



## A novel application of iron oxide nanoparticles for detection of hydrogen peroxide in acid rain

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### ABSTRACT

Determining the concentration of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is of great importance in food, pharmaceutical, environmental and clinical analyses. Horseradish peroxidase (HRP), an enzyme specifically catalyzing the oxidative reaction of H<sub>2</sub>O<sub>2</sub> to develop color reaction, has been widely used for measuring H<sub>2</sub>O<sub>2</sub> concentration. However, owing to the instability and high cost of this enzyme, discovering efficient mimics of peroxidase has been important to conquer these disadvantages of protein catalyst. Recently we have found that Fe<sub>3</sub>O<sub>4</sub> magnetic nanoparticles (Fe<sub>3</sub>O<sub>4</sub> MNPs) possess intrinsic peroxidase-like activity, which can catalyze oxidation of various peroxidase substrates in the presence of H<sub>2</sub>O<sub>2</sub>. Based on this finding, we developed a spectrometric method using Fe<sub>3</sub>O<sub>4</sub> MNPs as a catalyst to determine H<sub>2</sub>O<sub>2</sub> in rainwater. Our data show that the Fe<sub>3</sub>O<sub>4</sub> MNPs are efficient catalysts to determine H<sub>2</sub>O<sub>2</sub> in rainwater. Compared to HRP, the Fe<sub>3</sub>O<sub>4</sub> MNPs are reusable and economical and these characteristics make the particles a board range of applications in determining H<sub>2</sub>O<sub>2</sub> in the rainwater.

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### 1. Introduction

Acid rain (pH<5.5), causing the death of fish and plant, is a serious environmental problem over the world. High acidity of the rain results from the oxidation reaction of H<sub>2</sub>O<sub>2</sub> with dioxide and nitrogen oxides in atmosphere dissolved in hydrometeors to H<sub>2</sub>SO<sub>4</sub> [1]. Thus, the concentration of H<sub>2</sub>O<sub>2</sub> is an important indicator providing information of the environment. In order to accurately and rapidly determine H<sub>2</sub>O<sub>2</sub> in rainwater, many analytical methods have been developed including titrimetric [2], fluorometric [3,4], chemiluminescence [5], electrochemical [6] and spectrophotometric [7,8] methods. Among these methods, HRP is widely used for catalyzing the oxidation of various hydrogen donor in the presence of H<sub>2</sub>O<sub>2</sub>. Although the HRP is a specific and efficient reagent, its instability and high cost restrict the application. In the past decades, some HRP mimics have been developed, such as metal porphyrin compound, small biomolecules like hemin and hematin, porphyrin and cyclodextrin [9–12], and applied in determining H<sub>2</sub>O<sub>2</sub> in rainwater. However, the catalytic activity of these HRP mimetics is lower than natural HRP. Therefore, it is highly desirable to develop new mimetic enzymes or to further enhance the activity of existed mimetic enzymes.

Recently we found that the Fe<sub>3</sub>O<sub>4</sub> MNPs possessed an intrinsic peroxidase-like activity [13]. Based on this finding, we carried out the potential application of the Fe<sub>3</sub>O<sub>4</sub> MNPs, as a new catalyst, for the determination of H<sub>2</sub>O<sub>2</sub> in rainwater and developed Fe<sub>3</sub>O<sub>4</sub> MNPs-based

spectrometric method. Our data showed that this new method is efficient to determine H<sub>2</sub>O<sub>2</sub> in rainwater.

### 2. Experimental section

#### 2.1. Synthesis of Fe<sub>3</sub>O<sub>4</sub> MNPs

Fe<sub>3</sub>O<sub>4</sub> MNPs with diameters about 30 nm were prepared according to the method of Jiang [14]. Briefly, we dissolved FeSO<sub>4</sub>·7H<sub>2</sub>O (0.07 M) and FeCl<sub>3</sub>·6H<sub>2</sub>O (0.14 M) in deionized water. Then 3.5 M ammonium hydroxide (NH<sub>4</sub>OH) was dropped into the mixture solution with

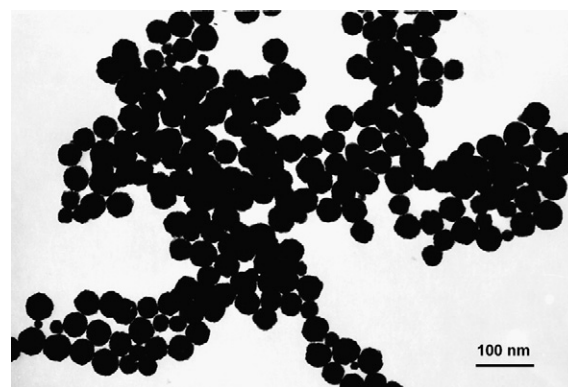


Fig. 1. TEM image of Fe<sub>3</sub>O<sub>4</sub> MNPs with an average diameter of 30 nm.

