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A Simple and Sensitive Fluorescence Quenching Method for the Determination of H_2O_2 Using Rhodamine B and Fe_3O_4 Nanocatalyst

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Abstract In pH 1.99 sodium acetate-HCl buffer solutions at 60 °C, Rhodamine B exhibited a strong fluorescence peak at 584 nm using an excitation wavelength of 548 nm. The fluorescence quenching occurred when Fe_3O_4 nanoparticles catalyzed H_2O_2 oxidation of Rhodamine B. Under the chosen conditions, the fluorescence intensity at 584 nm decreased when the concentration of H_2O_2 increased. The fluorescence quenching intensity is linear with the concentration of H_2O_2 in the range of 10–200 nmol/L. Thus, a new and simple and sensitive nanocatalytic fluorescence method was proposed for the determination of H_2O_2 in synthetic sample, with satisfactory results.

Keywords H_2O_2 · Fe_3O_4 nanoparticles · Nanocatalysis · Rhodamine B · Fluorescence quenching

Introduction

Hydrogen peroxide is widely applied in oxidation, bleaching, corrosion, disinfection and sterilization. Toxicology showed that human long-term exposure in hydrogen peroxide can cause human hair bleaching, respiratory inflammation and poisoning [1]. Therefore, the use range and the residue of hydrogen peroxide have been clearly limited by "Health standards for the use of food additives" in China [2]. According to the reports [3], the concentration of hydrogen peroxide, which is one of the main causes of acid rain, should not exceed 1.4 mg/m^3 in the air. As a result, it is of important significance to develop method for the determination of hydrogen peroxide. At present, several methods, such as high performance liquid chromatography [4, 5], electrochemical [6, 7], chemiluminescence [8], fluorescence [9], spectrophotometry [10, 11] and resonance scattering spectral methods [12, 13] have been proposed for the detection of hydrogen peroxide. High performance liquid chromatography, electrochemical and chemiluminescence methods are sensitive, but the process is complicated. The spectrophotometric method is simple and inexpensive but has poor sensitivity. In addition, enzymatic spectrophotometric and fluorescence spectrophotometry method have high sensitivity and selectivity using horseradish peroxidase as the catalyst. However, enzyme is instable and expensive. In recent years, people paid attention to the research of Fe_3O_4 nanoparticles, as the mimetic enzyme of horseradish peroxidase, because of its catalytic activity and stability and availability [14–18]. RhB was a commonly used reagent, and has been utilized in spectrophotometry, surface enhanced Raman scattering, fluorescence and resonance scattering analysis [19–22]. So far, it has not been reported that Fe_3O_4 nanoparticles catalyzed H_2O_2 oxidation of Rhodamine B fluorescence quenching. In this article, a new simple, rapid and sensitive fluorescence method has been proposed for the determination of H_2O_2, using Fe_3O_4 nanoparticles as the catalyst of the rhodamine B fluorescence quenching reaction.

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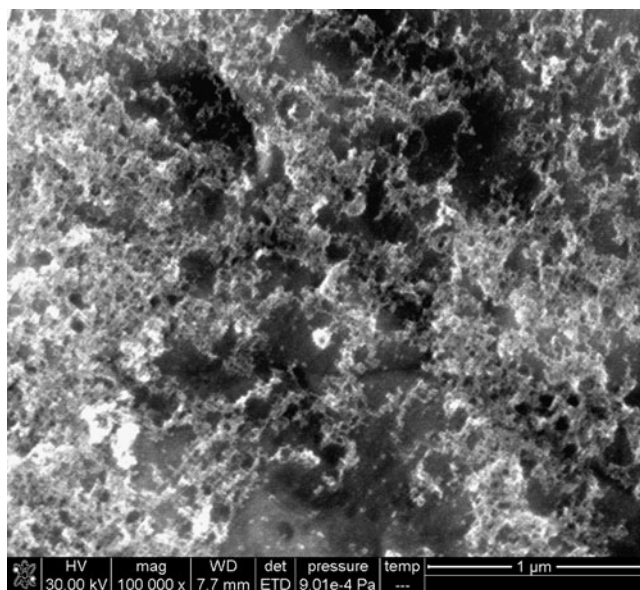e-mail: djohnson@tom.com

Fig. 1 Scanning electron microscope of the Fe₃O₄ nanoparticles

Experimental Section

Apparatus A Cary Eclipse fluorescence spectrophotometer (Varian, USA), 79-1 Magnetic Heating Stirrer (Jiangsu, China), SK8200LH Ultrasonic Reactor (Shanghai, China), FEI Quanta 200 FEG scanning electron microscope (FEI Co., Holand) were used.

