Spectrophotometric Determination of Hydrogen Peroxide Using *o*-Hydroxyhydroquinonephthalein, Titanium(IV) and Ethylenediaminetetraacetic Acid¹

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We reported² on a new method for the determination of hydrogen peroxide (denoted by H_2O_2) based on a fading of an *o*-sulphophenylfluorone (denoted by SPF)-titanium(IV) complex in the presence of ethylenediaminetetraacetic acid (denoted by EDTA). Although this method is highly sensitive, since the apparent molar absorptivity is $2.3 \times 10^5 \text{ l mol}^{-1} \text{ cm}^{-1}$, a heating procedure for a long time is necessary for a maximum color development. In a continuation of this investigation of an H_2O_2 determination, an attempt was made to obtain an *o*-hydroxyhydroquinonephthalein (denoted by QP)-titanium(IV) complex using QP instead of SPF so as to improve the QP-titanium(IV) complex method.

This paper deals with a simple and rapid procedure for determination of H_2O_2 by utilizing the QP-titanium(IV) complex. The proposed method was applied to an H_2O_2 determination in rain water. In addition, this method was also applied to assays of glucose and uric acid in human urine by combined use of an appropriate enzyme to produce H_2O_2 through enzymatic oxidation.

Experimental

Reagents and solutions

A stock solution ($1.0 \times 10^{-1} \text{ M}$, $1 \text{ M} = 1 \text{ mol dm}^{-3}$) of H_2O_2 was prepared by dissolving 1.1 cm^3 of 30% H_2O_2 (Mitsubishi Gas Chemical Co., Ltd.) with methanol to 100 cm^3 , standardized by iodometry and diluted with methanol, as required. A titanium(IV) solution ($1.5 \times 10^{-4} \text{ M}$) was prepared in deionized water, as described in a previous report.³ Solutions of all xanthene dyes, which had been synthesized according to methods described in the literature^{4,5}, were prepared in $5.0 \times 10^{-3} \text{ M}$ methanol solutions containing one drop of hydrochloric acid. An EDTA solution ($5.0 \times 10^{-2} \text{ M}$) was prepared by dissolving disodium ethylenediaminetetraacetate (Dojindo Lab.) with water. A surfactant solution was

obtained by dissolving 1.5 g of cetyltrimethylammonium chloride (CTAC) in water to 100 cm^3 . A 1.0% hydrochloric acid solution was used for pH adjustments. All other reagents and materials were of analytical grade, and were used without further purification.

Apparatus

A Shimadzu (Model UV-160) recording spectrophotometer with 1.0-cm silica cells was used. The pH measurements were made with a Horiba (F-7 AD) pH meter combining with a calomel glass electrode.

Standard procedure for the determination of H_2O_2

The following components were mixed in a 10-cm^3 volumetric flask: a solution containing $0.02\text{--}2 \mu\text{g}$ of H_2O_2 , 2.0 cm^3 of a 1.5% CTAC solution, 0.6 cm^3 of a $5.0 \times 10^{-2} \text{ M}$ EDTA solution, 0.5 cm^3 of a $1.5 \times 10^{-4} \text{ M}$ titanium(IV) solution, 2.0 cm^3 of a 1.0% hydrochloric acid solution and 0.5 cm^3 of a $5.0 \times 10^{-3} \text{ M}$ QP solution. The mixture was then made up to volume with water and transferred into a test tube. After keeping at room temperature for 25 min, the difference in the absorbance (ΔA) between the resultant solution and a reagent blank prepared under the same conditions, but without H_2O_2 , was measured at 530 nm against water.

Results and Discussion

Choice of dye and aminopolycarboxylic acid

In preliminary experiments, the effect of dyes was studied by measuring ΔA in a strongly acidic solution. Among the xanthene dyes (2,3,7-trihydroxy-6-fluorones and their analogues) tested (QP, SPF, salicylfluorone, *o*-nitrophenylfluorone, *o*-chlorophenylfluorone, *p*-fluorophenylfluorone, 3',4',5',6'-tetrachloro-QP, 4,5-dibromo-QP, Gallein, Pyrogallol Red and Xylenol Orange), QP was the most effective dye in terms of sensitivity. The effect of the amount of QP was examined, maintaining a

Fig. 1 Relationship between the standing time and ΔA in various concentrations of QP at room temperature: $[\text{H}_2\text{O}_2] = 1.0 \mu\text{g}/10 \text{ cm}^3$; $[\text{Ti}^{\text{IV}}] = 7.5 \times 10^{-6} \text{ M}$; $[\text{EDTA}] = 3.0 \times 10^{-3} \text{ M}$; $[\text{CTAC}] = 0.3\%$; pH, 1.7; QP concentration, $1.25 \times 10^{-4} \text{ M}$ (curve 1), $2.5 \times 10^{-4} \text{ M}$ (curve 2), $7.5 \times 10^{-4} \text{ M}$ (curve 3); reference, water.

fixed final concentration of titanium(IV) ($7.5 \times 10^{-6} \text{ M}$). The use of $2.5 \times 10^{-4} \text{ M}$ QP in the final concentration was the best with regard to the reaction rate and stability of color development at room temperature ($15 - 30^\circ \text{C}$). Upon using more than $5.0 \times 10^{-4} \text{ M}$ QP, although ΔA was almost identical, the value rapidly decreased. The reason was probably that the reaction rate of the titanium(IV)-QP-CTAC ternary complex was dependent on the QP concentrations. The results are shown in Fig. 1.