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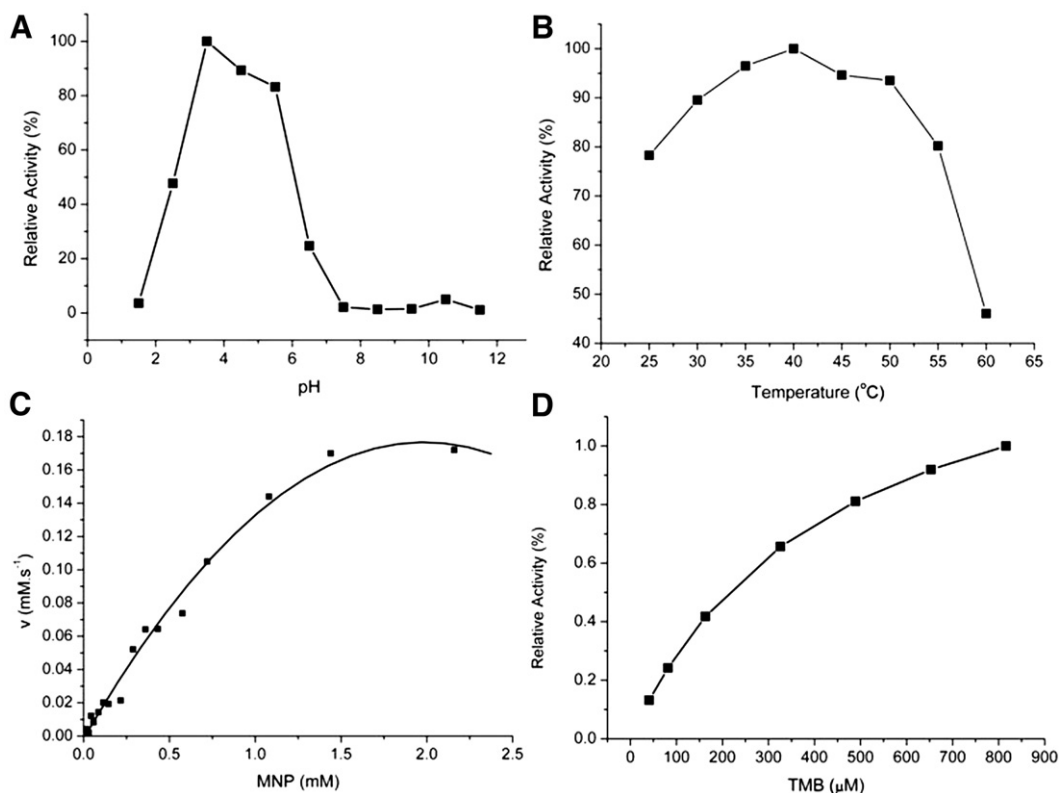


Fig. 2. The H<sub>2</sub>O<sub>2</sub> determination efficiency dependent on pH (A), temperature (B), initial concentrations of Fe<sub>3</sub>O<sub>4</sub> MNPs (C) and TMB substrate (D).

violently stirring. The Fe<sub>3</sub>O<sub>4</sub> MNPs were washed immediately with water for 5 times and then ethanol for 2 times.

### 2.2. Determination of H<sub>2</sub>O<sub>2</sub> in rainwater

The experiments for Fe<sub>3</sub>O<sub>4</sub> MNPs catalysis were carried out in 1.5 ml tube with 816 μM TMB and 2.9 μM H<sub>2</sub>O<sub>2</sub> in 500 μl rainwater, the reactions were initiated by adding 1.44 mM Fe<sub>3</sub>O<sub>4</sub> MNPs. The reaction produced a blue color and was measured by a Bio-Rad Microplate Reader 550 at 652 nm.

