Comparison among the Methods for Hydrogen Peroxide Measurements To Evaluate Advanced Oxidation Processes: Application of a Spectrophotometric Method Using Copper(II) Ion and 2,9-Dimethyl-1,10-phenanthroline

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Hydrogen peroxide (H_2O_2) in the range of several tens to several hundreds of micromoles per liter is usually added to the process water in advanced oxidation processes (AOPs). In this study, a spectrophotometric method using copper(II) ion and 2,9-dimethyl-1,10-phenanthroline (DMP) for measuring H_2O_2 concentration was compared with other methods [i.e., spectrophotometric methods using titanium oxalate and *N,N*-diethyl-*p*-phenylenediamine (DPD) and a fluorometric method using *p*-hydroxyphenyl acetic acid (POHPAA)]. Particular attention was paid to sensitivities and effects of coexisting substances. The most sensitive method was the fluorometric method, followed in order by DPD, DMP, and the titanium oxalate colorimetric method; their detection limits in 1-cm cells were 0.16, 0.77, 0.80, and 29 μM, respectively. Therefore, the DMP method was found to be reasonably sensitive when applied to AOPs. Also, the DMP reagent is commercially available, and the absorbance of $Cu(DMP)_2^+$, a reaction product of the DMP method, was not affected by reaction time. In the DMP method, copper(II)−DMP complexes react with humic acid, and colored chemicals are produced. However, the slopes of the calibration curves of H_2O_2 containing up to 10 mg of C L^{-1} from humic acid did not change significantly as compared to that in ultrapure water. The effect of chlorine on the DMP method was not observed up to at least 23 μM (0.8 mg of Cl L^{-1}) of free chlorine, although the DPD and fluorometric methods are known to be interfered by chlorine. From this study, it was concluded that the DMP method is suitable to be used in AOPs.

Introduction

Hydrogen peroxide (H_2O_2) is often used as a hydroxyl radical (HO•) generating agent in advanced oxidation processes
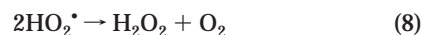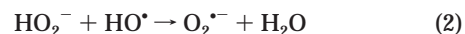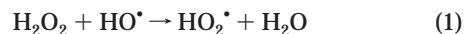
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(AOPs, e.g., the ozone/hydrogen peroxide process) (*1*). Hydrogen peroxide also acts as a hydroxyl radical scavenger and is generated as a reaction product of hydroxyl radicals and a decomposition byproduct of solute during the process (*2−4*):

$$H_2O_2 + HO^\bullet \rightarrow HO_2^\bullet + H_2O \tag{1}$$

$$HO_2^- + HO^\bullet \rightarrow O_2^{\bullet-} + H_2O \tag{2}$$

$$2HO^\bullet \rightarrow H_2O_2 \tag{3}$$

$$O_3 + M \rightarrow M_{oxide} (+ H_2O_2) \tag{4}$$

$$HO^\bullet + M \rightarrow R^\bullet \tag{5}$$

$$R^\bullet + O_2 \rightarrow ROO^\bullet \tag{6}$$

$$ROO^\bullet \rightarrow M'_{oxide} + HO_2^\bullet \tag{7}$$

$$2HO_2^\bullet \rightarrow H_2O_2 + O_2 \tag{8}$$

where $HO_2^\bullet$ is the hydroperoxyl radical, $O_2^{\bullet-}$ is the superoxide anion, M is the solute, $R^\bullet$ is the secondary radical, $ROO^\bullet$ is the peroxy radical. Therefore, the concentration of H_2O_2 in the process is related to the treatment efficiency. It is also known that an optimal H_2O_2 dose should exist in the O_3/H_2O_2 process and the optimal dose is dependent upon the characteristics of process water (e.g., components of organic compounds and their concentrations). On the other hand, as H_2O_2 is the only stable activating oxygen in the process, the time course of H_2O_2 concentration gives relevant information on the radical reactions in the processes. Thus, measurement of H_2O_2 concentration is important and useful to evaluate and analyze AOPs.

