in separation, recyclability and stability [17]. Therefore, developing enzyme mimetics with easy separation and recyclability, high catalytic activity, and favored stability becomes increasingly important. Recently, iron-containing nanomaterials have received a lot of attentions due to their magnetic property and high catalytic activity, such as iron oxide magnetic nanoparticles [14], ZnFe_2O_4 magnetic nanoparticles [18], $\text{Fe}_3\text{H}_9(\text{PO}_4)_6 \cdot 6\text{H}_2\text{O}$ hierarchical crystals [11], CoFe layered double hydroxide nanoplates [19], Fe (III)-based coordination polymer nanoparticles [20], graphene oxide- Fe_3O_4 magnetic nanocomposites [21], and FeSe-Pt@ SiO_2 nanospheres [22]. As reported, these iron-containing nanomaterials not only exhibited significant and efficient peroxidase-like activities, but also showed superior recoverability and reusability owing to their magnetic property, which greatly improved their application under various conditions, providing a new approach to develop more suitable peroxidase mimetics.

With distinct features of electron structure, chemical stability and ferromagnetism property, FeVO_4 has been widely used in sensors, lithium battery electrode materials and catalysts, attracting great interests recently [23]. However, the potential application prospect of FeVO_4 materials in the field of biology and immunoassay has not yet been fully developed or utilized. Owing to the magnetic property for easy recycle and high catalytic performance, FeVO_4 is expected to be a good candidate for peroxidase mimetics [24]. Hence, in this study, FeVO_4 materials with different morphologies were prepared via a facile hydrothermal method and were used to detect hydrogen peroxide. Analysis of enzyme catalytic properties including enzyme catalytic kinetics, catalytic mechanism, the relationship between morphology and catalytic performance, and repeatability and stability were also performed. Benefitting from the easy and convenient operation, sensitivity, high selectivity, and superior recoverability and reusability, FeVO_4 materials could have great potential applications in biotechnology, medicine and environmental protection in the future.

2. Experimental section

2.1. Reagents and materials

3,3',5,5'-tetramethylbenzidine (TMB) was purchased from Sigma-Aldrich (Shanghai, China). $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, NH_4VO_3 , NaOH, H_2O_2 (30 wt%), ethanol, isopropanol alcohol (IPA), dopamine hydrochloride, L-ascorbic acid, citric acid, glucose, and other chemicals were all of analytical grade and were obtained from Sinopharm Chemical Reagent Co., Ltd. (China). Lircon antiseptic liquid was purchased from Shandong Lierkang Disinfection Technology Co., Ltd. (Dezhou, China). All aqueous solutions were prepared with Milli-Q water (Millipore, USA).

2.2. Preparation of FeVO_4

FeVO_4 samples were synthesized through a facile hydrothermal method. In a typical procedure, 1.0 mmol of $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ was added into 30 mL of Milli-Q water under magnetic stirring. Meanwhile, 1.0 mmol of NH_4VO_3 was dissolved in 30 mL of Milli-Q water under magnetic stirring and 80 °C heating to obtain a transparent solution. Then the NH_4VO_3 solution was subsequently added dropwise into the $\text{Fe}(\text{NO}_3)_3$ solutions slowly under vigorous stirring to form a final suspension. Solution pH was then adjusted to 4.0 by adding 2.0 M NaOH. The suspension was stirred for another 30 min and then transferred into a 100 mL Teflon-lined autoclave. The autoclave was sealed, maintained at 180 °C for 16 h, and then cooled to room temperature naturally. The precipitates were collected and washed several times with Milli-Q water, then with absolute ethanol, followed by drying at 60 °C in air for 6 h, respectively. The final product was denoted as FeVO_4 -4 ('-4' stands for at pH 4.0). As control, some other FeVO_4 samples were obtained by adjusting the pH to 1.0, 7.0 and 10.0, which were denoted as FeVO_4 -1, FeVO_4 -7 and FeVO_4 -10, respectively.

2.3. Characterization

The crystal structure of the as-prepared samples was recorded by powder X-ray diffraction (XRD) measurements on a Rigaku Ultima IV powder X-ray diffractometer (Japan) under the following conditions: 40 kV, 30 mA, and graphite-filtered Cu K_α radiation ($\lambda = 0.15406$ nm). The structure and morphology of the as-prepared samples were observed by field-emission scanning electron microscopy (FESEM, Hitachi S-4800, Japan) and transmission electron microscopy (TEM, JEOL JEM-1200, Japan). The chemical compositions of the as-prepared samples was recorded by

FESEM that equipped with an energy-dispersive X-ray spectroscopy (EDX) at an acceleration voltage of 200 kV. The X-ray photoelectron spectroscopy (XPS) of the as-prepared samples was carried out with an X-ray photoelectron spectrometer equipped with an Al-anode (ESCALAB 250, Thermo Fisher Scientific Inc., USA). The total power dissipation of the X-ray source was 150 W (10 mA, 15 kV). Binding energies was measured by reference to the C 1s line at 284.60 eV. The hysteresis loop of the as-prepared samples was recorded by the vibrating sample magnetometer (VSM, Lakeshore 7400, USA). The specific surface areas of the samples were measured based on Brunauer-Emmett-Teller (BET) equation by an automatic nitrogen adsorption specific surface and pore size distribution analyzer (NOVA 4000e, Quantachrome Ins., USA) at 77 K after a pretreatment at 473 K for 2 h.

2.4. Peroxidase-like activity assay and optimal conditions for H₂O₂ detection

To test the peroxidase-like activity of the prepared FeVO₄ materials, a standard catalytic oxidation experiment was carried out at room temperature. The reaction mixture contained 150 μ L of 8.0 mM TMB, 300 μ L of 500 μ g/mL FeVO₄-4, 300 μ L of 10.0 mM H₂O₂, and 750 μ L of 50.0 mM PBS at pH 4.0. Reactions were monitored at 652 nm by an Ultrospec 2100 pro Amersham Biosciences UV-visible spectrophotometer right after all of the reagents were added and mixed. Control groups were FeVO₄-4 (500 μ g/mL) with TMB (8.0 mM), FeVO₄-4 (500 μ g/mL) with H₂O₂ (10.0 mM), and TMB (8.0 mM) with H₂O₂ (10.0 mM) in 50.0 mM PBS (pH 4.0), respectively. The effect of substrate H₂O₂ concentration (0~4.0 mM), FeVO₄-4 concentration (0~200 μ g/mL), pH (2~9), and temperature (15~40 °C) on the peroxidase-like activity of FeVO₄-4 NBs were also explored with the same operations.

