



Water-soluble fluorescent conjugated polymer-enzyme hybrid system for the determination of both hydroquinone and hydrogen peroxide

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ABSTRACT

In this paper, a sensitive and simple detecting system was developed for quantitative analysis of both hydroquinone (H_2Q) and hydrogen peroxide (H_2O_2), based on the successful combination of horse radish peroxidase (HRP) and water-soluble conjugate fluorescence polymers PPESO₃. In the presence of HRP and H_2O_2 , H_2Q could be oxidized to 1,4-benzoquinone (BQ), an intermediate, which plays the main role in the enhanced quenching of the photoluminescence (PL) intensity of PPESO₃. The quenching PL intensity of PPESO₃ (I_0/I) was proportional to the concentration of H_2Q and H_2O_2 in the range of 1.0×10^{-6} to 2.0×10^{-3} mol/L ($R^2 = 0.996$) and 6.0×10^{-6} to 2.0×10^{-3} mol/L ($R^2 = 0.999$), respectively. The detection limit for H_2Q and H_2O_2 was 5.0×10^{-7} mol/L and 1.0×10^{-6} mol/L, respectively. The present fluorescence quenching method was successfully applied for the determination of H_2Q in the lake water, rainwater, tap-water and chemical plant wastewater samples. Compared with previous reports, the fluorescence quenching approach described in this work is simple and rapid with high sensitivity, which has a potential application for detecting various analytes which can be translated into quinone.

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1. Introduction

Phenol compounds such as hydroquinone (H_2Q) are among the major components of pollutants produced by industrial wastewater and agricultural activities. H_2Q is a potentially carcinogenic substance, which causes severe effects on the central nervous system [1]. H_2Q has extremely detrimental effects on humans through oral, dermal, or respiratory tracks and thus represent a serious environmental problems and health hazards [2,3]. Due to the high toxicity and persistence of H_2Q in the environment, the sensitive and fast determination of H_2Q becomes an important subject. Many methods have been reported for the determination of H_2Q , including gas chromatography [4], chemiluminescence [5], amperometric detection and flow injection analysis [6]. However, compared with these methods, optical biosensors based on absorbance and fluorescence detection are more advanced because of several advantages [7,8]. For example, the response mechanism of optical detection is very classic, simple and reliable, and the reactions taking place in sample solutions are not disturbed because optical transducers do not change the component in the solutions. Recently, optical sensors based on fluorescence quenching have been paid more attention. Wu et al. detected phenol optically using an oxygen-sensitive luminescent dye [7]. Wang et al. used a quantum dots-enzyme system to detect phenolic compounds and H_2O_2

[9]. Zhu et al. used molecularly imprinted polymer microspheres prepared through precipitation polymerization for H_2Q recognition [1].

In recent years, conjugated polymers (CPs) have been intensively studied for chemical and biological sensor applications owing to their superior signal amplification and superquenching compared to quantum dots and small molecule dyes [10]. These amplification and superquenching properties are due to the conjugated polymer backbone [11], on which one single quencher molecule can cause an effective and fast quenching. Thanks to these amplification and superquenching properties, CPs show some interesting and useful properties i.e. strong light absorption, strong fluorescence, electroactivity, and good transport properties for charge carriers and excitons [12]. So far, various analytes, such as metal ions [13], small molecules [14], enzymes [15], proteins [16], DNA [17] and RNA [18] have been detected in aqueous solution or on solid substrates. Since CPs quenched by phenol have not been reported previously and indeed might have wide potential application in the development of phenol sensor, we developed a H_2Q sensor based on CPs (PPESO₃) quenching.

Based on the enzymatic reaction product of H_2Q can efficiently quench the photoluminescence (PL) intensity of PPESO₃, we established a sensitive and simple H_2Q sensor. Meanwhile, the PPESO₃ PL intensity can be quenched with the addition of H_2O_2 in the presence of H_2Q , thus detection of H_2O_2 can also be achieved. The detection limits for H_2Q compounds (5.0×10^{-7} mol/L) are low enough to detect the common levels of phenolic pollutants in wastewater and lower than the U.S. Environmental Protection Agency

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estimated wastewater discharge limit of 0.5 mg/L (at 10^{-6} mol/L level) [19].

