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Short Communication

Spectrophotometric determination of hydrogen peroxide with osmium(VIII) and *m*-carboxyphenylfluorone



SPECTROCHIMICA ACTA

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HIGHLIGHTS

GRAPHICAL ABSTRACT

- We present a simple spectrophotometric method for the determination of H₂O₂.
- This method is based on the fading of the color of an osmium(VIII)-MCPF complex.
- Beer's law was obeyed from 20– 406 ng mL⁻¹ with a relative standard deviation 0.33%.
- We performed the characterization of MCPF and obtained the crystal structure.

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ABSTRACT

Spectrophotometric determination of hydrogen peroxide was accomplished with osmium(VIII) and *m*-carboxyphenylfluorone (MCPF) in the presence of cetyltrimethylammonium chloride (CTAC). In the determination of hydrogen peroxide based on the fading of the color of osmium(VIII)-MCPF complex, Beer's law was obeyed in the range 20–406 ng mL⁻¹, with an effective molar absorption coefficient (at 580 nm) of 5.21×10^4 L mol⁻¹ cm⁻¹ and a relative standard deviation of 0.33% (*n* = 6). Further, we performed the characterization of MCPF and obtained the crystal structure.

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Introduction

Hydrogen peroxide (H_2O_2) is a source of oxygen radicals. A 2.5– 3.5% aqueous solution is listed in the Japanese Pharmacopoeia as external antiseptic [1], and a dilute solution used as bleach is approved for use as a food additive [2]. Although the toxicity of H_2O_2 is not high, skin may be severely damaged if exposed to H_2O_2 solutions with high concentrations (60% and higher). Intense inflammation of skin can occur if exposed to concentrations higher than 25%. Moreover, H_2O_2 causes intense pain if eyes are exposed

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to it. Acceptable H_2O_2 concentrations have been reported to be 1 ppm and 1.5 mg $mL^{-3}\sp(2)\sp(2)$.

Determination of H_2O_2 by several methodologies is described, including titrimetric determination [1], spectrophotometry [3–5], fluorophotometry [6–8] and chemiluminesence [9,10]. Spectrophotometry is a simple method, so we have reported spectrophotometry using EDTA, Ti(IV), salicylfluorone, and hexadecylpyridinium [11].

In a preliminary experiment, osmium(VII) formed a colored complex with *m*-carboxyphenylfluorone (MCPF) in the presence of cetyltrimethylammonium chloride (CTAC). Moreover, a decrease in absorbance was observed upon the addition of H_2O_2 . This phenomenon indicates the fundamental conditions for the determination of H_2O_2 using osmium(VIII) and MCPF for the purpose of

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enhancing the sensitivity and repeatability of the determination method. Furthermore, because a crystalline MCPF was obtained, structural analysis by X-ray diffractometry was performed.

Experimental

Synthesis of m-carboxyphenylfluorone

According to a method described in literature [12], 1,4-benzoquinone was dissolved in 20 mL of acetic anhydride and 1 mL of sulfuric acid, and the solution maintained at 30-50 °C for 30-60 min. The reaction mixture was poured into 1 L of water to afford a white precipitate that was filtered and washed with water. 1,2,4-Benzenetriol triacetate was obtained after drying the precipitate under reduced pressure for 5 h. In the synthesis of MCPF, 5 g of *m*-carboxybenzaldehyde and 15 g of 1,2,4,-benzenetriol triacetate were dissolved in 150 mL of 20% ethanol and 2 mL of concentrated sulfuric acid. The solution became red upon heating in a steam bath. This solution was subsequently allowed to stand in a cool, dark place for several weeks. The crude MCPF (4.0 g) obtained was collected and dissolved in a solution of 200 mL ethanol with 1 mL of hydrochloride acid, using a steam bath. Then, the resultant solution was concentrated under reduced pressure to half of its original volume. Next, the precipitate obtained by adding pure water into the concentrated reaction solution was redissolved in a 5:5:2 methanol/ethanol/water mixture. The solution was allowed to stand in a cool, dark place for several days. The precipitate was filtered and washed with pure water to yield pure MCPF (2.4 g) that was subsequently dried under reduced pressure. ¹H NMR (d₆-DMSO, 500 MHz) δ ppm: 8.20 (1H, d), 8.00 (1H, s), 7.80 (1H, t), 7.73 (1H, d), 6.73 (1H, s) 6.33 (1H, s). MS (SIMS): *m*/*z* = 365.