In addition, the peroxidase-like ability of FeVO₄ materials prepared under different pH values were also examined as well as the above FeVO₄-4 peroxidase-like activity assay experiments.

2.5. Analysis of active species

The active species generated in the reaction were detected by adding scavengers into the reaction solutions [25]. The concentration of scavenger IPA (a scavenger of hydroxyl radicals $\cdot$ OH)

was 10 mM. The specific procedure was the same as the FeVO₄-4 peroxidase-like activity assay experiments.

2.6. Steady-state kinetic study

Steady-state kinetic experiment was performed in a 1.5 mL reaction solution (50.0 mM PBS, pH = 4.0, 25 °C) with 100 µg/mL FeVO₄-4. TMB and H₂O₂ were involved in the reaction as substrates. The steady-state kinetic value was measured in time course mode at 652 nm [15, 26]. The Michaelis-Menten constant was calculated by using the Lineweaver-Burk double reciprocal according to the equation: $1/v = (K_m/V_{max})(1/[S]) + 1/V_{max}$ [27, 28].

2.7. H₂O₂ detection using FeVO₄-4

Colorimetric detection of H₂O₂ was set up as following: Firstly, 150 µL of 8.0 mM TMB and 300 µL of 500 µg/mL FeVO₄-4 were added into 750 µL of 50.0 mM PBS (pH = 4.0). Then, 300 µL of H₂O₂ with different concentrations (0~0.5 mM) was added to the reaction mixture. Time-scale color change was recorded on a UV-visible spectrophotometer (Ultrospec 2100 pro, Amersham Biosciences). In order to investigate the selectivity, 20.0 mM of dopamine hydrochloride, 20.0 mM of L-ascorbic acid, 20.0 mM of citric acid, and 20.0 mM of glucose were added in the control experiment. Method based on the FeVO₄-4 in the practical detection of H₂O₂ was evaluated using commercial antiseptic liquid (containing 0.79 M H₂O₂). The antiseptic solution was diluted 1000 times before the H₂O₂ detection experiment. The detection procedure was to the same as the above H₂O₂ determination experiments.

2.8. The stability and reusability of FeVO₄-4

The stability and reusability of FeVO₄-4 were tested using 300 µL of 10.0 mM H₂O₂, 300 µL of 500 µg/mL FeVO₄-4 and 150 µL of 8.0 mM TMB. Reagents were added into 750 µL of 50.0 mM PBS (pH = 4.0) and the color change was recorded with an UV-visible spectrophotometer (Ultrospec 2100 pro, Amersham Biosciences) for 10 min at room temperature. After each cycle, the FeVO₄-4 samples were collected by a magnet, washed three times with Milli-Q water and alcohol, dried at 60 °C for 15 min, and then reused in the next cycle. Finally, the crystal structure,

morphology and magnetism of the recycled FeVO₄-4 samples were analyzed by XRD, TEM and VSM as described in section 2.3.

3. Results and Discussions

3.1. Characterization of the as-prepared FeVO₄ samples

The crystal structure, crystallinity and purity of the as-prepared samples were examined by XRD measurements. **Fig. 1(A)** shows the XRD patterns of the as-prepared FeVO₄ samples. As shown in **Fig. 1(A)**, all of the diffraction peaks of FeVO₄-1 matched very well with the triclinic structure of FeVO₄, consistent with the literature value (JCPDS No. 25-0418). In addition, all the diffraction peaks of FeVO₄-4 can be indexed to the **orthorhombic phase FeVO₄ based on the reported data** (JCPDS No. 15-0294). Moreover, no impurity peaks were observed in FeVO₄-1 or FeVO₄-4 samples, indicating the pure phase of the samples. For FeVO₄-7 and FeVO₄-10 samples, all diffraction peaks were weak, which implied that the FeVO₄-7 and FeVO₄-10 samples had poor crystallinity and these products may be in amorphous forms. These results demonstrated that the pH value had significant effects on the crystal structure and crystallinity of FeVO₄, which might lead to different peroxidase-like activities of FeVO₄.

The chemical composition of the as-prepared FeVO₄-4 samples was examined by EDX. As shown in **Fig. 1(B)**, the EDX spectrum of FeVO₄-4 showed strong signals of Fe, V and O elements with no other elements, confirming the existence of Fe, V and O elements in FeVO₄-4. Besides, quantitative results showed the atomic ratio of Fe:V:O in FeVO₄-4 was 17:16:63, which agrees well with the theoretical stoichiometric ratio of 1:1:4 for Fe:V:O in FeVO₄-4, indicating that FeVO₄-4 is composed of Fe, V and O elements.

The morphologies and microstructures of the as-prepared FeVO₄ samples were examined by TEM and FESEM. As shown in **Fig. 2(A)**, FeVO₄-1 samples exhibited a long belt-like nanostructure with many irregular nanoparticles on its surface, and the average size of these nanobelts was about 3 μ m in length and 100 nm in width. Besides, FeVO₄-4 samples also showed a homogeneous and dispersed belt-like structure with many irregular nanoparticles on its surface (**Fig.**

2(B)). However, the high resolution TEM and FESEM images respectively shown in **Fig. 2(C)** and **Fig. 2(D)** reveal different morphologies and microstructures between FeVO₄-1 and FeVO₄-4. It can be clearly seen that the nanoparticles on the smooth surface of FeVO₄-4 nanobelts (NBs) were smaller and less than that of FeVO₄-1 NBs, and the length of FeVO₄-4 NBs was about 2 μm and shorter compared to that of FeVO₄-1 NBs. In addition, it can be seen in **Fig. 2(E)** that FeVO₄-7 samples showed as regular, uniform and thin nanoplates with average size about 20 nm. As for FeVO₄-10 samples, there were large amount of aggregates and irregular nanoparticles with amorphous state shown in **Fig. 2(F)**. Therefore, based on the TEM and FESEM observations, it can be deduced that the pH values in the preparation process had a significant influence on the morphologies and structures of FeVO₄, leading to the conversion from crystalline state to amorphous state as well as the decrease in size. These results indicated that pH values in the preparation process brought about different morphologies and structures of FeVO₄ samples, which further contributed to the distinct peroxidase activities.