So far, various methods have been used for the measurement of H_2O_2 in AOPs. Such methods use metallic compounds such as titanium oxalate, titanium tetrachloride (*5−8*) and cobalt(II) ion (*9*) that form colored complexes with H_2O_2, which can then be measured spectrophotometrically. It was reported, however, that the sensitivity of these methods was relatively low (*10*). The methods using peroxidase-catalyzed oxidation are also used in many studies. Le Lacheur et al. (*11*) and Benitez et al. (*12*) used a spectrophotometric method employing *N,N*-diethyl-*p*-phenylenediamine (DPD) (*10*). Schik et al. (*13*) used a fluorometric method employing *p*-hydroxyphenyl acetic acid (POHPAA) (*13−16*). Since these methods using peroxidase-catalyzed oxidation have high sensitivity, they have been applied to the determination of relatively low H_2O_2 concentrations, such as in environmental samples (*10, 13−17*). However, since H_2O_2 in the range of several tens to several hundreds of micromoles per liter is usually added to the process water in AOPs, a method that is reasonably sensitive in those ranges and is easy to use is desirable for AOPs.

In our previous research (*18*), a spectrophotometric method using copper(II) ion and 2,9-dimethyl-1,10-phenanthroline (DMP) (*19*) was applied. The procedure is simple and easy, and DMP reagent is commercially available. The principle is reduction of copper(II) with H_2O_2. The stoichiometry is as follows:

$$2Cu^{2+} + 4DMP + H_2O_2 \rightarrow 2Cu(DMP)_2^+ + O_2 + 2H^+ \tag{9}$$
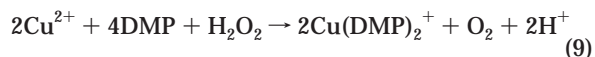
$Cu(DMP)_2^+$ is a bright yellow complex with maximal absor-

TABLE 1. Comparison of the Calibration Curves for the DMP Method, the Titanium Oxalate Colorimetric Method, the DPD Method, and the Fluorometric Method in Ultrapure Water

	H_2O_2 ranges in 10-mL volumetric flasks (μM)	H_2O_2 in sample solution (μM)[a]	detection limit (μM)[b]	molar absorptivity $\Delta A \Delta H_2O_2^{-1}$ (M^{-1} cm^{-1})	absorbance ranges in 1-cm cells[c]
DMP Method					
$n = 8$	1–50	2–500	0.80	14 300 ± 200[d]	0.01–0.7
Titanium Oxalate Colorimetric Method					
$n = 7$	20–1200	40–12 000	29	940 ± 100[d]	0.01–1
DPD Method					
$n = 5$	0.2–25	0.4–250	0.77	20 900 ± 900[d]	0.01–0.5
Fluorometric Method					
$n = 7$	0.1–6	0.2–60	0.16		

[a] H_2O_2 in the sample is diluted 2–10-fold in 10-mL volumetric flasks. [b] Describe the method to come up with the detection limit for each method with some references. [c] Absorbance is standardized by the blank solution. [d] 95% confidence interval.

bance at 454 nm and is stable under ordinary light (*19, 20*). The blank solution without H_2O_2 has a different color, and differences of the absorbance between the sample and blank solutions are approximately proportional to H_2O_2 concentration as long as the concentrations of the copper(II)–DMP complexes [e.g., Cu(DMP)$_2$$^{2+}$, Cu(DMP)$_2OH^+$, and Cu-(DMP)$^{2+}$] are high enough and H_2O_2 concentration is relatively low.

In this paper, the DMP method was compared with other methods (i.e., a titanium oxalate colorimetric method, a DPD method, and a fluorometric method using POHPAA) with special focus on the sensitivity for application to AOPs. The effects of coexisting organic compounds (i.e., humic acid, aldehydes, and carboxylate ions) and chlorine in the process were also investigated.

