Letters to the Editor

Spectrophotometric Determination of Hydrogen Peroxide Based on Fading of *o*-Sulfophenylfluorone-Titanium(IV) Complex¹

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*Osaka University of Pharmaceutical Sciences, Matsubara, Osaka 580, Japan***Keywords** Hydrogen peroxide, *o*-sulfophenylfluorone-titanium(IV) complex, spectrophotometry, ethylenediamine-tetraacetic acid

Of the hydrogen peroxide (H_2O_2) determinations, the methods²⁻⁷ which utilize the mixed ligand complex formation system, namely, H_2O_2 -M-R (M and R denote metal ion and organic reagent, respectively), have often been employed. These methods are simple, sensitive and inexpensive because of no need of enzyme. We speculated that a method which would utilize fading of M-R complex in the presence of ethylenediaminetetraacetic acid (EDTA) would serve as a very sensitive determination for H_2O_2 without serious interference of foreign metal ions. As a result, we decided to use *o*-sulfophenylfluorone-titanium(IV) (SPF-Ti(IV)) complex. Here, we report on a spectrophotometric method for the determination of H_2O_2 based on fading of a SPF-Ti(IV) complex.

Experimental

A stock solution (1.0×10^{-1} M) of H_2O_2 was prepared by dissolving 1.1 ml of 30% H_2O_2 (Mitsubishi Gas Chemical Co., Ltd.) with methanol to 100 ml, standardized by iodometry and diluted with methanol, as required. An SPF solution (1.0×10^{-3} M) and a Ti(IV) solution (1.0×10^{-3} M) were prepared in methanol and deionized water, respectively, as described in previous reports.^{8,9} An EDTA solution (1.0×10^{-1} M) was prepared by dissolving disodium ethylenediaminetetraacetate (Dojindo Lab.) with water. A surfactant solution was obtained by dissolving 1.0 g of Triton X-100 and 0.32 g of cetyltrimethylammonium chloride (CTAC, 1.0×10^{-2} M) in water to 100 ml. A 0.1 M citric acid-sodium citrate buffer solution was used for the pH adjustments. All other reagents and materials used were of analytical grade.

Results and Discussion

In order to examine the utility of a method for the determination of H_2O_2 based on fading of M-R

complex, color reactions between various dyes and M were studied by comparing the situations in the presence of H_2O_2 , EDTA and a surfactant. The dyes (R) used were SPF, phenylfluorone, *o*-hydroxyhydroquinonephthalein, salicylfluorone, *o*-chlorophenylfluorone, *o*-nitrophenylfluorone, *p*-carboxyphenylfluorone, 2',4'-disulfophenylfluorone, Pyrogallol Red, Pyrocatechol Violet, Chromazurol S, Xylenol Orange, Methylthymol Blue, 4-(2-pyridylazo)resorcinol, 1-(2-pyridylazo)-2-naphthol and Tiron. The M materials used were Ti(IV), Co(II), Fe(III), Cu(II), V(V), W(VI), Mo(VI), Ta(V) and Nb(V). From a comparison of various combinations of R and M, color reactions between xanthene dyes and Ti(IV) were found to be interfered with severely by micro amounts of H_2O_2 in the presence of EDTA and a cationic surfactant, and the decrease in absorbance of SPF-Ti(IV) complex was larger in proportion to the H_2O_2 concentration. On the other hand, neither decrease nor increase in absorbance of a xanthene dye-Ti(IV) complex exhibited in the absence of EDTA. CTAC was superior to other cationic surfactants in terms of sensitivity, and the coexistence of a nonionic surfactant (such as Triton X-100) together with CTAC was somewhat greater advantage than the use of CTAC alone in sensitivity and reproducibility. Several aminopolycarboxylic acids were examined including EDTA, iminodiacetic acid (IDA), nitrilotriacetic acid (NTA), ethylenediaminediacetic acid (EDDA), cyclohexanediaminetetraacetic acid (CyDTA), hydroxyethylenediaminetriacetic acid (EDTA-OH) and diethylenetriaminepentaacetic acid (DTPA). These materials could be arranged in the following order with respect to sensitivity: EDTA > EDTA-OH >> NTA, CyDTA. Thus, the fundamental conditions for the spectrophotometric determination of H_2O_2 were established based on fading of the SPF-Ti(IV)-CTAC (2:1:2, by the continuous variation and the molar ratio methods) complex.

The recommended procedure for the determination of H_2O_2 is as follows: in a 10-ml volumetric flask were mixed a solution containing 0.2–2 μg of H_2O_2 , 1.0 ml of the surfactant solution, 0.15 ml of a 1.0×10^{-3} M

Fig. 1 Absorption spectra of SPF-Ti(IV) and SPF solutions in the presence of H_2O_2 . $[\text{Ti(IV)}]=1.5 \times 10^{-5} \text{ M}$; $[\text{SPF}]=3.0 \times 10^{-5} \text{ M}$; $[\text{EDTA}]=2.0 \times 10^{-3} \text{ M}$; [surfactant]=1.0 ml of (1.0% Triton X-100+ $1.0 \times 10^{-2} \text{ M}$ CTAC solution)/10 ml; pH=5.5; Curves 1, 2 and 3: SPF-Ti(IV) solutions (H_2O_2 concentration (M): curve 1=0; curve 2= 3.0×10^{-6} ; curve 3= 6.0×10^{-6}). Curve 4: SPF solution. Reference: water.

Ti(IV) solution and 0.2 ml of a $1.0 \times 10^{-1} \text{ M}$ EDTA solution. The pH of the final solution was adjusted to 5.5 (optimal pH range between 5.0 and 6.6) with 3.0 ml of the buffer solution; then 0.3 ml of a $1.0 \times 10^{-3} \text{ M}$ SPF solution was added. The mixture was diluted to 10 ml with water, transferred into a test tube, mixed well and kept at 60°C for 50 min. After the solution had been cooled in water to room temperature, the difference of absorbance between sample and a reagent blank prepared in the same way was measured at 610 nm against water. Figure 1 shows the absorption spectra of SPF and SPF-Ti(IV) solutions in the presence and absence of H_2O_2 . The effective molar absorptivity was

$2.3 \times 10^5 \text{ l mol}^{-1} \text{ cm}^{-1}$ at 610 nm. This procedure is about 4–10 times more sensitive than other mixed ligand complex formation methods.^{2–7} The relative standard deviation (RSD) of this method was 1.0% ($n=5$) for $1.0 \mu\text{g}$ of H_2O_2 . The influence from the coexistence of foreign ions was considerably decreased. Various organic compounds, such as amino acids, organic acids and sugars, interfered very little with the determination of H_2O_2 . Overall recoveries of H_2O_2 from real samples (yogurt, shirasuboshi, soba and kamaboko) were within the range of 96.1–98.4%, with RSD values of less than 3.6%.

Though further investigations are necessary, especially on the elucidation of the reaction mechanism, we hope to apply the proposed method to biological and clinical fields.

References

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(Received January 14, 1991)

(Accepted February 18, 1991)