To further investigate the chemical states and surface compositions of the as-prepared FeVO₄-4 samples, XPS analysis was carried out, and the results are shown in **Fig. 3(A)~(D)**. It can be seen in **Fig. 3(A)** that only Fe, V, O, and C elements are detected in the typical XPS survey spectrum of FeVO₄-4, while the C 1s peak at around 284.6 eV can be attributed to the signal from carbon contained in the instrument as calibration. **Fig. 3(B)** shows the high-resolution XPS spectrum of Fe 2p. The peaks located at about 725.6 eV and 711.2 eV correspond to Fe 2p_{1/2} and Fe 2p_{3/2}, respectively. They can be assigned to a Fe³⁺ oxidation state [29, 30]. As shown in **Fig. 3(C)**, the V 2p orbital displays the splitting peaks at binding energies of around 524.7 eV and 517.1 eV, which are assigned to V 2p_{1/2} orbital and V 2p_{3/2} orbital, respectively, confirming the +5 valence of V in the sample [31, 32]. In addition, the O 1s binding energy at approximately 530.2 eV can be attributed to the oxygen species of lattice oxygen (O²⁻) of FeVO₄ [29-32], as shown in **Fig. 3(D)**. Therefore, the XPS results further confirmed the existence of Fe, V and O elements in FeVO₄, which is in good accordance with the XRD and EDX analysis.

The magnetism of FeVO₄-4 sample was determined by the hysteresis loop on VSM. It can be seen in **Fig. S1(A) (Supplementary Information)** that as a typical ferromagnetism material [23, 33], FeVO₄ exhibited good magnetism and apparent magnetic hysteresis at room temperature, and the coercivity, saturation magnetization and residual magnetization were about 750 Oe, 0.22 emu/g and 0.071 emu/g, respectively. **Fig. S1(B)** shows the hysteresis loop of FeVO₄-4 sample after using 10 cycles, and there was no significant change in magnetism compared to the sample before catalytic reaction (**Fig. S1(A)**), indicating good and stable magnetism of the FeVO₄-4 sample.

The BET specific surface area is known as an important influencing factor on the catalytic activity of nanomaterials and can be measured by nitrogen adsorption method [34]. The BET specific surface areas were measured as 56.16 m²/g, 67.97 m²/g, 32.53 m²/g, and 16.61 m²/g for FeVO₄-1, FeVO₄-4, FeVO₄-7, and FeVO₄-10, respectively. What's more, the pore diameter, pore volume, and size distribution of these samples are shown in **Table S1** and **Fig. S2 (Supplementary Information)**. It was obvious that FeVO₄-4 NBs exhibited a much higher BET specific surface area than that of FeVO₄-1, FeVO₄-7 and FeVO₄-10. This result was in agreement with the morphologies observed by the TEM and FESEM images (**Fig. 2**). A larger BET specific surface area will provide more active sites and better absorption property for the catalyst, enhancing the catalytic activity apparently. Therefore, it was reasonable to expect that the as-prepared FeVO₄-4 NBs would exhibit excellent peroxidase mimetic catalytic activity.

3.2. Peroxidase-like activity of FeVO₄-4 NBs

To investigate the peroxidase-like activity of the as-prepared FeVO₄-4 NBs, catalytic oxidation of the typical peroxidase substrate TMB in the absence or presence of H₂O₂ was tested. FeVO₄-4 NBs acted as the peroxidase mimic. As illustrated in **Scheme 1**, H₂O₂ reduction reaction took place in the presence of a chromogenic electron donor TMB, promoted by the partial electron transferring to the surface of FeVO₄-4 NBs. Subsequently, the TMB was oxidized through one electron transfer and the solution changed to blue. This peroxidase mimic catalytic activity of FeVO₄-4 NBs would lead to its potential application in environmental and biological detection. In addition, the

UV-visible absorption spectra of the reaction systems were recorded on a UV-visible spectroscopy. As seen in **Fig. 4(A)**, the $\text{H}_2\text{O}_2 + \text{FeVO}_4\text{-4}$ system, the $\text{TMB} + \text{FeVO}_4\text{-4}$ system, and the $\text{H}_2\text{O}_2 + \text{TMB}$ system all showed no absorption, while the $\text{H}_2\text{O}_2 + \text{TMB} + \text{FeVO}_4\text{-4}$ system showed an obvious absorption peak at 652 nm, indicating that $\text{FeVO}_4\text{-4}$ could act as a peroxidase to oxidize TMB to produce oxidized TMB with blue color in the presence of H_2O_2 . In addition, **Fig. 4(B)** showed the color changes in different systems. It can be seen that the $\text{H}_2\text{O}_2 + \text{FeVO}_4\text{-4}$ system, the $\text{TMB} + \text{FeVO}_4\text{-4}$ system, and the $\text{H}_2\text{O}_2 + \text{TMB}$ system were all colorless. However, the $\text{H}_2\text{O}_2 + \text{TMB} + \text{FeVO}_4\text{-4}$ system presents an apparent color variation and showed a deep blue color, which was in agreement with the UV-visible absorption spectra shown in **Fig. 4(A)**.