2. Experiment

2.1. Chemicals

1,4-Diethynylbenzene and Tetrakis(triPhenylPhosphine)palladium ($(PPh_3)_4Pd$) were purchased from Aldrich Chemical Co. and Hangzhou Kaida Metal Catalyst & Compounds Co. Ltd. (Hangzhou, China), respectively. 2,5-Diiodohydroquinone, dioxane (Tianjin Guangfu Institute of elaborate chemical industry) and 1,3-propanesultone (J&K Chemical) were used as received. Horse radish peroxidase (HRP) was obtained from Sino-American Biotechnology Co. Ltd. H_2Q and benzoquinone (BQ) were purchased from Tianjin Guangfu Fine Chemical Research Institute. All the other chemicals, including CuI , Na_2HPO_4 , NaH_2PO_4 , H_2O_2 methanol, acetone, ether and DMF were obtained from Beijing Chemical Co. Ltd. Stock solutions of 0.1 mol/L H_2O_2 , benzoquinone, hydroquinone, and HRP were freshly prepared daily. All chemicals used were of analytical reagent grade without further purification. The water used in all experiments had a resistivity of 18 M Ω /cm. All the water used in the experiments was deaerated by purging with N_2 for 30 min.

2.2. Instrumentation

All fluorescence measurements were carried out in a 1 cm path-length quartz cuvette at ambient temperature with a Shimadzu RF-5301 spectrometer. In all optical experiments, the excitation wavelength was 400 nm and the fluorescence intensity referred to the maximum emission of PPESO₃ at 520 nm.

2.3. Experimental method

The synthesis of PPESO₃ was according to the previous paper [20]. For BQ quenching experiment, 1 mL of 2.0 μ mol/L PPESO₃ in PBS (pH 7.0) solution and 1 mL of BQ solution with different concentrations from 2.0 μ mol/L to 6.0 mmol/L were added to a quartz cuvette. The final volume of every sample is 2.0 mL and the final concentration of PPESO₃ is 1.0 μ mol/L. Then the mixture was shaken evenly and the spectral information was recorded by spectrofluorophotometer. For the assay of H_2Q , the conjugated polyelectrolyte PPESO₃ was diluted to 1.0 μ mol/L with PBS (pH 7.0), followed by the addition of 2.0 μ g/mL HRP and 3.0 mmol/L H_2O_2 . Then, various amounts of H_2Q were added, with the ultimate concentration of H_2Q covered a relatively wide range (1.0×10^{-6} to 2.0×10^{-3} mol/L). The resulting solution (2.0 mL) was shaken evenly kept at room temperature for 14 min before recording the spectral information by spectrofluorophotometer. The method of detecting H_2O_2 was same as that of H_2Q . As H_2Q is easily oxidized under environmental conditions, so deoxidation water were used in each H_2Q detecting experiment. For real samples detection, the system can be used to determine both H_2Q and BQ. Combination of the system and the hybrid without H_2O_2 and/or HRP would be useful to detect H_2Q and BQ individually. The molecular structures of BQ, H_2Q and PPESO₃ are shown in Scheme S1 (in supplementary material).

For real samples detection, we chose four kinds of water samples (lake water, rainwater, chemical plant wastewater and tap-water) and all the water samples were diluted 10 times with 0.02 mol/L PBS (pH 7.0). The diluted water samples were added with different concentrations of H_2Q (0.1 mmol/L, 1.0 mmol/L, 2.0 mmol/L) to prepare the spiked samples. Real samples detection was carried out using the procedure described above.