Experimental Section

Apparatus. A spectrophotometer (UV-2500, Shimadzu Co.) and a spectrofluorophotometer (RF-5000, Shimadzu Co.) were used with 1-cm cells. All glassware was thoroughly washed with tap water and rinsed with ultrapure water, then heated, and dried at 80 °C for 8 h. Experiments were conducted at room temperature (25 ± 2 °C) under fluorescent lights.

Reagents. Deionized distilled water, prepared by an ultrapure water system (CPW-200, Advantec Toyo Co.), was further purified by a compact ultrapure water system (EASY pure RF, Barnstead Co.) and was used within a few hours of preparation. The resistivity of the ultrapure water was greater than 18.1 MΩ cm. Hydrogen peroxide solution, 30 wt % (Wako Chemical Co.), was diluted with the ultrapure water to the required concentration. The concentration of the purchased solution was determined by measuring UV absorbance at 240 nm (*10*). One gram of DMP reagent (Sigma Chemical Co.), which was used as received, was dissolved in 100 mL of ethanol (Wako Chemical Co.) and was stored in a brown bottle at 4 °C. Copper(II) sulfate solution (0.01 M) was prepared by dissolving copper(II) sulfate pentahydrate (Wako Chemical Co.) in ultrapure water. A phosphate buffer solution (0.1 M) was prepared from K$_2$HPO$_4$ and NaH$_2$PO$_4$ (Wako Chemical Co.) with pH adjusted to 7.0 by H$_2$SO$_4$ (1 N) and NaOH (1 N). Humic acid and peroxidase (P8375, typeIV) were obtained from Aldrich Chemical Co. and Sigma Chemical Co., respectively, and all other reagents used were analytical grade.

Procedure. All experiments involving the measurement of H_2O_2 were conducted in 10-mL volumetric flasks to maintain similar conditions among the four methods. The concentrations of reagents in the final solution were identical to those of the corresponding original papers.

DMP Method. The procedure as described by Baga et al. (*19*) was used with slight modification. One milliliter each

of DMP, copper(II) sulfate, and phosphate buffer (pH 7.0) solutions was added to a 10-mL volumetric flask and was mixed. A measured volume of sample was added to the volumetric flask, and then the flask was filled up with ultrapure water. After mixing, the absorbance of the sample (at 454 nm) was measured. The blank solution was prepared in the same manner but without H_2O_2. Using the difference in absorbance between the sample and blank solutions, a calibration curve was generated.

From the calibration curve, H_2O_2 concentrations were calculated as follows:

$$\Delta A_{454} = \epsilon[H_2O_2] \times V/10$$

where ΔA_{454} is the difference of the absorbance between sample and blank solutions at 454 nm, ϵ is the slope of the calibration curve (M^{-1} cm^{-1}), [H_2O_2] is the H_2O_2 concentration (M), and V is the sample volume (mL) (typically 1–5 mL).

Other Methods. The photometric method using titanium oxalate (Wako Chemical Co.) as described by Wagner et al. (*21*) was used, and the absorbance of the colored complex was measured at 385 nm. The DPD method as described by Bader et al. (*10*) was used in this study, and the absorbance was measured at 551 nm. However, the DPD reagent (Sigma Chemical Co.) concentration in the final solution was 350 μM in our study [higher than that of Bader et al. (*10*)]. The fluorometric method using POHPAA reagent (Sigma Chemical Co.) as described by Weinberg et al. (*16*) was used. Glycine and ethylenediaminetetraacetic acid, reagents for reducing the effects of residual oxidants (i.e., ozone and chlorine) and metal cations, were not added since no interferences would be expected for experiments in ultrapure water. Catalase, which Weinberg et al. (*16*) used to correct for the effect of organic hydroperoxidase, was also not used for the same reason. The effect of organic hydroperoxidase was not included in this study.