In addition, the peroxidase mimetic catalytic activities of the as-prepared FeVO_4 samples were studied and compared by the catalytic oxidation of TMB in the presence of H_2O_2 . It can be seen in **Fig. 4(C)** that $\text{FeVO}_4\text{-4}$ NBs exhibited the best peroxidase mimetic catalytic activity among the as-prepared samples and had the greatest absorption at 652 nm, followed by $\text{FeVO}_4\text{-1}$, $\text{FeVO}_4\text{-7}$, and $\text{FeVO}_4\text{-10}$. What's more, the color changes of TMB with different FeVO_4 were observed in **Fig. 4(D)**. The $\text{FeVO}_4\text{-4}$ NBs system showed the most conspicuous blue color in comparison with that of $\text{FeVO}_4\text{-1}$, $\text{FeVO}_4\text{-7}$, and $\text{FeVO}_4\text{-10}$. These results indicated that the FeVO_4 prepared under different pH values exhibited distinct peroxidase mimetic catalytic activities. The most superior peroxidase mimetic catalytic activity of $\text{FeVO}_4\text{-4}$ NBs could be mainly attributed to the good crystalline, stable nanobelt structure and large BET specific surface area, making it a highly efficient colorimetric sensor for H_2O_2 detection. Furthermore, these results provided a strategy and direction in developing novel peroxidase mimetic nanomaterials with a good crystalline and large BET specific surface area.

3.3. Active species analysis and catalytic mechanism study

As is known, the active $\cdot\text{OH}$ radicals could be easily generated in the H_2O_2 containing system [13]. Therefore, in order to probe the peroxidase mimetic catalytic mechanism, radical trapping experiments were conducted by adding $\cdot\text{OH}$ scavengers IPA into the $\text{FeVO}_4\text{-H}_2\text{O}_2\text{-TMB}$ system [25],

where IPA would easily react with $\cdot\text{OH}$, leading to the decrease of the absorption at 652 nm and a color fading of the system. It can be seen clearly in **Fig. S3 (Supplementary Information)** that an obvious decrease in absorption and color fading was observed with the addition of IPA, confirming that $\text{FeVO}_4\text{-4 NBs}$ could catalytically activate H_2O_2 to generate $\cdot\text{OH}$ radicals that subsequently oxidized TMB to produce a TMB oxide with blue color. Hence, the peroxidase mimic catalytic mechanism of $\text{FeVO}_4\text{-4 NBs}$ was proposed according to the above results, as shown in **Scheme 1**. $\text{FeVO}_4\text{-4 NBs}$ could react with H_2O_2 to form the strong oxidizing $\cdot\text{OH}$ radicals, which could further oxidize TMB, and thus presented the peroxidase-like activity [13]. Therefore, these results indicated that $\cdot\text{OH}$ radicals played key roles in $\text{FeVO}_4\text{-4 NBs}$ peroxidase mimetic catalytic reaction.

3.4. Optimal conditions for H_2O_2 detection

To further analyze the peroxidase mimetic activities of $\text{FeVO}_4\text{-4 NBs}$, the catalytic ability of $\text{FeVO}_4\text{-4 NBs}$ under different $\text{FeVO}_4\text{-4 NBs}$ concentrations and H_2O_2 concentrations were measured. **Fig. S4(A) and Fig. S4(B) (Supplementary Information)** show that the peroxidase-like catalytic reaction rate increased with increasing the concentration of $\text{FeVO}_4\text{-4 NBs}$, and the solution color also deepened gradually. For the convenience of operation, the concentration of 100 $\mu\text{g/mL}$ for $\text{FeVO}_4\text{-4 NBs}$ was used in the following experiments. **Fig. S4(C)** shows the time course-dependent absorbance changes by adding H_2O_2 with different concentrations. The catalytic reaction rate increased with the increase of H_2O_2 concentrations, and there was no inhibition in the catalytic reaction at high H_2O_2 concentration. Previous reports showed that excess concentration of H_2O_2 could inactive HRP and inhibit the catalytic activity of HRP [35], which did not happen in $\text{FeVO}_4\text{-4}$ peroxidase mimetic system. Therefore, the enzyme catalytic activity of the $\text{FeVO}_4\text{-4 NBs}$ was more stable than that of HRP at high H_2O_2 concentrations. The optimal H_2O_2 concentration was chosen as 2.0 mM in the following experiments. In addition, **Fig. S4(D)** shows the different color presentations with different H_2O_2 concentration in the reaction system, and the increase of H_2O_2 concentration displayed color variation from light to dark blue, which further demonstrated the feasibility for H_2O_2 detection via a colorimetric method.

In addition, similar to natural enzymes, the catalytic activity of FeVO₄-4 NBs is closely dependent on the pH values, temperatures and other experimental conditions [36, 37]. Hence, influences of pH and temperature on the catalytic activity of FeVO₄-4 NBs were tested. **Fig. S5(A)** shows that the maximum absorption for FeVO₄-4 NBs catalytic system appeared at pH 4.0, while the pH value above or lower than 4.0 would lower the peroxidase-like activity. Thus, the optimal pH was 4.0. Moreover, **Fig. S5(B)** shows a temperature-dependent assay in the range of 15 °C to 40 °C. It is apparent that the catalytic activity was affected by the temperature, and the catalytic activity increased first with elevating temperature and then declined when temperature was higher than 25 °C. Therefore, for the convenience of operation, 25 °C was selected as the experimental temperature.