Reagents A 0.025 mg/mL Rhodamine B (RhB, Shanghai Reagent Factory, China) solution solution was used. A pH 1.99 sodium acetate-HCl (Shantou Xilong Chemical Factory, China) buffer solution was prepared as follows: A 1.0 mL 1.0 mol/L sodium acetate and 1.05 mL 1 mol/L HCl was added to a 50 mL volumetric flask, diluted to 50 mL, and was stored in a refrigerator at 4 °C. Hydrogen peroxide (30%, w/v solution) was purchased from Shantou Xilong chemical factory (Guangdong, China), and its concentration (0.420 mol/L) was titrated by a 0.4000 mol/L potassium permanganate standard solution. A 1.000×10^{-6} mol/L H_2O_2 working solution was obtained by diluting the stock solution (0.420 mol/L) with water. A Fe_3O_4 nanoparticles were prepared by adding 1.475 g $FeSO_4 \cdot 7H_2O$ (Sinopharm Chemical Reagent Co. Ltd, China) and 2.025 g $FeCl_3 \cdot 6H_2O$ (Sinopharm Chemical Reagent Co. Ltd, China) to 30 mL Erlenmeyer flask at 80 °C water bath with vigorous stirring. Then 5 mL 28% ammonia (Shantou xilong chemical factory, China) was added rapidly into the mixture. The solution was heated under stirring for another 90 min, during the time its color changed from grass-green to orange-yellow, and finally to black quickly, indicating the formation of Fe_3O_4 nanoparticles. The solution was cooled to room temperature while stirring continuously. Then the precipitates were filtered and washed with water several times. At last, Fe_3O_4 nanoparticle

suspension should be diluted to 100 mL, and stored in a refrigerator at 4 °C. The nanoparticle concentration, calculated by $FeCl_3$, was 8.7 g/L, with a size of 20 nm. And the suspension was diluted to 0.87 g/L for use. All reagents were used as analytical grade, and all solutions were prepared with doubly distilled water.

Procedure Into a graduated test tube, 150 μL of 0.025 mg/mL RhB, 100 μL of pH 1.99 sodium acetate-HCl buffer solutions, 200 μL of 0.87 g/L Fe_3O_4 suspension, and different amount of H_2O_2 was added, diluted to 2.0 mL with water and mixed well. The mixture reacted at 60 °C for 20 min, then it was cooled by flowing water, and the Fe_3O_4 nanoparticles were separated from the solution using a magnet. The fluorescence spectrum was recorded on a spectrofluorophotometer and the fluorescence intensity at 584 nm (F_{584nm}) was measured with the excitation wavelength of 548 nm. Meanwhile, a reagent blank (F_0) was recorded, and a value of $\Delta F_{584nm}(=F_0 - F_{584nm})$ was calculated.