Results and Discussion

Comparison of Methods. Table 1 summarizes the results of calibration curves obtained from all four methods (i.e., DMP method, titanium oxalate colorimetric method, DPD method, and fluorometric method) when H_2O_2 was added to the ultrapure water. The ranges of H_2O_2 concentrations used in this study were selected based upon the differences in the sensitivities of the methods that were reported in the literature (*10, 13, 19*). The H_2O_2 concentration in the 10-mL volumetric flask was 2–10 times smaller than those in the sample solution because of dilution in the volumetric flasks.

The detection limit for the titanium oxalate colorimetric method was 29 μM, and the slope of the calibration curve (i.e., molar absorptivity) was 940 ± 100 M^{-1} cm^{-1} (95% confidence interval). The slope obtained by Wagner et al.
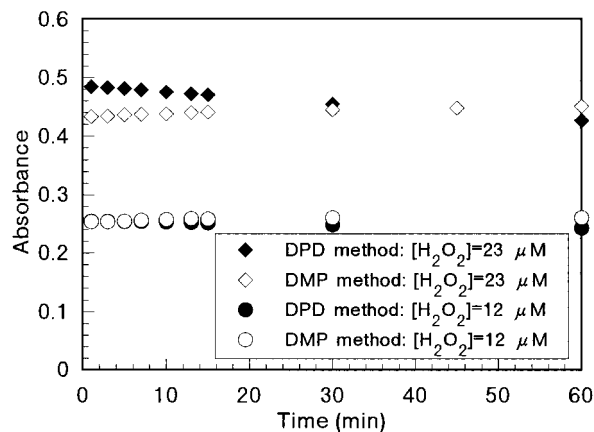
FIGURE 1. Time course of the absorbance of the reaction products of the DMP method and the DPD method.

(21) was 1005 M^{-1} cm^{-1}. Since the absorbance of 100 μM H_2O_2 was about 0.08, the reasonable concentration ranges for the method seemed to be more than several hundreds of micromoles per liter although the method was capable of measuring several tens of micromoles per liter of H_2O_2.

The detection limit of the DPD method was 0.77 μM, and a linear calibration curve could be used in the range of 0.2–25 μM (absorbance = 0.01–0.5) in a 1-cm cell. According to Bader et al. (10), the detection limit was 0.06 μM (0.2 μg L^{-1}) in a 10-cm cell, and the slope of the calibration curves was 21 000 ± 500 M^{-1} cm^{-1}. This value is consistent with that of our study (20 900 ± 900 M^{-1} cm^{-1}). In this study, the concentration range was extended from 0.2 to 50 μM, but the calibration curve deviated slightly from linearity when the concentration was above 35 μM. The DPD method was sensitive to reaction time, and the absorbance decreased gradually with time (Figure 1). It was also observed that higher H_2O_2 concentrations led to a more rapid decline of the absorbance. Consequently, the operational range was considered to be 0.2–25 μM.

The fluorometric method was the most sensitive method in this study, and the detection limit was 0.16 μM. However, due to the high sensitivity, solutions with high H_2O_2 concentrations had to be diluted prior to reaction. Thus, the method is more suitable for measuring H_2O_2 at lower concentration ranges (e.g., less than several μM). This method is often used by automatic measuring systems (13, 14, 17). In the case of manual measurement, this method is more tedious than other methods because it requires a 5 min reaction time and pH adjustment of the samples before and after the reaction.