3.5. Steady-state kinetics analysis

The peroxidase-like catalytic mechanism of FeVO₄-4 NBs was further investigated using steady-state kinetics with H₂O₂ and TMB as substrates [10, 38], as shown in **Fig. 5**. The kinetic data were collected by varying the concentration of one substrate while keeping the other substrate concentration constant. The typical Michaelis-Menten curves were recorded by varying the concentration of TMB or H₂O₂ while keeping the other one constant, as shown in **Fig. 5(A)** and **Fig. 5(B)**. A series of steady-state reaction rates were calculated and applied to the Lineweaver-Burk double reciprocal plot (**Fig. 5(C)** and **Fig. 5(D)**) according to the Michaelis-Menten equation [10, 22, 39-41]: $1/v = (K_m/V_{max}) \times (1/[S]) + 1/V_{max}$, where v was the initial velocity, K_m was the Michaelis-Menten constant, V_{max} was the maximal reaction velocity, and $[S]$ was the concentration of the substrate. The K_m and V_{max} for HRP were listed in **Table 1**. K_m is commonly identified as an indicator of enzyme affinity to substrates, and the smaller the value of K_m , the stronger affinity between the enzyme and the substrate. Hence, the $K_m(\text{H}_2\text{O}_2)$ of FeVO₄-4 NBs was significantly lower than that of the natural enzyme HRP (**Table 1**), indicating that FeVO₄-4 NBs had a stronger binding affinity for H₂O₂ than HRP. On the other hand, the $K_m(\text{TMB})$ of FeVO₄-4 NBs was higher than that of HRP (**Table 1**), presenting that FeVO₄-4 NBs had a lower binding affinity for TMB

than HRP. Additionally, the peroxidase-like catalytic reaction based on FeVO₄-4 NBs followed the typical Michaelis-Menten behavior [10, 11] towards both substrates: TMB and H₂O₂. And the double-reciprocal plots (**Fig. 5(C)** and **Fig. 5(D)**) exhibit the characteristic parallel lines of a ping-pong mechanism, indicating that FeVO₄-4 NBs bound and reacted with the first substrate and then released the first product before reacting with the second substrate, which is similar to that of HRP [14, 26].

3.6. Analytical application in the detection of H₂O₂

A colorimetric method for detection of H₂O₂ was carried out based on the intrinsic peroxidase-like catalytic activity of FeVO₄-4 NBs. Since the absorbance at 652 nm of the oxidized TMB is in proportion to the H₂O₂ concentration, H₂O₂ could be conveniently detected by the naked eye or using a UV-visible spectrometer. **Fig. 6(A)** shows the time-course dependent absorbance changes at 652 nm of oxidized TMB in the presence of H₂O₂ with different concentrations. As shown in **Fig. 6(A)**, the reaction rate of the enzymatic reaction increased with increasing the concentration of H₂O₂. **Fig. 6(B)** presents a typical H₂O₂ concentration-response curve under the optimal conditions. **Fig. 6(B)** showed that the range of detection was from 0.002 to 0.1 mM. In addition, the curve was linear in the range from 2 to 40 μ M and the linear fitting equation was $A_{652\text{nm}} = 1.655 \times 10^{-4} + 0.3761C \text{ (mM)}$, with a correlation coefficient of 0.9969. Based on the IUPAC rules, the detection limit in spectrometric method is defined as the concentration of the analyte giving signals equivalent to the blank signal plus three times the standard deviation of the blank signals ($S/N = 3$). Hence, the detection limit of H₂O₂ was calculated to be 0.2 μ M. Moreover, as shown in the inset of **Fig. 6(B)**, the color variation for H₂O₂ response was also obvious by visual observation as low as 0.2 μ M. Therefore, this proposed H₂O₂ detection method on the basis of FeVO₄-4 NBs was friendly to visual observation, offering a convenient approach to detect H₂O₂ by naked eye even at low concentrations. Furthermore, compared to other nanomaterials with peroxidase-like activity reported previously, as shown in **Table 2**, FeVO₄-4 NBs exhibited a reasonable linear range for H₂O₂ detection and the detection limit was lower than that of some

reported nanomaterials [1, 4, 15, 19], further confirming that the optical biosensing system built in this study is an excellent platform for the detection of H_2O_2 .

3.7. Selectivity and real sample analysis

To evaluate the selectivity of the $\text{FeVO}_4\text{-4-TMB-H}_2\text{O}_2$ optical detection system, some other substances including 20.0 mM of dopamine hydrochloride (DA), 20.0 mM of L-ascorbic acid (AA), 20.0 mM of citric acid (CA), and 20.0 mM of glucose were selected as detection targets and tested with the procedure as stated above. As shown in **Fig. 7(A)**, though the concentrations of other substances were 10 times higher than that of standard H_2O_2 (2.0 mM), there was no obvious absorbance at 652 nm or color change. This shows the high selectivity of the $\text{FeVO}_4\text{-4 NBs-based H}_2\text{O}_2$ colorimetric detection system. In addition, the applicability of $\text{FeVO}_4\text{-4-TMB-H}_2\text{O}_2$ optical detection system was tested by using commercial antiseptic liquid (0.79 M H_2O_2) diluted 1000 times as the real sample). It can be seen in **Fig. 7(B)** that the diluted commercial antiseptic liquid showed an evident absorbance at 652 nm, and the color was obvious to naked eye. The concentration of H_2O_2 in the commercial antiseptic liquid was calculated to be about 0.76 ± 0.04 M based on our analysis. This concentration was closed to the actual concentration of the commercial antiseptic liquid (0.79 M) and literature reports (0.81 M) [16]. Therefore, these results confirmed the favorable applicability of the $\text{FeVO}_4\text{-4 NBs-based H}_2\text{O}_2$ colorimetric detection system.

3.8. Stability and reusability of $\text{FeVO}_4\text{-4 NBs}$

The stability and reusability of $\text{FeVO}_4\text{-4 NBs}$ were explored by conducting the peroxidase mimetic experiments successively for 10 cycles. After each cycle, the $\text{FeVO}_4\text{-4}$ samples were collected by magnet due to **their magnetic properties**, and after being washed with Milli-Q water and ethanol for several times and dried, $\text{FeVO}_4\text{-4}$ samples were reused in the next cycle. **Fig. 8(A)** shows that there was no significant loss in solution absorbance values during the ten successive cycles with every cycles lasting for 10 min, indicating the excellent stability and reusability of $\text{FeVO}_4\text{-4 NBs}$. The combination of a RSD of only 1.87% and the almost same color change in each