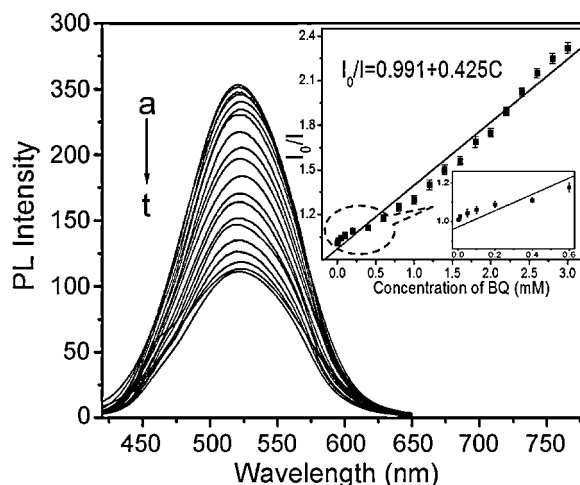


Fig. 1. The PL spectra of 1.0 μ mol/L PPESO₃ in different concentrations of BQ. a–u represent the concentrations of BQ of 0.001, 0.006, 0.01, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8 and 3.0 mmol/L, respectively. The inset shows the linear relationship between I_0/I and concentration of BQ. The excitation wavelength was 400 nm.

3. Results and discussion

3.1. Superquenching of water-soluble PPESO₃ by 1,4-benzoquinone

1,4-benzoquinone (BQ) as a classic electron acceptor could shuttle the electron from the conduction band to the valence band of the excited luminescence material, resulting in the quenching of PL intensity [21]. In aqueous solution, PPESO₃ exhibited a strong green emission at around 520 nm. When BQ was added to PPESO₃ PBS solution, even at very low concentration, the fluorescence of PPESO₃ was quenched remarkably. The quenching was enhanced gradually with the increasing quantity of BQ. And the efficiency of fluorescence quenching can be evaluated quantitatively by the Stern–Volmer equation [22].

$$\frac{I_0}{I} = 1 + K_{SV}[Q]$$

where I and I_0 are the fluorescence intensities with and without the quencher, respectively, $[Q]$ is the quencher concentration, and K_{SV} represents the Stern–Volmer constant. From the slope of the Stern–Volmer plot (Fig. 1 inset), K_{SV} is calculated to be 4.25×10^2 L/mol, which shows that BQ is an effective quencher by accepting electron from PPESO₃. A linear relationship between I_0/I and concentration of BQ was gained from 1.0×10^{-6} to 3.0×10^{-3} mol/L BQ. The PL intensity of PPESO₃ was quenched more than 95% by 8.0 mmol/L BQ (not shown in the figure).

The relationship between the reaction time and PL intensity of PPESO₃ was shown in Fig. 2. It can be seen that upon addition of BQ, the PL intensity of PPESO₃ quenched immediately due to electron transfer quickly from PPESO₃ to BQ, then the fluorescence intensity remained nearly unchanged with further increase in reaction time, which indicated that it was very rapid to reach equilibrium for the interaction between BQ and PPESO₃.

To distinguish between static and dynamic mechanisms, temperature dependence of Stern–Volmer equation should be addressed. Fig. S1 (in supplementary material) shows the Stern–Volmer plot for fluorescence quenching system of PPESO₃–BQ at two different temperatures. The K_{SV} is 1.10×10^3 L/mol at 313 K while the K_{SV} is 4.25×10^2 L/mol at 288 K. The results show that the Stern–Volmer quenching constant is in proportion to

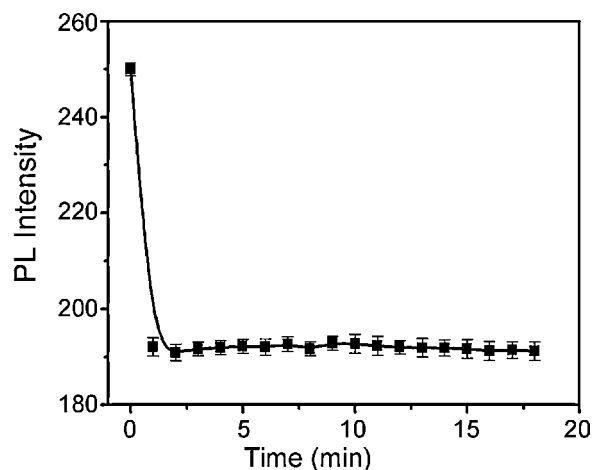
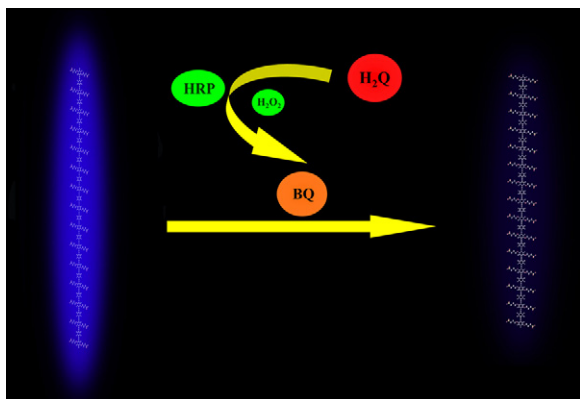