## 3. Results and discussion

### 3.1. Preparation and characterization of Fe<sub>3</sub>O<sub>4</sub> MNPs

Firstly, we prepared Fe<sub>3</sub>O<sub>4</sub> MNPs using the method of Jiang [14]. The size and shape of Fe<sub>3</sub>O<sub>4</sub> MNPs were examined by TEM. It was showed that the particles appeared spherical

with an average diameter of 30 nm (Fig. 1). Upon observation, we could also found that the size distribution of Fe<sub>3</sub>O<sub>4</sub> MNPs was significantly restricted in a narrowed range.

### 3.2. The H<sub>2</sub>O<sub>2</sub> determination is pH and temperature dependent

In order to utilize the catalytic characterization of Fe<sub>3</sub>O<sub>4</sub> MNPs in determining of H<sub>2</sub>O<sub>2</sub> concentration in rainwater, we first tested whether the catalytic activity of Fe<sub>3</sub>O<sub>4</sub> MNPs were dependent on pH and temperature. The result suggested that the optimal pH of the Fe<sub>3</sub>O<sub>4</sub> MNPs catalysis was about pH 3.5 (Fig. 2A). However, the activity was remarkably decreased at pH 6 and completely lost at pH 8, which was consistent with the feature of HRP [13].

In addition, we measured the catalytic activity of Fe<sub>3</sub>O<sub>4</sub> MNPs under a range of temperatures from 25 to 60 °C (Fig. 2B). Data inferred that the relative activity of particles remained higher than 80% until heating to 60 °C. The optimal temperature was approximately 37 °C. This evidence implied that the Fe<sub>3</sub>O<sub>4</sub> MNPs had a broad application in determining H<sub>2</sub>O<sub>2</sub> in acid rain under different temperatures.

### 3.3. Optimal concentration of Fe<sub>3</sub>O<sub>4</sub> MNPs and TMB substrate in H<sub>2</sub>O<sub>2</sub> determination

To test the optimal concentration of Fe<sub>3</sub>O<sub>4</sub> MNPs for H<sub>2</sub>O<sub>2</sub> determination, a range of Fe<sub>3</sub>O<sub>4</sub> concentration from 0 to 2.16 mM was used in the reaction system. The result

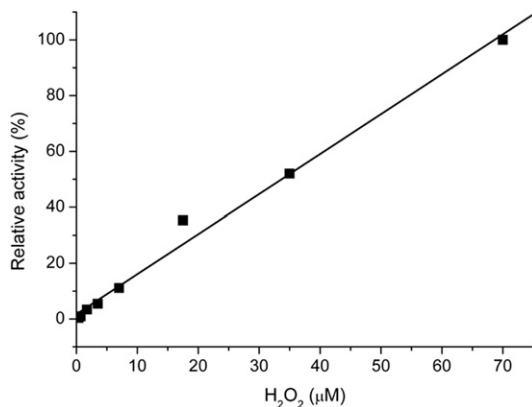


Fig. 3. The linear range of the reaction of Fe<sub>3</sub>O<sub>4</sub> MNPs in determination H<sub>2</sub>O<sub>2</sub>.

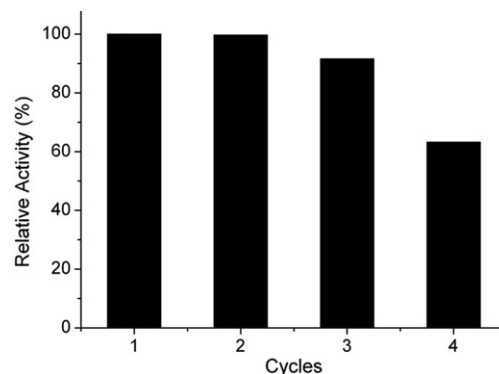


Fig. 4. Reusability of the Fe<sub>3</sub>O<sub>4</sub> MNPs in H<sub>2</sub>O<sub>2</sub> determination.

**Table 1**  
Results of the analyses of H<sub>2</sub>O<sub>2</sub> in rainwater samples

Sample	Detected (mM)	Added (mM)	Found (mM)	Recovery (%)
Rainwater 1	5.2(±2.9%)	2.9	2.8	96
Rainwater 2	2.8(±3.2%)	2.9	3	97

inferred that the initial velocity was increased along with the increase of Fe<sub>3</sub>O<sub>4</sub> MNPs concentration from 0 to 1.44 mM. After the concentration of Fe<sub>3</sub>O<sub>4</sub> MNPs reached 1.44 mM, the initial velocity was no longer increased (Fig. 2C). Therefore, we took 1.44 mM as optimal concentration of Fe<sub>3</sub>O<sub>4</sub> MNPs for the following reactions.