Results and Discussion

Principle In pH 1.99 sodium acetate-HCl buffer solutions at 60 °C, Fe_3O_4 nanoparticles in mean size of 20 nm (Fig. 1) catalyzed H_2O_2 to form hydroxyl radical ·OH, which can oxidize RhB conjugated molecule to form colorless and non-fluorescence product [23]. With the H_2O_2 increased, more RhB will be oxidized by more ·OH, so that the fluorescence intensity becomes weaker. The fluorescence quenching intensity is linear with the
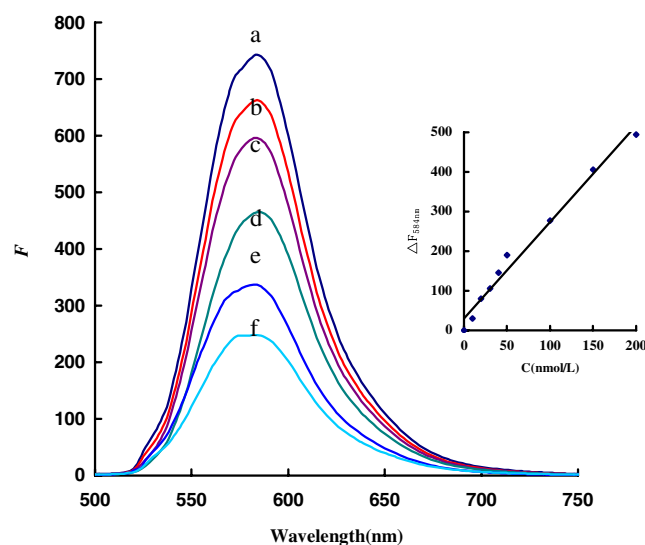

Fig. 2 Fluorescence spectra. **a** pH 1.99 sodium acetate-HCl+ 1.88 μg/mL RhB +87.0 mg/L Fe_3O_4); **b** a+20 nmol/L H_2O_2; **c** a+ 40 nmol/L H_2O_2; **d** a+100 nmol/L H_2O_2; **e** a+150 nmol/L H_2O_2; **f** a+200 nmol/L H_2O_2
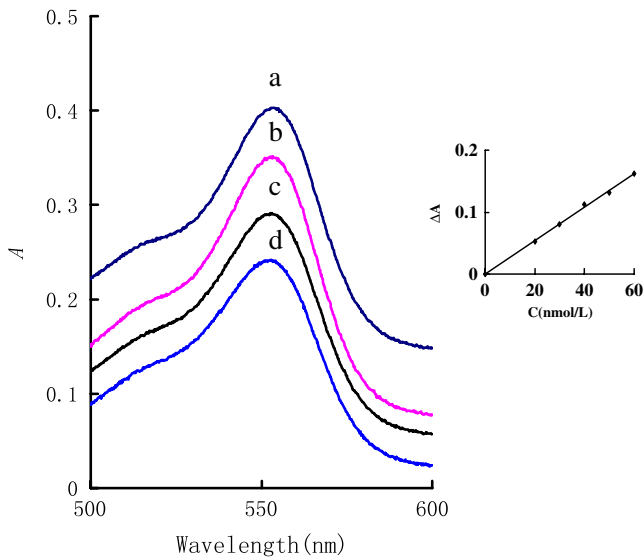
Fig. 3 Absorbption spectra. **a** pH 1.99 sodium acetate –HCl+ 1.88 µg/mL RhB+87.0 mg/L Fe_3O_4; **b** a+20 nmol/L H_2O_2; **c** a+ 40 nmol/L H_2O_2; **d** a+60 nmol/L H_2O_2

concentration of H_2O_2. Based on this, a new simple and sensitive fluorescence method has been proposed for the determination of H_2O_2.

Fluorescence Spectra In the medium of sodium acetate - HCl, RhB emits strong fluorescence. Results showed that the maximum excited wavelength of RhB was at 548 nm and the maximum fluorescence wavelength was at 584 nm. In the presence of Fe_3O_4 as the catalyst and H_2O_2 as the oxidant, RhB can be oxidized to form colorless and non-fluorescence product, and the fluorescence intensity at 584 nm decreases. Owing to the Rayleigh scattering of Fe_3O_4 nanoparticles, that affected the fluorescence record
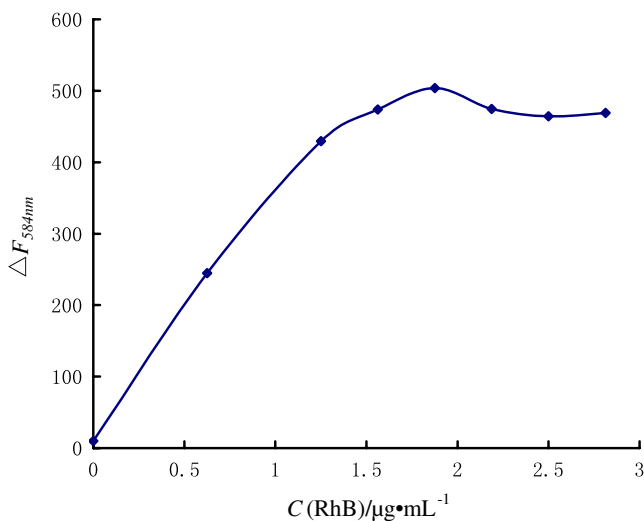