Fig. 2. The relationship between PL intensity of 1.0 $\mu\text{mol/L}$ PPESO₃ and the reaction time in the presence of 1.0 mmol/L BQ.

temperature, which suggests that dynamic mechanisms should be responsible for the fluorescence quenching of PPESO₃ by BQ [23].

3.2. Quenching by hydroquinone in the presence of HRP and H₂O₂

H₂Q can be oxidized into the form of BQ with the catalyzed oxidation action of HRP and H₂O₂ [9]. Taking into account the results in above section, BQ as a quencher could cause a significant quenching of the PL intensity of PPESO₃. It is supposed that the quantification of H₂Q can be achieved by measuring the fluorescence quenching of PPESO₃ in the presence of HRP and H₂O₂ (Scheme 1). In order to testify the detecting scheme, several control experiments have been carried out. PPESO₃ mixed with H₂O₂, H₂Q and HRP separately and the mixtures of two or three were investigated and the results were shown in Fig. 3. It can be seen that neither H₂O₂ nor H₂Q can quench the PL intensity of PPESO₃. The influence of sole HRP on the PL intensity of PPESO₃ was also very slight which can be ignored. The PL quenching of PPESO₃ could not be observed even when both H₂O₂ and H₂Q exist (Fig. 3). It implied that H₂Q cannot be oxidized into BQ without the presence of HRP in the system, thus PL quenching of PPESO₃ by BQ would not happen. However, upon sequential addition of HRP, H₂O₂ and H₂Q into an aqueous solution of PPESO₃, fluorescence superquenching can be observed remarkably. These results indicate that under catalyzed action of HRP, BQ which is intermediate produced in the oxidation of H₂Q could induce fluorescence quenching of PPESO₃. The formation of BQ is essential to quench the fluorescence emission of PPESO₃.



Scheme 1. Schematic illustration of PPESO₃ fluorescence quenching by H₂Q in the presence of H₂O₂ and HRP.

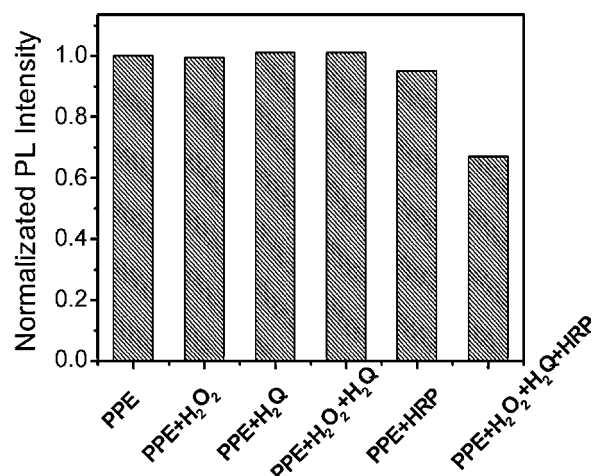


Fig. 3. The effect of HRP as catalyst on the oxidation of H₂Q in the absence/presence of HRP/H₂O₂ in 1.0 $\mu\text{mol/L}$ PPESO₃.

These results were consistent with Scheme 1 proposed above which affords the feasible and easy route for detection of H₂Q.