The calibration curve of the DMP method was linear in the range of 1–50 μM (absorbance = 0.01–0.7), and the slope was 14 300 ± 200 M^{-1} cm^{-1}. Baga et al. (19) also reported that the calibration curves were linear in the range of 1–120 μM (absorbance = 0.01–1.8) in 1-cm cells and the slope was 15 000 M^{-1} cm^{-1}. The absorbance of the blank solution was about 0.08 and had a yellowish green color. The detection limit was 0.80 μM. The H_2O_2 concentration range that can be detected by the DMP method dictates its applicability to AOPs (Table 1). Additionally, $Cu(DMP)_2^+$ is stable under a fluorescent light regardless of the H_2O_2 concentration (Figure 1); therefore, measurement time is flexible (within 15 min after reaction). Preparation of the necessary reagents is easy because the DMP reagent is highly soluble in ethanol solution. Furthermore, Baga et al. (19) showed that the DMP method was applicable over a pH range of 5–9, although buffer solution (pH 7.0) was used in this study. Therefore, it was concluded that the DMP method was suitable to measure H_2O_2 in AOPs considering its reasonable sensitivity and simplicity.
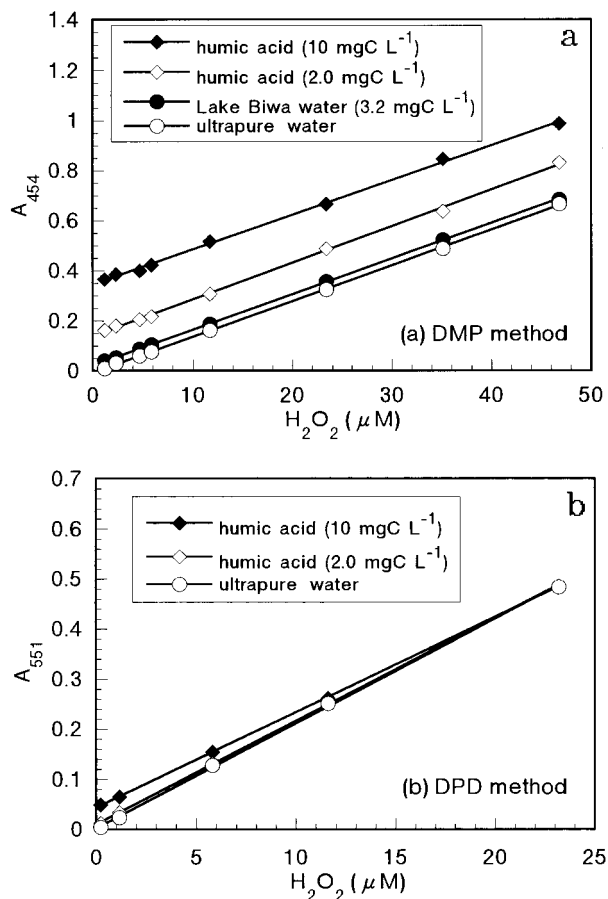

FIGURE 2. Calibration curves in the presence of humic acid. Absorbance was standardized by the differences for the blank solution in ultrapure water. (a) DMP method, (b) DPD method.

Effects of Coexisting Organic Compounds and Chlorine. Figure 2 shows the effect of humic acid on the measurements of H_2O_2 concentration by the DMP method and the DPD method, respectively. The slopes of the calibration curves of the DMP method for ultrapure water, Lake Biwa water (45% of the water was added in final solution), 2.0 mg of C L^{-1} humic acid, and 10 mg of C L^{-1} humic acid were 14 300 ± 200, 14 200 ± 100, 14 600 ± 500, and 13 900 ± 500 M^{-1} cm^{-1}, respectively, for the 95% confidence interval. The slopes of the calibration curves of the DPD method in ultrapure water, 2.0 mg of C L^{-1} humic acid, and 10 mg of C L^{-1} humic acid were 20 900 ± 900, 20 600 ± 600, and 19 000 ± 400 M^{-1} cm^{-1}, respectively. From the results, the slopes of the DMP method did not change significantly in the presence of humic acid, but the DPD method was negatively interfered with 10 mg of C L^{-1} humic acid. Also, in the DMP method, absorbance of the solution containing humic acid greatly increased as compared with that of ultrapure water (Figure 2a). The sum of the absorbance of the blank solution with ultrapure water and that of the humic acid solution itself was lower than the absorbance of blank solution with humic acid (sum of absorbance of the blank solution with ultrapure water and 10 mg of C L^{-1} humic acid solution = 0.188, absorbance of blank solution with 10 mg of C L^{-1} humic acid = 0.353). Thus, it indicated that the copper(II)–DMP complexes reacted with humic acid, which absorbs light. With increasing concentration of humic acid, the slopes of the calibration curves for the DMP method show a decreasing trend. However, three slopes were not significantly different from one another with humic acid concentrations of less than 10 mg of C L^{-1}. From these results, it can be concluded that the copper(II)–DMP complexes react with H_2O_2 and