The effect of aminopolycarboxylic acids was examined: EDTA, nitrilotriacetic acid (NTA), triethylenetetraminehexaacetic acid (TTHA), hydroxyethylenediaminetriacetic acid (EDTA-OH), cyclohexanediaminetetraacetic acid (CyDTA), diethylenetriaminepentaacetic acid (DTPA), ethylenediamineacetic acid (EDAA) and glycoletherdiaminetetraacetic acid (GEDTA). These materials could be arranged in the following order with respect to sensitivity: $\text{EDTA} > \text{NTA}$, $\text{TTHA} > \text{DTPA} > \text{EDTA-OH}$, CyDTA , $\text{GEDTA} > \text{EDAA}$. On the other hand, no decrease in the absorbance of the QP-titanium(IV) complex was exhibited in the absence of these materials.

On the basis of these results, further experiments were carried out with $7.5 \times 10^{-6} \text{ M}$ titanium(IV), $2.5 \times 10^{-4} \text{ M}$ QP and $3.0 \times 10^{-3} \text{ M}$ EDTA in the final volume, considering the H_2O_2 determination limit and stability of color development at room temperature.

Optimization of conditions

A study of the effect of the pH showed that the best pH range is from 1.0 to 2.2, using 1.0% hydrochloric acid.

In order to stabilize the color development and to increase the sensitivity, various surfactants were ex-

amined: CTAC, cetylpyridinium chloride, Zephiramine, Tween 20, Brij 35, Triton X-100, polyvinyl alcohol, sodium dodecylsulfate, Aerosol OT and Swanol AM-101. CTAC, as a cationic surfactant, worked best as a dispersion agent. The effect of the CTAC concentrations on ΔA was examined. The use of an approximate 0.3% CTAC in the final concentration was the most suitable in terms of the sensitivity and stability of color development at room temperature.

The same ΔA was obtained even when the order in the addition of the reagents was varied.

Based on the above-mentioned experimental results, a standing time of 25 min at room temperature was selected for all of the measurements.

Calibration curve

A calibration curve for H_2O_2 was constructed by the standard procedure. A good linear relationship was observed over the $0.02 - 2 \mu\text{g}$ range of H_2O_2 in a final volume of 10 cm^3 . The apparent molar absorptivity was $1.6 \times 10^5 \text{ l mol}^{-1} \text{ cm}^{-1}$ at 530 nm. This procedure is about 3 - 10 times more sensitive than other methods⁶⁻¹¹, but is slightly less sensitive than the SPF-titanium(IV)² and oxo[5,10,15,20-tetra(4-pyridyl)porphyrato]titanium(IV) method.¹² The relative standard deviations (RSD) for five runs of 0.2 and $1.0 \mu\text{g}$ of H_2O_2 were 2.8% and 1.2%, respectively; it was 8.3% even at concentrations as low as $0.02 \mu\text{g}$, which was the lowest concentration that could be determined.

Table 1 Effect of foreign substances

Substance (added as)	Amount ^a	$\Delta A_{530 \text{ nm}}$	Rec., %
None	—	0.485	100.0
Fe^{III} (sulfate)	5	0.429	88.5
Cu^{II} (nitrate)	5	0.417	86.0
Ca^{II} (chloride)	100	0.485	100.0
Mg^{II} (chloride)	100	0.485	100.0
BrO_3^- (potassium)	10	0.407	83.9
F^- (sodium)	10	0.390	80.4
Cl^- (sodium)	100	0.485	100.0
NO_3^- (sodium)	100	0.485	100.0
SO_4^{2-} (sodium)	100	0.485	100.0
H_2PO_4^- (potassium)	100	0.485	100.0
Ascorbic acid	100	0.453	93.4
Caffeine	100	0.485	100.0
Glucose	100	0.485	100.0
Uric acid	100	0.485	100.0
Creatinine	100	0.485	100.0
Urea	100	0.485	100.0
Glycine	100	0.485	100.0
Lauroyl peroxide	10	0.470	96.9
<i>t</i> -Butyl hydroperoxide	10	0.485	100.0
Cumen hydroperoxide	10	0.485	100.0
Human serum albumin	(100 μg)	0.485	100.0

H_2O_2 taken, $1.0 \mu\text{g}/10 \text{ cm}^3$; $[\text{Ti}^{\text{IV}}]$, $7.5 \times 10^{-6} \text{ M}$; $[\text{QP}]$, $2.5 \times 10^{-4} \text{ M}$; $[\text{EDTA}]$, $3.0 \times 10^{-3} \text{ M}$; $[\text{CTAC}]$, 0.3%; pH, 1.7; reference, water.

a. Molar ratio. Substance/ H_2O_2 .