3.3. Optimization of experimental parameters for H₂Q detection

In this study, the relationship between the quenching effect and the incubation time was investigated. Fig. 4 shows the fluorescence quenching efficiency of H₂Q on PPESO₃ with different incubation time. When 1.0 mmol/L H₂Q was added to the system of 2.0 $\mu\text{g/mL}$ HRP, 1.0 mmol/L H₂O₂ in 0.02 mol/L PBS buffer (pH 7.0), the PL intensity of PPESO₃ decreased gradually from 220.7 to 102.8 with the incubation time changed from 0 to 14 min. When incubation time was longer than 14 min, the quenching effect reaches a plain and remained unchanged. The results proved that the processes of catalyzed oxidation of H₂Q to BQ and PPESO₃ quenched by BQ finished in 14 min. In this study, the incubation time of 14 min is adopted.

Effect of HRP concentration on the PL intensity of PPESO₃ was investigated at three different concentrations of H₂Q and H₂O₂ (1.0, 2.0, 5.0 mmol/L) (Fig. 5). The concentration of H₂Q was equal to that of H₂O₂ because the same mole of them was consumed in the reaction [9]. We found that the quenching effect was enhanced by increasing quantity of H₂Q and H₂O₂. Taking 2.0 $\mu\text{g/mL}$ HRP for

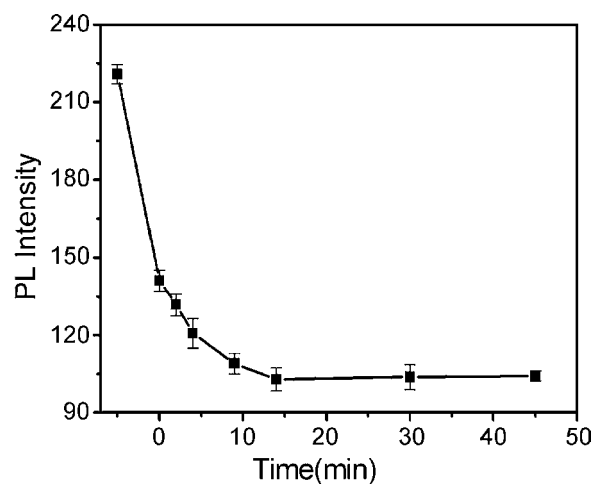


Fig. 4. The relationship between the PL intensity of 1.0 $\mu\text{mol/L}$ PPESO₃ and the incubation time in the presence of 1.0 mmol/L H₂O₂ and 2.0 $\mu\text{g/mL}$ HRP with the addition of 1.0 mmol/L H₂Q.

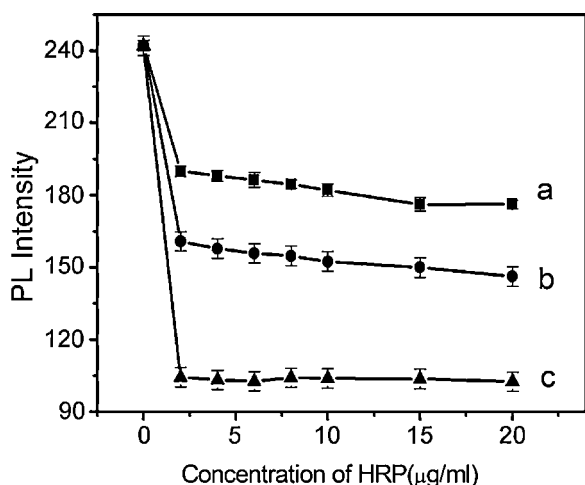


Fig. 5. Effect of different HRP concentrations on the fluorescence quenching of 1.0 $\mu\text{mol/L}$ PPESO₃ by H₂Q. With the addition of 1.0 mmol/L H₂Q and 1.0 mmol/L H₂O₂ (a); with the addition of 2.0 mmol/L H₂Q and 2.0 mmol/L H₂O₂ (b); with the addition of 5.0 mmol/L H₂Q and 5.0 mmol/L H₂O₂ (c).

example, the PL intensity of PPESO₃ has only 20% decrease when the concentration of H₂Q is 1.0 mmol/L. While it decreased to 160.8 and 104.4 from 240.0 when increasing the concentration of H₂Q to 2.0 mmol/L and 5.0 mmol/L respectively. On the other hand, the PL intensity of PPESO₃ decreased dramatically after adding a small amount of HRP and maintained the same when the concentration of HRP reached 2.0 $\mu\text{g/mL}$ for a given concentration of H₂Q and H₂O₂. These results indicate that the quantity of HRP did not depend on the concentration of H₂Q and H₂O₂. Thus, 2.0 $\mu\text{g/mL}$ HRP is adopted in following experiments.