reaction system confirmed the good reproducibility of FeVO₄-4 NBs reaction system, as seen in **Fig. 8(B)**. In addition, by taking advantages of the magnetic property, the FeVO₄-4 NBs catalysts could be easily recycled (inset of **Fig. 8(C)**). XRD and TEM were used to further analyze the crystal structure and morphology of FeVO₄-4 NBs after ten cycled experiments. It can be seen in **Fig. 8(C)** that the XRD pattern of FeVO₄-4 NBs after ten successive cycles shows no significant change in peak intensity and crystal structure. **Fig. 8(D)** shows that there was no apparent morphology change for FeVO₄-4 NBs, though a little impurity appeared on the surface of the nanobelts, showing its good stability in crystal structure and morphology. Furthermore, it can be seen in **Fig. S1(B)** that the hysteresis loop of FeVO₄-4 after ten successive cycles shows no significant change in magnetism compared to the sample before catalytic reaction (**Fig. S1(A)**), demonstrating the good and stable magnetism of FeVO₄-4. These results indicated that FeVO₄-4 NBs exhibited an excellent stability, reusability and recoverability during the peroxidase mimic catalytic reaction, which favored a long-term and practical use under various conditions for analysis and detection.

4. Conclusions

In summary, series of FeVO₄ materials with different morphologies were successfully prepared through a facile hydrothermal method and were studied as peroxidase mimics. The different pH values during the preparation process led to different crystal structures, morphologies and peroxidase-like activities of the as-prepared FeVO₄ products. FeVO₄-4 NBs exhibited the best intrinsic peroxidase-like activity compared to other FeVO₄ materials owing to its nanobelt structure combined with a large BET specific surface area. On account of its excellent peroxidase mimic activity of FeVO₄-4 NBs, a novel ultrasensitive system for optical detection of H₂O₂ was successfully established, and the detection limit of H₂O₂ could reach 0.2 μM. Besides, FeVO₄-4 NBs exhibited a good selectivity, reproducibility, long-term stability, and easy recovery property benefited from its chemical stability and magnetic property. Furthermore, the ·OH radicals played a major role in the peroxidase mimetic catalytic reaction for FeVO₄-4 NBs. This work provides a novel, fast response, low cost, accessible and highly sensitive system for visual detection of H₂O₂,

making FeVO₄-4 NBs a bright prospect in biomedical analysis and other related areas.

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References

- [1] H. Wei, E. Wang, Fe₃O₄ magnetic nanoparticles as peroxidase mimetics and their applications in H₂O₂ and glucose detection, *Anal. Chem.* 80 (2008) 2250-2254.
- [2] N. Wang, J.C. Sun, L.J. Chen, H. Fan, S.Y. Ai, A Cu₂(OH)₃Cl-CeO₂ nanocomposite with peroxidase-like activity, and its application to the determination of hydrogen peroxide, glucose and cholesterol, *Microchim. Acta* 182 (2015) 1733-1738.
- [3] K. Zhao, W. Gu, S. Zheng, C. Zhang, Y. Xian, SDS-MoS₂ nanoparticles as highly-efficient peroxidase mimetics for colorimetric detection of H₂O₂ and glucose, *Talanta* 141 (2015) 47-52.
- [4] F.M. Qiao, L.J. Chen, X. Li, L. Li, S.Y. Ai, Peroxidase-like activity of manganese selenide nanoparticles and its analytical application for visual detection of hydrogen peroxide and glucose, *Sensor. Actuat. B: Chem.* 193 (2014) 255-262.
- [5] W.J. Shi, H. Fan, S.Y. Ai, L. Zhu, Pd nanoparticles supported on nitrogen, sulfur-doped three-dimensional hierarchical nanostructures as peroxidase-like catalysts for colorimetric detection of xanthine, *RSC Adv.* 5 (2015) 32183-32190.
- [6] R. Andre, F. Natalio, M. Humanes, J. Leppin, K. Heinze, R. Wever, H. C. Schroder, W.E.G. Muller, W. Tremel, V₂O₅ nanowires with an intrinsic peroxidase-like activity, *Adv. Funct. Mater.* 21(2011) 501-509.

- [7] Z. Yang, H. Ji, 2-Hydroxypropyl- β -cyclodextrin polymer as a mimetic enzyme for mediated synthesis of benzaldehyde in water, *ACS Sustainable. Chem. Eng.* 1 (2013) 1172-1179.
- [8] A. Khorshid, R.R. Amin, Y.M. Issa, Fabrication of a novel highly selective and sensitive nano-molar Cu (I), Cu (II) membrane sensors based on thiosemicarbazide and acetaldehydethiosemicarbazone copper complexes, *J. Chemica Acta* 1 (2013) 52-58.
- [9] X. Chen, X. Zhou, J. Hu, Pt-DNA complexes as peroxidase mimetics and their applications in colorimetric detection of H₂O₂ and glucose, *Anal. Methods* 4 (2012) 2183-2187.
- [10] L.J. Chen, B. Sun, X. Wang, F.M. Qiao, S.Y. Ai, 2D ultrathin nanosheets of Co-Al layered double hydroxides prepared in L-asparagine solution: enhanced peroxidase-like activity and colorimetric detection of glucose, *J. Mater. Chem. B* 1 (2013) 2268-2274.
- [11] T. Zhang, Y. Lu, G. Luo, Synthesis of hierarchical iron hydrogen phosphate crystal as a robust peroxidase mimic for stable H₂O₂ detection, *ACS Appl. Mater. Interfaces* 6 (2014) 14433-14438.
- [12] H. Wei, E. Wang, Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes, *Chem. Soc. Rev.* 42 (2013) 6060-6093.
- [13] L. Li, L. Ai, C. Zhang, J. Jiang, Hierarchical {001}-faceted BiOBr microspheres as a novel biomimetic catalyst: dark catalysis towards colorimetric biosensing and pollutant degradation, *Nanoscale* 6 (2014) 4627-4634.
- [14] L. Gao, J. Zhuang, L. Nie, J. Zhang, Y. Zhang, N. Gu, T. Wang, J. Feng, D. Yang, S. Perrett, X. Yan, Intrinsic peroxidase-like activity of ferromagnetic nanoparticles, *Nat. Nanotechnol.* 2 (2007) 577-583.
- [15] Y. Song, K. Qu, C. Zhao, J. Ren, X. Qu, Graphene oxide: intrinsic peroxidase catalytic activity and its application to glucose detection, *Adv. Mater.* 22 (2010) 2206-2210.
- [16] Z.B. Xiang, Y. Wang, P. Ju, D. Zhang, Optical determination of hydrogen peroxide by exploiting the peroxidase-like activity of AgVO₃ nanobelts, *Microchim. Acta* 183 (2016) 457-463.
- [17] Y. Lin, J. Ren, X. Qu, Catalytically active nanomaterials: a promising candidate for artificial enzymes, *Acc. Chem. Res.* 47 (2014) 1097-1105.

