A Novel Fluorescent Reagent for Analysis of Hydrogen Peroxide

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8-(4,6-Dichloro-1,3,5-triazinoxy) quinoline (DTQ) was evaluated as a new fluorescent reagent for determining hydrogen peroxide. It was found that the fluorescence intensity of DTQ in alkaline medium could be dramatically enhanced upon addition of H_2O_2. Based on this effect, a simple and selective method for the spectrofluorimetric determination of hydrogen peroxide was established. The relative standard deviation of the method was found to be 1.1% for 9 replicate determinations of a 4.6×10^{-6} mol/L hydrogen peroxide solution. The linear range was 2.3×10^{-7}—2.3×10^{-5} mol/L with a detection limit of 2.2×10^{-8} mol/L ($S/N = 3$). The method was attempted to determine hydrogen peroxide in synthetic human serum samples with satisfactory results.

Keywords 8-(4,6-dichloro-1,3,5-triazinoxy) quinoline, hydrogen peroxide, serum, fluorescence

Introduction

The oxidation of many clinical substances in body fluids produces a quantity of hydrogen peroxide, so the determination of trace hydrogen peroxide is of considerable importance in clinical chemistry.[1] Further, the monitoring of hydrogen peroxide is also necessary to environmental science since it is a key species in the reactions of the troposphere, being involved in important reactions such as the catalyzed or uncatalyzed aqueous phase oxidation of SO_2 and the ultraviolet-enhanced aqueous phase oxidation of organic species.[2] Up to now, various methods for analysis of H_2O_2 have been developed, including electroanalysis,[3,4] photometry,[5,6] luminescence[7,8] and fluorescence.[9,10] Among them, the most widely used method involves the peroxidase-catalyzed reactions between hydrogen peroxide and hydrogen-donating substances, since enzymatic determinations of H_2O_2 are of unusual sensitivity, selectivity and simplicity.

In connection with a program of investigating spectroscopic probes, several new cyanuric chloride derivatives have been prepared in our laboratory,[11,12] among which 8-(4,6-dichloro-1,3,5-triazinoxy)quinoline (DTQ) will be presented here as a new fluorescent reagent for quantitative analysis of hydrogen peroxide, though it had been successfully used for *in situ* fluorescent labeling of volatile methamine.[12] It was found that the fluorescence intensity of DTQ at 440 nm was largely increased upon the addition of trace H_2O_2, and the increase was linear to the concentration of H_2O_2 in certain ranges. Based on this, a new fluorescence method for analysis of H_2O_2 was established, which is rather sensitive. This method has been successfully attempted to determine H_2O_2 added in serum.

Experimental

Instruments and materials

Fluorescence spectra were recorded as raw data with F-2500 spectrofluorimeter in 10 × 10 mm quartz cells (Hitachi Ltd., Tokyo). A model 25 pH-meter (Shanghai, China) was used for pH measurements.

Cyanuric chloride and 8-hydroxyquinoline were obtained from Acros and Merck, respectively, which were used as received. Hydrogen peroxide was purchased from the Tianjin East Chemical Reagent, Co. The solution of hydrogen peroxide was prepared in distilled water daily. DTQ (Fig. 1) was synthesized according to our previous procedure,[12] and its solution was prepared in dry acetone at a concentration of 1 mg/mL. It should be pointed out that the stock solution of DTQ should be kept in a cold and dry place to avoid hydrolysis. All the other reagents were of analytical grade.

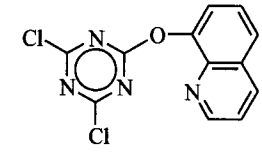

Fig. 1 Structure of DTQ.

Procedure

To a 10-mL tube, 2 mL of phosphate buffer (0.1 mol/L Na_2HPO_4-NaH_2PO_4, pH = 8.8) and 40 μL of hydrogen peroxide solution (4.62×10^{-6} mol/L) were added, followed by addition of 20 μL of DTQ solution (1 mg/mL). The mixture was diluted to 4 mL with distilled water and then a portion of the solution was transferred to a quartz cell. The relative fluo-

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rescence ΔF was measured against a reagent blank without hydrogen peroxide prepared under the same conditions.

Results and discussion

Fluorescent properties

Fig. 2 shows the fluorescence spectra of DTQ before and after reaction with hydrogen peroxide. It can be seen that DTQ has an excitation peak at 313 nm and an emission peak at 440 nm with a shoulder at 390 nm, respectively. The introduction of trace hydrogen peroxide resulted in a large increase in fluorescence intensity. The mechanism for this effect is unclear and may be rather complex, but a feasible explanation may be that the possible decomposition or redox reaction of H_2O_2 affects the fluorescence properties of the system. Based on such enhancement the conditions for the determination of hydrogen peroxide were investigated.