To know whether the catalytic activity of the Fe<sub>3</sub>O<sub>4</sub> MNPs was dependent on TMB concentration, the experiments were performed by adding a range of TMB concentrations from 40.8 μM to 816 μM (Fig. 2D). The results indicated that the activity of Fe<sub>3</sub>O<sub>4</sub> MNPs was TMB concentration dependent and with the increase of TMB concentration the Fe<sub>3</sub>O<sub>4</sub> MNPs activity increase and reached the maximal at 816 μM.

#### 3.4. Performance of the system for H<sub>2</sub>O<sub>2</sub> measurement

Under the optimal conditions described above, we first obtained the incubation time of becoming the maximum and constant absorbance of this reaction. In this method, the absorbance at 652 nm reached a plateau after 30 min which was comparable to the previous method [15]. Thus, we chose 1 h as a sufficient incubation time further to investigate.

To test the linear range of Fe<sub>3</sub>O<sub>4</sub> MNPs in H<sub>2</sub>O<sub>2</sub> determination, all experiments were carried out under the optimal conditions. The reactions were initiated by adding different concentration of H<sub>2</sub>O<sub>2</sub>. The calibration graph of the absorbance versus the H<sub>2</sub>O<sub>2</sub> concentration was linear in the range from 0 to 70 μM (Fig. 3) and the limit of detection (LOD) was  $1.75 \times 10^{-7}$ . To the best of our knowledge, some enzymes (like HRP) or mimetic enzymes (like Hb) used for determining H<sub>2</sub>O<sub>2</sub> almost showed the linear range from 0–100 μM and the LOD of these catalysts was around  $1 \times 10^{-7}$  [4]. Thus, these results demonstrated that the Fe<sub>3</sub>O<sub>4</sub> MNPs could be used as a promising catalyst, which had both advantages of a wide linear range and a low detection limit.

#### 3.5. Fe<sub>3</sub>O<sub>4</sub> MNPs are reusable

H<sub>2</sub>O<sub>2</sub> determination experiments were repeated for 4 rounds using the same MNPs to examine the reusability of Fe<sub>3</sub>O<sub>4</sub> MNPs. After each experiment the MNPs were simply collected by a magnet then regenerated by sonication and washing with deionized water. After 3 rounds of recycle, the MNPs still remained almost 100% catalytic activity (Fig. 4). Due to the low expense of MNPs preparation, the characteristic of reusability further decreased the cost per treatment.

#### 3.6. Application of Fe<sub>3</sub>O<sub>4</sub> MNPs in determining H<sub>2</sub>O<sub>2</sub> in rainwater

The system was applied to determine H<sub>2</sub>O<sub>2</sub> concentration in rainwater samples collected on different days in Institute of Biophysics, Chinese Academy of Science

(Beijing, China). Before analysis, the rainwater samples were treated through 0.22 μM filter. In order to evaluate the validity of the proposed method for determination of H<sub>2</sub>O<sub>2</sub> in rainwater, recovery studies were carried out using standard addition method. All the experiments were carried out under the optimal conditions. As shown in Table 1, the recovery of this system was higher than 95%, which indicated that the Fe<sub>3</sub>O<sub>4</sub> MNPs were suitable to determine the concentration of H<sub>2</sub>O<sub>2</sub> in rainwater.

## 4. Conclusion

Our results demonstrate that Fe<sub>3</sub>O<sub>4</sub> MNPs can be used as a promising catalyst to determine H<sub>2</sub>O<sub>2</sub> in rainwater. The linear range and the limit of determination are comparable with HRP. There are significant advantages of using Fe<sub>3</sub>O<sub>4</sub> MNPs in determining H<sub>2</sub>O<sub>2</sub>: firstly, the Fe<sub>3</sub>O<sub>4</sub> MNPs are cheap and efficient; moreover, the Fe<sub>3</sub>O<sub>4</sub> MNPs could be recycled and regenerated; thirdly, the Fe<sub>3</sub>O<sub>4</sub> MNPs were magnetic controllable. These attractive features endowed them broad applications in determining H<sub>2</sub>O<sub>2</sub> in rainwater by constructing a system of biosensor.

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