- [18] L. Su, J. Feng, X.M. Zhou, C.L. Ren, H.H. Li, X.G. Chen, Colorimetric detection of urine glucose based ZnFe_2O_4 magnetic nanoparticles, *Anal. Chem.* 84 (2012) 5753-5758.
- [19] Y. Zhang, J. Tian, S. Liu, L. Wang, X. Qin, W. Lu, G. Chang, Y. Luo, A.M. Asiri, A.O. Al-Youbi, X. Sun., Novel application of CoFe layered double hydroxide nanoplates for colorimetric detection of H_2O_2 and glucose, *Analyst* 137 (2012) 1325-1328.
- [20] J. Tian, S. Liu, Y. Luo, X. Sun, Fe (III)-based coordination polymer nanoparticles: peroxidase-like catalytic activity and their application to hydrogen peroxide and glucose detection, *Catal. Sci. Technol.* 2 (2012) 432-436.
- [21] Y. Dong, H. Zhang, Z.U. Rahman, L. Su, X. Chen, J. Hu, X. Chen, Graphene oxide- Fe_3O_4 magnetic nanocomposites with peroxidase-like activity for colorimetric detection of glucose, *Nanoscale* 4 (2012) 3969-3976.
- [22] F.M. Qiao, Z. Wang, K. Xu, S.Y. Ai, Double enzymatic cascade reactions within FeSe-Pt@SiO_2 nanospheres: synthesis and application toward colorimetric biosensing of H_2O_2 and glucose, *Analyst* 140 (2015) 6684-6691.
- [23] V.D. Nithya, R.K. Selvan, C. Sanjeeviraja, D.M. Radheep, S. Arumugam, Synthesis and characterization of FeVO_4 nanoparticles, *Mater. Res. Bull.* 46 (2011) 1654-1658.
- [24] T. Lin, L. Zhong, L. Guo, F. Fu, G. Chen, Seeing diabetes: visual detection of glucose based on the intrinsic peroxidase-like activity of MoS_2 nanosheets, *Nanoscale* 6 (2014) 11856-11862.
- [25] L. Ye, J. Chen, L. Tian, J. Liu, T. Peng, K. Deng, L. Zan, BiOI thin film via chemical vapor transport: photocatalytic activity, durability, selectivity and mechanism, *Appl. Catal. B: Environ.* 130-131 (2013) 1-7.
- [26] D.J. Porter, H.J. Bright, The mechanism of oxidation of nitroalkanes by horseradish peroxidase, *J. Biol. Chem.* 258 (1983) 9913-9924.
- [27] M. Liu, H. Zhao, S. Chen, H. Yu, X. Quan, Interface engineering catalytic graphene for smart colorimetric biosensing, *ACS Nano* 6 (2012) 3142-3151.
- [28] L. Ai, L. Li, C. Zhang, J. Fu, J. Jiang, MIL-53(Fe): a metal-organic framework with intrinsic

- peroxidase-like catalytic activity for colorimetric biosensing, *Chem. Eur. J.* 19 (2013) 15105-15108.
- [29] E.B. Ali, J.C. Bernede, A. Barreau, Thin films of FeVO₄ obtained by annealing under room atmosphere of Fe and V layers sequentially deposited, *Mater. Chem. Phys.* 63 (2000) 208-212.
- [30] A. Dixit, P. Chen, G. Lawes, J.L. Musfeldt, Electronic structure and polaronic excitation in FeVO₄, *Appl. Phys. Lett.* 99 (2011) 141908.
- [31] L. Chen, Q. Zhang, R. Huang, S.F. Yin, S.L. Luo, C.T. Au, Porous peanut-like Bi₂O₃-BiVO₄ composites with heterojunctions: one-step synthesis and their photocatalytic properties, *Dalton Trans.* 41 (2012) 9513-9518.
- [32] Z.Q. He, Y.Q. Shi, C. Gao, L.N. Wen, J.M. Chen, S. Song, BiOCl/BiVO₄ *p-n* heterojunction with enhanced photocatalytic activity under visible-light irradiation, *J. Phys. Chem. C* 118 (2014) 389-398.
- [33] Z. He, J.I. Yamaura, Y. Ueda, Flux growth and magnetic properties of FeVO₄ single crystals, *J. Solid State Chem.* 181 (2008) 2346-2349.
- [34] L. Zhang, W. Wang, Z. Chen, L. Zhou, H. Xu, W. Zhu, Fabrication of flower-like Bi₂WO₆ superstructures as high performance visible-light driven photocatalysts, *J. Mater. Chem.* 17 (2007) 2526-2532.
- [35] J.A. Nicell, H. Wright, A model of peroxidase activity with inhibition by hydrogen peroxide, *Enzyme Microb. Tech.* 21 (1997) 302-310.
- [36] K. Chattopadhyay, S. Mazumdar, Structural and conformational stability of horseradish peroxidase: effect of temperature and pH, *Biochemistry* 39 (2000) 263-270.
- [37] P.D. Josephy, T. Eling, R.P. Mason, The horseradish peroxidase-catalyzed oxidation of 3, 5, 3', 5'-tetramethylbenzidine. Free radical and charge-transfer complex intermediates, *J. Biol. Chem.* 257 (1982) 3669-3675.
- [38] L.J. Chen, K. Sun, P. Li, X.Z. Fan, J.C. Sun, S.Y. Ai, DNA-enhanced peroxidase-like activity of layered double hydroxide nanosheets and applications in H₂O₂ and glucose sensing, *Nanoscale* 5 (2013) 10982-10988.