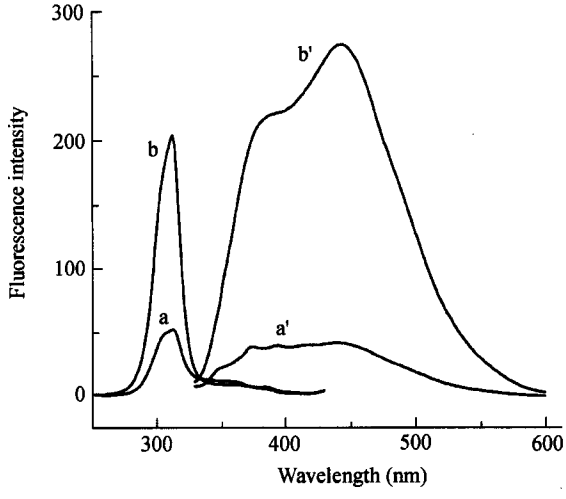

Fig. 2 Fluorescence spectra of DTQ and DTQ-H_2O_2 system. (a) and (a′) are the excitation and emission spectra of DTQ, respectively; (b) and (b′) are the excitation and emission spectra of DTQ-H_2O_2 system, respectively. Conditions: [DTQ] = 8.5×10^{-6} mol/L, [H_2O_2] = 9.24×10^{-6} mol/L, Na_2HPO_4-NaH_2PO_4 buffer (0.1 mol/L, pH 8.8).

Effect of pH

As is well known, the reaction medium is one of the most important factors that influence the fluorescence of a system, either by changing the intensity or by shifting the spectrum. In order to obtain the maximum efficiency in the production of fluorescence at 440 nm, different reaction media were examined. Fig. 3 shows the results obtained in the solution of Na_2CO_3-$NaHCO_3$ (0.1 mol/L), $Na_2B_4O_7$-NaOH (0.1 mol/L), NH_3-NH_4Cl (0.1 mol/L) and Na_2HPO_4-NaH_2PO_4(0.1 mol/L), respectively. It is seen from this figure that the maximum intensity can be obtained in 0.1 mol/L Na_2HPO_4-NaH_2PO_4 buffer with pH 8.0—9.0. A reaction medium of Na_2HPO_4-NaH_2PO_4 buffer with pH 8.8 was thus selected for the present system.

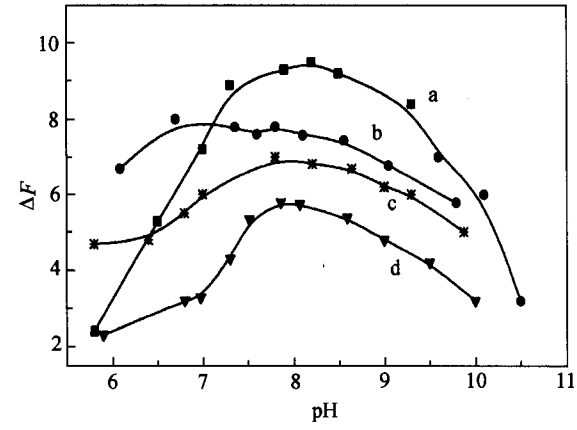

Fig. 3 Effects of reaction medium and pH. Buffers: (a) Na_2HPO_4-NaH_2PO_4; (b) $Na_2B_4O_7$-NaOH; (c) Na_2CO_3-$NaHCO_3$; (d) NH_3-NH_4Cl. Conditions: [DTQ] = 3.4×10^{-5} mol/L, [H_2O_2] = 4.62×10^{-6} mol/L.

Effect of DTQ *concentration*

The effect of DTQ concentration was tested in detail. It was found that the relative fluorescence intensity reached a maximum when the concentration of DTQ was in the range of 1.7×10^{-5}—3.4×10^{-5} mol/L. So, a concentration of 1.7×10^{-5} mol/L DTQ may be chosen for the experiments.

Effect of surfactant

The effect of surfactant was also checked. The experimental results showed that most of the surfactants such as Triton X-100, Zeph, BDHAC and SDS enhanced the fluorescence intensity of both the test solution and the reagent blank solution, but no obvious increase of signal to noise was observed. So a surfactant was not used in this system.