The pH value can influence the HRP activity and stability of the conjugation via electrostatic interaction. So the effect of the pH of buffer solution was studied in this work. We investigated the quenching effect with different concentrations of H₂Q in PBS with pH from 4.0 to 9.0. PPESO₃ fluorescence intensity at 520 nm was recorded and the results were shown in Fig. S2 (in supplementary material). It can be seen that the I_0/I at 520 nm increased gradually with the change of pH value from 5.0 to 7.0. The maximal I_0/I which means the largest quenching appeared at pH 4.0 and 7.0. The inset graph shows that the fluorescence emission peak of PPESO₃ at pH 7.0 is more symmetrical distribution than that of at pH 4.0. Considering the bioactivity of HRP and symmetrical fluorescence emission peak of PPESO₃, we chose PBS of pH 7.0 in this study.

3.4. Detection of H₂Q via the HRP–H₂O₂–PPESO₃ system

The quenching effect of H₂Q concentration on the PL intensity of PPESO₃ in HRP–H₂O₂ system was further investigated under the optimized condition. Fig. 6 shows the fluorescence spectra of PPESO₃ in the presence of 3.0 mmol/L H₂O₂, 2.0 $\mu\text{g/mL}$ HRP and a series of different H₂Q concentrations. It can be seen that the PL intensity of PPESO₃ decreased gradually upon increasing concentration of H₂Q, while no obvious changes of spectral widths. We illustrate the corresponding plot showing a linear relationship between I_0/I and H₂Q concentrations in the range of 1.0×10^{-6} to 2.0×10^{-3} mol/L with a correlation coefficient $R^2 = 0.996$. The linear regression equation is $I_0/I = 0.960 + 1.185 C$ (H₂Q) (mmol/L) and the detection limit (3 σ) for H₂Q was found to be 5.0×10^{-7} mol/L, which was better than the reported electrochemical sensor of 1.0×10^{-6} mol/L [1], and had the similar performance as CdTe quantum dots-enzyme hybrid system of 5.0×10^{-7} mol/L [9]. The detection limits for H₂Q are low enough to detect the common levels of phenolic pollutants in wastewater and lower than the U.S.

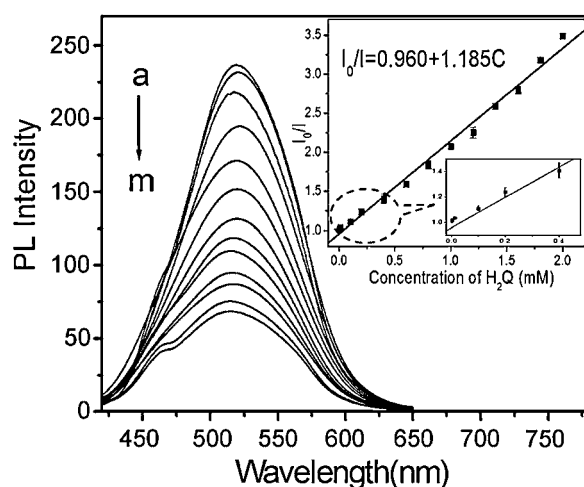


Fig. 6. The effect of H₂Q concentration on the PL intensity of 1.0 $\mu\text{mol/L}$ PPESO₃ in the presence of 3.0 mmol/L H₂O₂ and 2.0 $\mu\text{g/mL}$ HRP. a–n represent the concentrations of H₂Q of 0.001, 0.01, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 mmol/L, respectively. The inset shows the linear relationship between I_0/I and the concentration of H₂Q.

Environmental Protection Agency estimated wastewater discharge limit of 0.5 mg/L (at 10^{-6} mol/L level) [19].