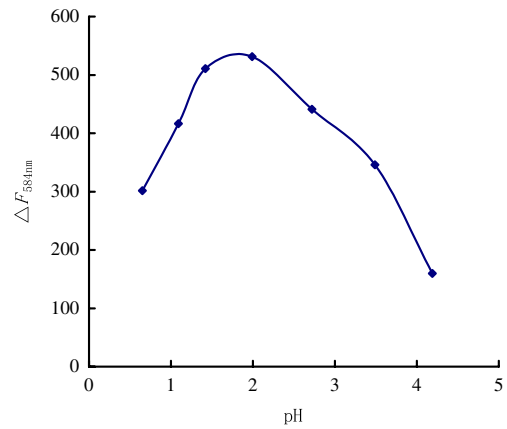Fig. 4 Effect of RhB concentration. pH 1.99 sodium acetate-HCl +87.0 mg/L Fe_3O_4 +200 nmol/L H_2O_2

Fig. 5 Effect of pH. 1.88 µg/mL RhB+200 nmol/L H_2O_2+87.0 mg/L Fe_3O_4

and should be separated from the system by a magnet. In the optimum conditions, as the concentration of H_2O_2 increased, the fluorescence intensity at 584 nm decrease linearly (Fig. 2). Thus, a fluorescence wavelength of 584 nm was chosen for use.

Absorption Spectra There is a maximum absorption peak at 553 nm for RhB (Fig. 3). As the concentration of H_2O_2 increased, the absorbance decrease at 553 nm, and the decreased absorbance is linear with the concentration of H_2O_2 in the range of 20–60 nmol/L, and its regress equation is $\Delta A_{553nm}=0.0027C-0.0003$, correlation coefficient is 0.9986, detection limit is 8.8 nmol/L H_2O_2. However, this spectrophotometric method is not sensitive and the detection range is narrow.

Effect of RhB Concentration As shown in Fig. 4, it can be seen that the ΔF_{584nm} values of the system increased gradually with the concentration of RhB at first, because more RhB molecules were oxidized by the hydroxyl radical. Then the system reached its maximum ΔF_{584nm} value when the concentration was 1.88 µg/mL RhB,
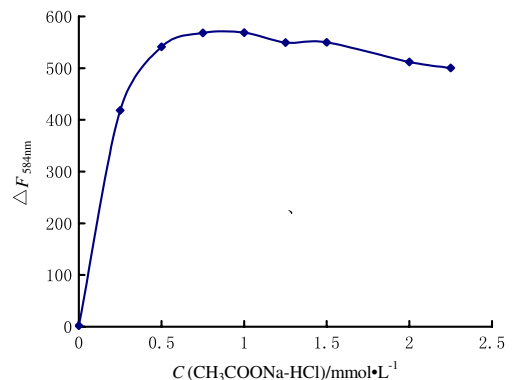

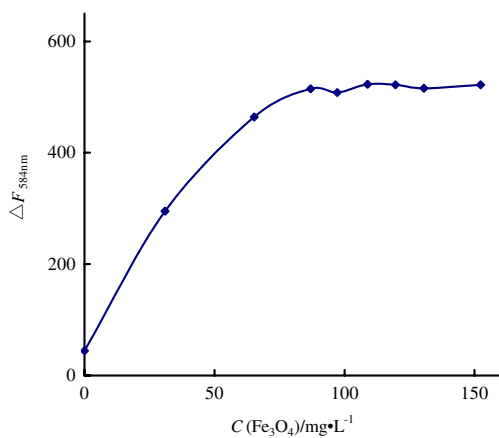Fig. 6 Effect of sodium acetate-HCl concentration. 1.88 µg/mL RhB+200 nmol/L H_2O_2+87.0 mg/L Fe_3O_4

Fig. 7 Effect of Fe$_3$O$_4$ concentration. pH 1.99 sodium acetate –HCl+ 1.88 μg/mL RhB+200 nmol/L H$_2$O$_2$

because there are enough RhB molecules to be oxidize in the system. Therefore, 1.88 μg/mL RhB was chosen for the system.