Effect of temperature

Since DTQ is a dichlorotriazine derivative easy to hydrolyze, the reaction temperature would affect its stability and thereby the fluorescence of the system. Fig. 4 shows such an effect of temperature (T). With the increase of temperature, the fluorescence intensity decreases, but the fluorescence intensity almost did not change any more at $T > 40$ ℃. It is understandable that a low temperature causes a strong fluorescence due to the decrease of collisional quenching. Although the relative fluorescence intensity had a maximum at $T = 0$ ℃, this temperature was not the selected temperature in our experiments because 0 ℃ is critical and inconvenient. Rather, the determination was performed at room temperature [(25 ± 3) ℃] and within 5 min (a longer waiting time of ≥10 min caused a decrease of ≥5% in fluorescence) after test solution was prepared.

Linearity

Under the optimum conditions mentioned above, the rel-

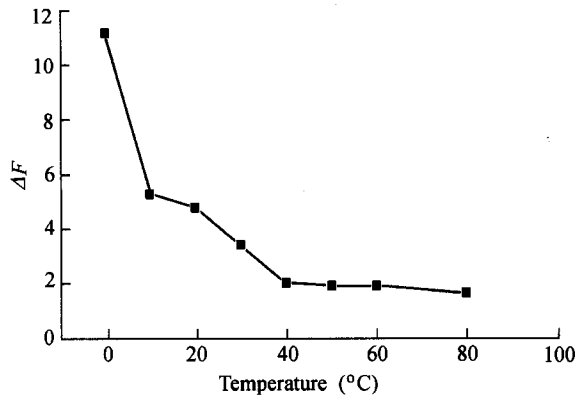

Fig. 4 Effect of heating on the fluorescence of the system. Conditions: [DTQ] = 3.4×10^{-5} mol/L, [H_2O_2] = 4.62×10^{-6} mol/L, Na_2HPO_4-NaH_2PO_4 (0.1 mol/L, pH 8.8), reaction time: 5 min.

ative fluorescence intensity was directly proportional to the hydrogen peroxide concentration over the range 2.3×10^{-7}—2.3×10^{-5} mol/L with a detection limit of 2.2×10^{-8} mol/L ($S/N = 3$). The regression equation was determined to be $F = 0.090[C] + 12.46$ ($r = 0.999$, $n = 5$, 2.3×10^{-6}—2.3×10^{-5} mol/L H_2O_2); $F = 0.323[C] + 3.244$ ($r = 0.996$, $n = 5$, 2.3×10^{-7}—2.3×10^{-6} mol/L H_2O_2), where F is the relative fluorescence intensity and [C] is the hydrogen peroxide concentration ($\times 10^{-7}$ mol/L). The relative standard deviation was 1.1% for 9 replicate determinations of 9.24×10^{-7} mol/L hydrogen peroxide.

Influence of foreign substances

The influence of foreign substances on the determination of 9.24×10^{-7} mol/L hydrogen peroxide was studied. The tolerance limit was estimated with the criteria of relative error less than ±5% in fluorescence signal. The results are listed in Table 1. It can be seen that most species in body fluids have no effect on fluorescence signal even in the absence of any masking reagent, suggesting that this method is selective.

Table 1 Effects of diverse substances on the determination of H_2O_2 (1 μmol/L)

Foreign substances	Maximum tolerance ratio of ion to H_2O_2 (molar ratio)
K^+, Cl^-, SO_4^{2-}	> 1000
Cu^{2+}	500
Ba^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}	200
Cd^{2+}	170
Co^{2+}, Cr^{3+}	100
Pb^{2+}, Zn^{2+}	50
Ag^+, Al^{3+}	20
Fe^{3+}	10
Ni^{2+}	8
Albumin	50 mg/mL

Analysis of samples

In order to test the applicability of the proposed method, the determination of hydrogen peroxide added in serum was attempted and the results are given in Table 2. The recoveries of hydrogen peroxide from serum are 91%—107%, implying that DTQ can be used for determining H_2O_2.

Table 2 Determination of trace amount of H_2O_2 added in serum

Sample[a]	H_2O_2 added ($\times 10^{-6}$ mol/L)	H_2O_2 found ($\times 10^{-6}$ mol/L)[b]	Recovery (%)
Serum I	9.24	9.87 ± 0.02	106.8
	13.86	12.71 ± 0.01	91.7
	28.48	26.17 ± 0.05	92.0
Serum II	9.24	8.61 ± 0.03	93.2
	13.86	14.28 ± 0.04	103.0
	28.48	29.90 ± 0.02	105.0

[a] Serum samples were provided by Zhongguancun hospital of Beijing. [b] Analytical results were expressed as mean of three determinations ± S.D.

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