Table 2 Determination of H₂O₂ in rain water

Sample	H ₂ O ₂ found ^a , ppb		RSD, %	Recovery ^b , %
	Proposed method	POPHA method		
A	167	185	2.5	106.6
B	884	863	1.3	97.0
C	262	260	2.9	103.1
D	604	572	1.6	102.3
E	133	147	3.7	96.8

a. Average of 5 determinations.

b. H₂O₂ taken, 0.5 µg/10 cm³.*Interference from foreign substances*

For an assessment of its usefulness in environmental and clinical assays, the influence of many foreign substances on the determination of H₂O₂ was examined. Inorganic ions such as sodium, potassium, calcium, magnesium, chloride, sulfate and nitrate ions did not noticeably affect the accuracy of the determination, even when present in large excess compared with H₂O₂. A decrease in ΔA was obtained with iron(III), copper(II), and fluoride ions in 5- to 10-fold amounts of H₂O₂. Most of the organic compounds showed only a minor effect on the H₂O₂ determinations. Ascorbic acid gave rise to small negative errors, even when present in large quantities. The presence of organic peroxides, such as lauroyl peroxide, *t*-butyl hydroperoxide and cumen hydroperoxide, scarcely interfered. The results are summarized in Table 1.

Determination of H₂O₂ in rain water

The proposed method was used to determine H₂O₂ in rain water. The results are shown in Table 2. H₂O₂ at ppb levels was determined. The overall recoveries of H₂O₂ from each sample were within 96–107%, with RSD values of less than 3.7%. The obtained results were in good agreement with those obtained by the *p*-hydroxyphenylacetic acid (POPHA) method.¹³

Application to the determination of glucose and uric acid

The present method was applied to assays of glucose

and uric acid using an appropriate combination of enzymes to produce H₂O₂ through enzymatic oxidation. Glucose and uric acid can produce H₂O₂ by glucose oxidase and uricase, respectively, and can be determined by monitoring the produced H₂O₂. The linear ranges of glucose and uric acid determinations were up to 20 and 10 µg, respectively.

The method was successfully applied to assays of human urinary glucose and uric acid in combinations with each enzyme. No preconcentration was required to determine glucose and uric acid by the present method because of its high sensitivity for H₂O₂. The human urine samples were measured by the present method and by the glucose oxidase (GOD) method (a clinical kit, glucose B-test Wako, Wako Pure Chem.) as well as by the 3-methyl-*N*-(β -hydroxyethyl)aniline (MHEA) method (a clinical kit, uric acid B-test Wako, Wako Pure Chem.). The results are given in Table 3.

Composition and reaction mechanism

The titanium(IV)-to-QP ratio and titanium(IV)-to-CTAC ratio determined by Job's method of continuous variation were 1:4 and 1:4, respectively. Thus, the formed ternary complex may be expressed as Ti(QP)₄(CTAC)₄. On the other hand, titanium(IV):H₂O₂:EDTA was 1:1:1 with or without CTAC and the experimental result was in agreement with the literature value.¹⁴ Furthermore, we found that the formation reaction rate of the H₂O₂-titanium(IV)-EDTA ternary complex was considerably slow compared with that of the titanium(IV)-QP-CTAC ternary complex. The reaction mechanism may be due to the formation of a H₂O₂-titanium(IV)-EDTA complex following consumption of the titanium(IV)-QP-CTAC complex. However, a further investigation is necessary to confirm this conclusion regarding the composition of the titanium(IV)-QP-CTAC ternary complex.

References

1. A part of this work was presented at the 112nd Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, May 1992.

Table 3 Determination of glucose and uric acid in human urine^a (µg cm⁻³)

Sample	Proposed method		Other method		RSD, %	Recovery ^b , %
	Glucose	Uric acid	Glucose ^c	Uric acid ^d		
A	66.1		66.8		3.7	97.5
		250.2		245.3	2.6	100.9
B	75.7		70.7		3.3	98.4
		350.2		351.6	2.9	103.2
C	148.5		155.6		2.3	101.4
		270.5		274.0	3.1	96.1

a. Average of 5 determinations. b. Glucose taken, 10 µg/10 cm³; uric acid taken, 5 µg/10 cm³. c. GOD method. d. MHEA method.

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