3.5. Detection of H₂O₂ via the HRP–H₂Q–PPESO₃ system

According to the conclusion above, H₂Q can be quantitatively analyzed in the HRP–H₂O₂–PPESO₃ system. Similarly, H₂O₂ can also be detected by quenching PPESO₃ in the presence of HRP and H₂Q, so a detection method for H₂O₂ can be developed using the HRP–H₂Q–PPESO₃ system. The detection of H₂O₂ was carried out by adding different concentrations of H₂O₂ to PPESO₃ aqueous solution in the presence of 3.0 mmol/L H₂Q and 2.0 $\mu\text{g/mL}$ HRP in 20 mmol/L PBS buffer (pH 7.0). The results indicated that successive addition of H₂O₂ resulted in remarkable fluorescence quenching of PPESO₃ (Fig. 7). The 5.0 mmol/L H₂O₂ exhibits good quenching effects on the PL intensity of PPESO₃ with the quenching extent above 90% (not shown in the figure). The inset in Fig. 7 shows the relationship between I_0/I and the concentration of H₂O₂. A linear calibration plot was obtained in the range of

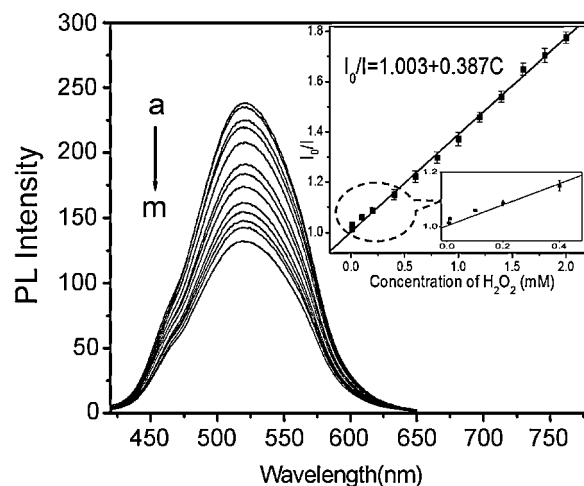


Fig. 7. The effect of H₂O₂ concentration on the PL intensity of 1.0 $\mu\text{mol/L}$ PPESO₃ in the presence of 3.0 mmol/L H₂Q and 2.0 $\mu\text{g/mL}$ HRP. a–n represent the concentrations of H₂O₂ of 0.006, 0.01, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 mmol/L, respectively. The inset shows the relationship between I_0/I and the concentration of H₂O₂.

Table 1

The interference of coexisting ions on the fluorescence quenching of $1.0 \mu\text{mol/L}$ PPES O_3 by H_2Q in the HRP/ H_2O_2 system.

| Coexisting substance | Tolerable concentration ratios | $\Delta I/I$ (% , $n=3$) ^a |
|----------------------|--------------------------------|--|
| Na^+ | 180 | 2.9 |
| K^+ | 200 | 0.58 |
| Zn^{2+} | 60 | 3.1 |
| Pb^{2+} | 80 | −0.92 |
| Cd^{2+} | 50 | 0.44 |
| Al^{3+} | 100 | −4.8 |
| NO_3^- | 120 | 3.0 |
| Cl^- | 200 | 0.60 |
| Glucose | 200 | 1.08 |
| Benzoic acid | 100 | −4.7 |
| Sodium benzoate | 150 | 4.92 |

^a $\Delta I = I_0 - I$, where I_0 and I are the fluorescence intensities of PPES O_3 –HRP– H_2O_2 – H_2Q system in the absence and presence of interfering species.

6.0×10^{-6} to 2.0×10^{-3} mol/L, the linear regression equation is $I_0/I = 1.003 + 0.387C(\text{H}_2\text{O}_2)$ (mmol/L) with a correlation coefficient $R^2 = 0.999$. The detection limit (3 σ) for H_2O_2 is 1.0×10^{-6} mol/L, which is close to the early reported (5.0×10^{-7} mol/L) [24]. According to the experiment result above, it is confirmed that the high sensitivity of the proposed H_2O_2 assay is mainly derived from the amplified fluorescence quenching of water-soluble conjugated polymers. And this quenching method may be extensively used to detect biological system with H_2O_2 producing process in the future.