Effect of pH Value The catalytic activity of Fe$_3$O$_4$ nanoparticles is dependent on pH. The pH value is too high or too low that was not benefit to form hydroxyl radical. The effect of pH value (pH 0.65–4.19) on the ΔF_{584nm} was studied in this paper as in Fig. 5. The ΔF_{584nm} value of system reached their maximum when the pH value was 1.99. Thus, pH 1.99 was selected for the system.

Moreover, the influence of sodium acetate-HCl concentration on ΔF_{584nm} was considered in Fig. 6. The ΔF_{584nm} value reached its maximum when the concentration of sodium acetate was in the range of 5.0×10^{-4}–1.5×10^{-3} mol/L. Therefore, a concentration of 1.0×10^{-3} mol/L sodium acetate -HCl was selected for use.

Effect of Fe$_3$O$_4$ Concentration Fe$_3$O$_4$ nanoparticle is the catalyst. In the absence of the catalyst, there is no thydroxyl radical and the quenching reaction did not take place. When
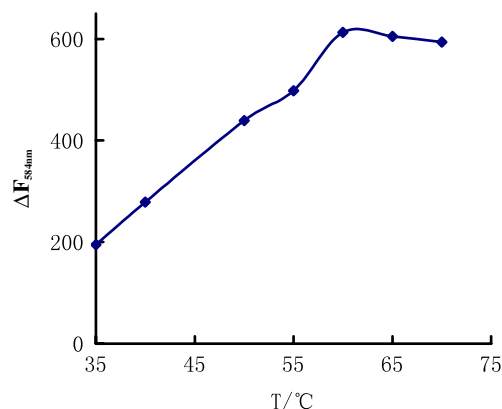

Fig. 8 Effect of reaction temperature. pH 1.99 sodium acetate -HCl +1.88 μg/mL RhB+87.0 mg/L Fe$_3$O$_4$+200 nmol/L H$_2$O$_2$

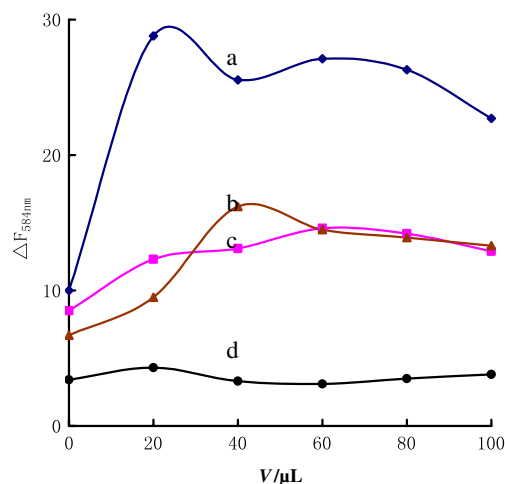
Fig. 9 Effect of nanoparticle on the RhB-H$_2$O$_2$ system. **a** 2.4×10^{-4} mol/L Au; **b** 2.4×10^{-4} mol/L Au:Se=5:1(calculated by Au); **c** 2.4×10^{-4} mol/L Ag; **d** 1.0×10^{-4} mol/L Pt

the nanocatalyst increased the thydroxyl radicals increased, and the quenching enhanced. The effect of the concentration of Fe$_3$O$_4$ nanoparticles concentration on ΔF_{584nm} was tested (Fig. 7). Results showed that the ΔF_{584nm} value was nearly 0 when there was no Fe$_3$O$_4$ nanoparticles in the solution. It could be seen that, when Fe$_3$O$_4$ concentration was less than 87.0 mg/L, with the increasing of Fe$_3$O$_4$ concentration, the formed thydroxyl radicals increased, and ΔF_{584nm} increased greatly. However, if Fe$_3$O$_4$ concentration was more than 87.0 mg/L, the fluorescence quenching intensity tends to maximum value. Thus, a concentration of 87.0 mg/L Fe$_3$O$_4$ was chosen for use.