- [39] L.J. Chen, N. Wang, X. Wang, S.Y. Ai, Protein-directed in situ synthesis of platinum nanoparticles with superior peroxidase-like activity, and their use for photometric determination of hydrogen peroxide, *Microchim. Acta* 180 (2013) 1517-1522.
- [40] J. Mu, Y. Wang, M. Zhao, L. Zhang, Intrinsic peroxidase-like activity and catalase-like activity of Co_3O_4 nanoparticles, *Chem. Commun.* 48 (2012) 2540-2542.
- [41] P. Ju, Y.H. Xiang, Z.B. Xiang, M. Wang, Y. Zhao, D. Zhang, J.Q. Yu, X.X. Han, BiOI hierarchical nanoflowers as novel robust peroxidase mimetics for colorimetric detection of H_2O_2 , *RSC Adv.* 6 (2016) 17483-17493.

Figure captions:

Fig. 1. XRD patterns of the FeVO_4 samples prepared under different pH values (A) and the EDX spectrum of FeVO_4 -4 (B).

Fig. 2. TEM images of FeVO_4 -1 (A), FeVO_4 -4 (B, C), FeVO_4 -7 (E), and FeVO_4 -10 (F); FESEM image of FeVO_4 -4 (D).

Fig. 3 XPS spectra of FeVO_4 -4: survey spectrum (A), high resolution XPS spectra of Fe 2p (B), V 2p (C), and O1s (D).

Fig. 4. UV-visible absorption spectra (A) and color changes (B) of different reaction systems (a. $\text{H}_2\text{O}_2 + \text{FeVO}_4$ -4, b. TMB + FeVO_4 -4, c. $\text{H}_2\text{O}_2 + \text{TMB}$, and d. $\text{H}_2\text{O}_2 + \text{TMB} + \text{FeVO}_4$ -4); UV-visible absorption spectra (C) and color changes (D) of in the presence of different FeVO_4 samples (a. FeVO_4 -1, b. FeVO_4 -4, c. FeVO_4 -7, and d. FeVO_4 -10).

Fig. 5. Steady-state kinetic assays of FeVO_4 -4 NBs. The reaction velocity (V) was measured using 100 $\mu\text{g/mL}$ of FeVO_4 -4 in 25 mM PBS (pH = 4.0) at room temperature. The TMB concentration was varied and the concentration of H_2O_2 was 10.0 mM (A), the H_2O_2 concentration was varied and the concentration of TMB was 0.8 mM (B), and the double-reciprocal plots of peroxidase-like activity of FeVO_4 -4 NBs with a fixed concentration of one substrate relative to varying concentration of the other substrate (C, D).

Fig. 6. Time-dependent absorbance changes at 652 nm in the presence of different concentrations of H_2O_2 in 25 mM PBS (pH = 4.0) with 100 $\mu\text{g/mL}$ $\text{FeVO}_4\text{-4}$ NBs at room temperature (A) and a dose-response curve for H_2O_2 detection (B). Inset: the linear calibration plot for H_2O_2 and color changes.

Fig. 7. (A) Selectivity analysis for $\text{FeVO}_4\text{-4}$ NBs detection system with 20.0 mM dopamine hydrochloride (DA), 20.0 mM L-ascorbic acid (AA), 20.0 mM citric acid (CA), and 20.0 mM glucose as the detection targets by monitoring the absorbance at 652 nm (Inset: related color changes); (B) Real sample detection (antiseptic liquid was diluted 1000 times) compared to 2.0 mM H_2O_2 by monitoring the absorbance at 652 nm (Inset: related color changes).

Fig. 8. Stability and reusability experiments of $\text{FeVO}_4\text{-4}$ NBs detection system conducted at 100 $\mu\text{g/mL}$ $\text{FeVO}_4\text{-4}$ NBs in 25 mM PBS (pH 4.0) with 2.0 mM H_2O_2 and 0.8 mM TMB as substrates (A, B); XRD pattern (C) and TEM image (D) of $\text{FeVO}_4\text{-4}$ NBs after ten cycles. Inset: the related color changes in ten cycles and the recovery of $\text{FeVO}_4\text{-4}$ NBs by magnet.

Scheme 1. Schematic illustration of peroxidase-like activity of $\text{FeVO}_4\text{-4}$ NBs.

Table 1. Comparison of K_m and $V_{\max}$ between $\text{FeVO}_4\text{-4}$ NBs and HRP for H_2O_2 and TMB.

Table 2. Comparison of mimetic enzyme activity in the linear range and detection limit for H_2O_2 detection between $\text{FeVO}_4\text{-4}$ NBs and other mimetic enzymes.

Table 1. Comparison of K_m and V_{max} between FeVO₄-4 NBs and HRP for H₂O₂ and TMB.

Catalyst	Substance	K_m (mM)	V_{max} (M/s)	Reference
FeVO ₄	H ₂ O ₂	0.0732	2.72×10^{-8}	This work
FeVO ₄	TMB	0.691	2.51×10^{-8}	This work
HRP	H ₂ O ₂	0.214	2.46×10^{-8}	[4]
HRP	TMB	0.275	1.24×10^{-8}	[4]

Table 2. Comparison of mimetic enzyme activity in the linear range and detection limit for H₂O₂ detection between FeVO₄-4 NBs and other mimetic enzymes.

Mimetic enzyme	Linear range (μM)	Detection limit (μM)	Reference
FeVO ₄	2~40	0.2	This work
Fe ₃ O ₄	5~100	3.0	[1]
Co-Al LDH	10~50	10.0	[10]
Fe ₃ H ₉ (PO ₄) ₆ ·6H ₂ O	57.4~525.8	1.0	[11]
BiOBr	0.5~30	0.3	[13]
CoFe	1~20	0.4	[19]