3.6. Interference study

The detection selectivity of PPES O_3 –HRP– H_2O_2 system for H_2Q was further evaluated with various coexistence ions added. Interference investigation was carried out at a H_2Q concentration of 10^{-4} mol/L with various coexistence substrates added. The ions which usually exist in the wastewater and the water-soluble glucose, benzoic acid and sodium benzoate with chemical structures similar to H_2Q were chosen as the interference ions. The tolerance of each coexistence substrate was taken as the highest concentration yielding a relative error less than $\pm 5\%$. As shown in Table 1, the results showed that the tolerable concentration ratios of coexisting substance to 10^{-4} mol/L H_2Q was over 180-fold for Na^+ , 200-fold for K^+ , Cl^- , 100-fold for Al^{3+} ; 60-fold for Zn^{2+} , 80-fold for Pb^{2+} , 50-fold for Cd^{2+} , 120-fold for NO_3^- , 200-fold for glucose, 100-fold for benzoic acid and 150-fold for sodium benzoate. It can be seen that the proposed fluorometric method displays a high selectivity for the determination of H_2Q .

Table 2

Analytical results of H_2Q in real water samples.

| Samples | Added (mmol/L) | Found (mmol/L) ^a | Recovery (%) |
|---------------------------|----------------|-----------------------------|--------------|
| Lake water | 0.100 | 0.104 ± 0.029 | 104 |
| | 1.000 | 1.003 ± 0.010 | 100 |
| | 2.000 | 2.073 ± 0.022 | 104 |
| Rain water | 0.100 | 0.178 ± 0.044 | 108 |
| | 1.000 | 1.039 ± 0.023 | 104 |
| | 2.000 | 2.011 ± 0.025 | 101 |
| Chemical plant wastewater | 0.100 | 0.956 ± 0.048 | 96 |
| | 1.000 | 0.940 ± 0.023 | 94 |
| | 2.000 | 1.967 ± 0.015 | 99 |
| Tap-water | 0.100 | 0.109 ± 0.027 | 109 |
| | 1.000 | 1.070 ± 0.035 | 107 |
| | 2.000 | 2.056 ± 0.030 | 103 |

^a Values are mean of three replicates (\pm S.D.).

3.7. Real samples detection

In order to evaluate the feasibility of the method in the determination of H_2Q in real samples, four kinds of water samples (lake water, rainwater, chemical plant wastewater and tap-water) were analyzed in this study. The results were listed in Table 2. It can be seen that the coexistent substances did not interfere with the determination of H_2Q , the results showed satisfactory recoveries in the range of 94–109%, which indicates that the HRP– H_2O_2 system permits the quantitative determination of H_2Q in the presence of some coexistent substances.

4. Conclusions

In this work, we developed a sensitive and facile fluorescent sensor for detecting H_2Q and H_2O_2 based on the superquenching of fluorescent conjugated polymer. We could successfully detect H_2Q and H_2O_2 with the detection limit of 5.0×10^{-7} mol/L and 1.0×10^{-6} mol/L in the linear range of 1.0×10^{-6} to 2.0×10^{-3} mol/L and 6.0×10^{-6} to 2.0×10^{-3} mol/L respectively. The sensitivity for the detection of H_2Q in this study was much higher than that of previous optical methods. It is the first report regarding water-soluble fluorescent conjugated polymer employed for the detection of phenol compounds under the HRP catalyzed action. This method may provide a basis for the future development of fluorescent conjugated polymer sensor, which may widely be used in detecting H_2Q or H_2O_2 industrial water, environmental analysis, pharmaceuticals, food industry, and so on. Furthermore, with the advantages of simplicity, rapidness, highly efficiency quenching, non-toxicity and easy operation, the fluorescent conjugated polymer has great potential in the development of sensors for widely detecting various analytes with quinone producing process.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.talanta.2011.08.053](https://doi.org/10.1016/j.talanta.2011.08.053).

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