Effect of Reaction Temperature and Time Catalyic reaction ia a kinetic process. General, reaction temperature increased the velocity enhanced. Too high temperature was not benefit to form thydroxyl radicals. The effect of reaction temperature between 35 °C and 75 °C on the ΔF_{584nm} was considered. Figure 8 indicated that the ΔF_{584nm} changed greatly with the temperature. When temperature was 60 °C, the ΔF_{584nm} value reached its maximum. So a reaction temperature of 60 °C was selected. In addition, the effect of time on ΔF_{584nm} showed that the system needed 20 min to reach the maximum ΔF_{584nm}. Therefore, a reaction time of 20 min was selected in this assay.

Table 1 Determination results of H$_2$O$_2$ in synthetic samples

Sample	True value /μmol·L^{-1}	Determined value /μmol·L^{-1}	Recovery /%	RSD /%
1	1.0	0.920±0.07	92.0	7.6
2	2.0	1.89±0.11	94.5	5.8
3	4.0	3.69±0.23	92.2	6.2

Influence of Foreign Substances The influence of 11 foreign ions on the nanocatalytic reaction determination of 100 nmol/L H_2O_2 was examined. The tolerance limit is defined as the molar ratio of [Ion]/[H_2O_2] that giving a relative error not more than ± 10%. The results showed that 100,000 times of Zn^{2+}, 20,000 times of Hg^{2+}, 10,000 times of Ba^{2+}, 2,000 times of Al^{3+}, 1,000 times of Mg^{2+} and NH_4^+, 500 times of Ca^{2+}, 300 times of Ni^{2+}, 250 times of Cu^{2+}, 25 times of F^- and 20 times of CO_3^{2-} have no interference on the determination. Furthermore, the catalytic effect of four metal nanoparticles on the CH_3COONa-HCl-RhB-H_2O_2 system was considered, such as Au, Ag, Au:Se (5:1) and Pt. Figure 9 indicated that the ΔF_{584nm} changed weakly as the concentration of four metal nanoparticles increased. It can be seen that the method has good selectivity.

Working Curve According to the procedure, the ΔF_{584nm} value is proportional to the H_2O_2 concentration (C) in the range of 10–200 nmol/L. The regression equation is $\Delta F_{584nm} = 2.44C + 27.6$, the correlation coefficient is 0.9936, the detection limit is 5.8 nmol/L H_2O_2. This method has higher sensitivity, better selectivity, easier availability and lower cost than the reported methods [4–13].

Analysis of Synthetic Samples Three synthetic samples were prepared in this assay. Sample 1, 2 and 3 include 1.0×10^{-6} mol/L H_2O_2-2.0×10^{-3} mol/L Mg^{2+}-2.0×10^{-2} mol/L Ba^{2+}-1.0×10^{-3} mol/L Ca^{2+}, 2.0×10^{-6} mol/L H_2O_2-4.0×10^{-3} mol/L Hg^{2+}-3.0×10^{-4} mol/L Ni^{2+}-4.0×10^{-4} mol/L NH_4^+ and 4.0×10^{-6} mol/L H_2O_2-0.1 mol/L Mg^{2+}-2.0×10^{-4} mol/L Cu^{2+}-4.0×10^{-4} mol/L Al^{3+}, respectively. The concentration of H_2O_2 in three samples was determined five times respectively. The results were listed in Table 1, the recovery was in the range of 92.0%–94.5% and relative standard deviation (RSD) were in the range of 5.8%–7.6%.

Conclusion

Based on the nanocatalysis of Fe_3O_4 on the H_2O_2 oxidization of RhB fluorescence quenching, a new method has been proposed for the determination of trace H_2O_2 with high sensitivity, good selectivity, easy availability and low cost. This assay was applied to the detection of H_2O_2 in synthetic sample with satisfactory results.

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