humic acid independently. The amount of copper(II)−DMP complexes added is enough if the concentration of humic acid is less than 10 mg of C L^{-1}, and the DMP method is capable of measuring the H$_2$O$_2$ concentration in the presence of the appropriate concentration of humic acid. On the other hand, the fluorometric method was interfered by humic acid. As the concentrations of humic acid increased, the slopes of the calibration curves greatly decreased. These results are consistent with those of Zepp et al. (*22*).

The effects of aldehydes and carbonate ions, decomposition byproducts of high molecular weight organic compounds in AOPs, on the measurements of H$_2$O$_2$ by the DMP method were also investigated. Formaldehyde and acetaldehyde are reducing chemicals but did not interfere with the DMP method up to 0.4 and 0.5 mM, respectively. Acetate and formate ions also did not interfere with the DMP method up to 0.5 mM. The effect of oxalate ion was not observed up to 0.05 mM, but when compared to ultrapure water, the absorbance of 0.5 mM oxalate ion was lower. The decrease of absorbance was approximately 0.02 for 0.5 mM oxalate ion and approximately 0.03 for 1.0 mM oxalate ion. Since the absorbance of the blank solution also decreased to the same degree, the slope of the calibration curve did not change significantly (e.g., for 1.0 mM oxalate ion solution, the slope was 14 700 ± 400 M^{-1} cm^{-1}). Therefore, it was concluded that the slopes of the calibration curves of the DMP method did not change significantly by coexisting organic compounds, although the absorbance of blank solution changed in some cases. Furthermore, the absorbance could be corrected for negative and positive effects with the absorbance of blank solution at 454 nm.

Chlorine reacts with H$_2$O$_2$, and the rate of reaction is relatively high [e.g., rate constant between H$_2$O$_2$ and hypochlorite (OCl^{-1}) $= 3.4 \times 10^3$ M^{-1} s^{-1}] (*13*). Therefore, high H$_2$O$_2$ concentration does not coexist with chlorine. However, some reagents used for the measurement of H$_2$O$_2$ react with chlorine, and signals (i.e., absorbance and fluorescence) are produced in the absence of H$_2$O$_2$. The fluorometric method is interfered by chlorine, and the intensity of fluorescence increases with an increase in chlorine concentration. It was reported that the fluorescence intensity of the sample solution containing 2.8 μM (0.1 mg of Cl L^{-1}) HOCl/OCl$^-$ as free chlorine was equivalent to that of 66 μM (2250 μg L^{-1}) H$_2$O$_2$ (*13*). DPD also reacts with chlorine (*23*), and it is used in the Standard Methods procedure for the determination of chlorine (*24*). On the other hand, the DMP method was not interfered significantly by chlorine up to at least 23 μM (0.8 mg of Cl L^{-1}) free chlorine. One reason attributed to this may be due to the fact that in the DMP method H$_2$O$_2$ behaves as a reductant, but in other methods (i.e., the DPD method and the fluorometric method) H$_2$O$_2$ behaves as an oxidant. Thus, the DMP method is advantageous for lower effects from oxidants.

Comparison of Commercial DMP Reagents. Experiments were conducted with a few commercially available DMP reagents. The blank solution in ultrapure water has a yellowish green color that differs from that of Cu(DMP)$_2{}^+$ (i.e., bright yellow). And, the absorbance of the blank solution at 454 nm was about 0.08. However, blank solutions of some commercially available reagents had a bright yellow color and high absorbance (e.g., above 0.40). Thus, it is desirable to check DMP reagents before use.

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