Reagents and chemicals

A H₂O₂ solution was prepared using suitably diluted reagentgrade 30% H₂O₂. An osmium(VIII) standard stock solution $(1.0 \times 10^{-3} \text{ mol L}^{-1})$ was prepared in water. The working solution was prepared by suitable dilution of this stock solution. A $1.0 \times 10^{-3} \text{ mol L}^{-1}$ solution of MCPF, which had been synthesized according to a method described in the literature, was prepared in methanol, and a 5.0% aqueous solution of CTAC was prepared by direct dissolution in water. A pH 4.2 buffer solution was prepared by mixing appropriate amounts of 0.2 mol L⁻¹ CH₃COOH and CH₃COONa solutions. Reagent-grade chemicals were utilized throughout the study. Ultrapure water was prepared immediately before use, by purifying deionized water using a Milli-Q Labo sys-



Fig. 1. Absorption spectra of MCPF-osmium(VIII)–H₂O₂ solutions. Parameters: H₂O₂: 1.8 × 10⁻⁵ mol L⁻¹; Os(VIII): 0.5 × 10⁻⁴ mol L⁻¹; CTAC: 1.5%; MCPF: 1.0 × 10⁻⁴ mol L⁻¹; pH: 3.3; Curve A: MCPF–Os(VIII)–H₂O₂ solution; Curve B: MCPF–Os(VIII) solution; Curve C: Curve A minus Curve B

tem. A Shimadzu spectrophotometer (Model UV-160) with 1.0 cm matched silica cell was used for absorbance measurements. All pH measurements were conducted using a Horiba (F-11) pH meter in combination with a calomel glass electrode.

Standard procedure for the determination of H₂O₂

The following components were mixed in a 10 mL volumetric flask: a solution containing 0.20–4.08 µg of H_2O_2 , 3.0 mL of 5.0% CTAC solution, 3.0 mL of 0.2 M CH₃COOH-CH₃COONa buffer solution (pH 3.3), 1.0 mL of a 1.0×10^{-3} mol L⁻¹ MCPF solution, and 0.5 mL of a 1.0×10^{-3} mol L⁻¹ Os(VIII) solution. The mixture was diluted to 10 mL with water, transferred to a test tube, mixed well, and maintained at room temperature for 20 min. The absorbance of the resulting solution was measured at 580 nm against a reagent blank without osmium ions.

Result and discussion

Optimization of experimental conditions

Analysis of the effect of pH showed that the optimal pH was approximately 3.3, which was achieved using 3.0 mL of 0.2 mol L^{-1} CH₃COOH/CH₃COONa solution. After investigating the effects of several acidic media, including CH₃COOH/CH₃COONa, HCl/Na₃ (C₃H₅O(COO)₃), HCl/Glycine/NaCl, HCl/C₆H₄(COOH)(COOK), and HCl/Na₂B₄O₅(OH)₄·8H₂O solutions, the effect of dispersing agents on the H₂O₂-osmium(VII)-MCPF reaction was examined using cationic surfactants (CTAC, stearyltrimethylammonium chloride, cetylpyridinium chloride, and dimethylbenzyltetradecylammonium chloride), an anionic surfactant (sodium dodecylsulfate), an amphoteric surfactant (Swanol AM-101), and non-ionic surfactants (methylcellulose, polyethylene glycol mono-*p*-isooctylphenyl ether, poly(N-vinylpyrrolidone), polyvinyl alcohol, and polyoxyethylene sorbitan monolaurate). A maximum and constant absorbance difference (ΔAbs) value was obtained by adding more than 3.0 mL of 5.0% CTAC to 10 mL of MCPF-osmium(VIII)-H₂O₂ solution. The recommended procedure for the assay of H₂O₂ is as follows: The following components were mixed in a 10 mL volumetric flask: a solution containing 0.20-4.08 µg of H₂O₂, 3.0 mL of 5.0% CTAC solution, 3.0 mL of 0.2 M CH₃COOH-CH₃COONa buffer

 Table 1

 Effect of foreign substances on ⊿Abs at 580 nm and percent recovery.

Substance	Molar ratio (substance/	⊿Abs at	Recovery
	H ₂ O ₂)	380 IIII	(%)
None	-	0.313	100
Ca(II)	100	0.313	100
Mg(II)	100	0.313	100
Zn(II)	100	0.313	100
Fe(II)	1	0.227	73
Fe(III)	10	0.267	85
Cu(II)	100	0.313	100
NaCl	1000	0.313	100
NaH ₂ PO ₄	100	0.313	100
Na ₂ SO ₄	1000	0.324	103
KNO ₃	1000	0.313	100
NaF	100	0.313	100
Citric acid	10	0.216	69
Tartaric acid	10	0.313	100
Oxalic acid	1	0.237	76
Ascorbic	10	0.276	88
acid			
Glucose	1000	0.313	100
EDTA	10	0.313	100

 $\begin{array}{ll} H_2O_2: & 0.6\times 10^{-5} \mbox{ mol } L^{-1}; & Os(VIII): & 0.5\times 10^{-4} \mbox{ mol } L^{-1}; & CTAC: & 1.5\%; & MCPF: \\ 1.0\times 10^{-4} \mbox{ mol } L^{-1}; & pH \ 3.3, \mbox{ Reference: } MCPF-Os(VIII) \mbox{ solution.} \end{array}$



Fig. 2. Crystal structure of MCPF.

solution (pH 3.3), 1.0 mL of a 1.0×10^{-3} mol L⁻¹ MCPF solution, and 0.5 mL of a 1.0×10^{-3} mol L⁻¹ Os(VIII) solution. The mixture was diluted to 10 mL with water, transferred to a test tube, mixed well, and maintained at room temperature for 20 min. The absorbance of the resulting solution was measured at 580 nm against a reagent blank without H₂O₂.

Absorption spectra, calibration curve, sensitivity, and accuracy

The absorption spectra of the MCPF-osmium(VIII)-H₂O₂ and MCPF-osmium(VIII) solutions were acquired according to standard procedures, and these are shown in Fig. 1. The calibration curve produced using the standard procedure showed a good linearity in the H_2O_2 concentration range 20–406 ng mL⁻¹, with a correlation coefficient (r) of 1.000. The effective molar absorption coefficient (ε) was $5.21 \times 10^4 \,\text{L}\,\text{mol}^{-1}\,\text{cm}^{-1}$. The relative standard deviation (RSD) of the results were lower than 0.33% (n = 6).

Effect of foreign substance

The effect of foreign substances on the proposed method was examined using 0.2 μ g of H₂O₂ solution made up to 10 mL. The results are summarized in Table 1. Calcium(II), magnesium(II), zinc(II), copper(II), sodium chloride, monobasic sodium phosphate, potassium nitrate, sodium fluoride, or glucose at molar quantities exceeding 100 times that of H₂O₂ showed no effects on the determination of H₂O₂. Tartaric acid and EDTA showed no effects at molar quantities 10 times higher than that of H₂O₂. A small positive error was observed in the presence of sodium sulfate, whereas negative errors were observed with iron(II), iron(III), citric acid, oxalic acid, and ascorbic acid.

Crystallization of MCPF

The crystal structure of MCPF, illustrated in Fig. 2, consists of planar xanthene and benzene rings linked by a C9-C6' single bond to form a dihedral angle, in the same way as previously reported for o-sulfophenylfluorone [13]. Furthermore, the two carboxyl groups of the benzoic acid moiety appear to be located on either side, because the carboxyl group may be oriented on the right or left side owing to the spin of the C9–C6' single bond.

Conclusion

In conclusion, we have found a simple spectrophotometric method for the determination of hydrogen peroxide by using osmium(VIII), MCPF, and CTAC, a cationic surfactant, in the acetate buffer solution. The determination range is the same as that for our previously reported method, but the repeatability of the new method was better. Although further examination is needed, MCPF is a catechol-type compound and has adjacent hydroxyl group. Therefore, it is oxidized by H₂O₂, and reactivity with Os(VIII) is expected to decrease. However, in this report, we showed the fundamental conditions for the determination of H₂O₂, and we performed structural analysis of MCPF by X-ray diffractometry.

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