Title

A more selective and accurate method than indigo method for measuring dissolved ozone concentration (*p*-vinylbenzoic acid method)

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Highlights

Ozone reacts *p*-vinylbenzoic acid (*p*-VBA) to form *p*-formylbenzoic acid (*p*-FBA).

The ozone concentration can be determined from the *p*-FBA concentration.

p-VBA method is more accurate than the indigo method.

p-VBA method can be used in the presence of oxidants other than ozone.

p-VBA method does not require calibration of the quantitative reagent.

ABSTRACT

Dissolved ozone concentration measurement is crucial for ozone treatment. In the most used conventional indigo method, the ozone concentration is measured by the decrease in absorbance due to the loss of the C–C double bond of indigotrisulfonic acid. However, measurement of ozone concentration is difficult when water contains substances that react with C–C double bonds other than ozone. To address this concern, we developed a novel breakthrough method to measure ozone concentration by measuring the *p*-formylbenzoic acid (*p*-FBA) produced after the reaction of *p*-vinylbenzoic acid and ozone. The formation of *p*-FBA was almost not caused by other substances (hypochlorous acid, hypobromous acid, permanganate ion and hydrogen peroxide), and its yield to ozone was maintained at 1 in river water, treated wastewater and seawater. In addition, the experimental error is smaller with the new method than with indigo. Furthermore, the new method does not require cumbersome calibration unlike indigo method because highly pure forms of *p*-FBA are commercially available. *p*-FBA can be separated by liquid chromatography and detected with highly sensitive ultraviolet and mass spectrometric detectors, and hence easily analyzed simultaneously with other substances. Our new method contributes to extensive ozone treatment and ozonation management.

KEYWORDS

ozonolysis, *p*-formylbenzoic acid, hypochlorous acid, hypobromous acid, permanganate ion, seawater

1. Introduction

Ozone treatment is applied in various fields, such as water and wastewater, seawater, food, medical care, sewage sludge, and pulp bleaching (Holah et al., 2016; Loeb et al., 2012; Paul and Liu, 2012; Tripathi et al., 2020). In practice and research in these fields, the measurement of dissolved ozone concentration is crucial.

In the indigo method (Bader and Hoigné, 1981), which is currently used for measuring dissolved ozone concentration, water containing ozone is mixed with an indigotrisulfonic acid (indigo) solution adjusted to pH 2 using phosphate buffer. Ozone reacts with the C–C double bond in indigo in a 1:1 molar ratio. The concentration of indigo before and after the reaction is measured using absorption spectrophotometry at a wavelength of 600 nm, and the ozone concentration is calculated. The indigo method has long supported ozone treatment, but has five limitations.

Firstly, the reaction of substances other than ozone, such as hypochlorous acid (Yoshizawa, 2019), hypobromous acid (Penru et al., 2013) and permanganate ion (Gregory and Carlson, 2001), hydrogen peroxide (Bader and Hoigné, 1981), with the C–C double bond can result in an overestimation of ozone concentration. Considering that a large amount of hypobromous acid is produced during the ozonation of seawater, there is currently no method to selectively detect ozone concentration (Buchan et al., 2005) in seawater. Secondly, the purity of commercially available indigo is less than 80% and contains many impurities. It is reported that even with the same manufacturer, the measured molar absorption coefficients of indigo are different on different dates of manufacture (Gordon and Bubnis, 2002). Thus, calibration of molar absorption coefficients is needed, but it is cumbersome and beset with uncertainties because of the high volatility of dissolved ozone. In addition, some ozone treatment facilities do not have the capacity to perform this calibration. Thirdly, the stoichiometry of ozone and indigo is dependent on pH. If the pH of the solution is above 4 in any fraction during mixing, the amino group in indigo reacts with ozone and a fixed stoichiometry is not maintained (Bader and Hoigné, 1981). Fourth, the concentration of the indigo reagent and mixing ratio of the indigo solution to ozone water should be optimized according to the ozone concentration (Bader and Hoigné, 1981). Fifth is the effect of the absorbance of the sample measured at a wavelength of 600 nm. As ozone is effective in removing color, it is often applied to highly colored water. Therefore, the dissolved ozone concentration is often measured with a high background absorbance at a wavelength of 600 nm. It may be possible to remove ozone separately with a reducing agent that does not interfere with absorbance at a wavelength of 600 nm and subtract it as background noize. However, this operation is cumbersome and introduces additional measurement errors.

To summarize, it is desirable to develop a novel measurement that overcomes these drawbacks to selectively, accurately, and conveniently measure dissolved ozone concentrations in various fields. Therefore, we propose a new method to measure ozone concentration using the reaction of *p*-vinylbenzoic acid (*p*-VBA) with ozone to produce *p*-formylbenzoic acid (*p*-FBA) in equal amounts to the reacted ozone.

It is expected that oxidants other than ozone react with *p*-VBA to produce little or no *p*-FBA. *p*-VBA (FUJIFILM Wako Pure Chemical Corporation, date accessed Jul 20 2021, n.d.) and *p*-FBA (Apollo Scientific Ltd, date accessed Jul 20 2021) are commercially available with guaranteed purities of 97% or higher and are suitable for quantification. *p*-VBA has no sites that react quickly with ozone, other than vinyl groups, regardless of the pH. *p*-FBA can be separated by liquid, ion, or gas (Santander et al., 2013) chromatography and detected with high sensitivity by ultraviolet (UV) and mass spectrometry (MS) detectors. *p*-FBA measurement, carried out using column separation, is not affected by coexisting substances. The *p*-VBA method differs significantly from the indigo method, as stated above, and its measurement error can also be significantly lower. In addition, it is common to measure the concentration not only of dissolved ozone but also of pollutants at any moment in ozone treatment experiments. According to the Globally Harmonized System of Classification and Labelling of Chemicals, *p*-VBA and *p*-FBA are relatively safe reagents. On the aspect that indigo method can be performed with a spectrophotometer, it is superior to the *p*-VBA method. However, based on the price at the purchase for this paper (FUJIFILM Wako Pure Chemical Corporation; Tokyo Chemical Industry Co. , Ltd.), *p*-VBA is 7,392 JPY per 5 g and indigo is 28,215 JPY per 5 g. Taking molar mass and reagent purity into account, the price of *p*-VBA is 1/22nd that of indigo. In the aspect of reagent cost, *p*-VBA method is less expensive than indigo method, even considering the purchase of *p*-FBA.

The structure of *p*-VBA was chosen, as the C–C double bond reacts quickly and stoichiometrically with ozone and a benzene ring is required for sensitive UV detection. It may seem possible to use a reaction in which vinylbenzene reacts with ozone to form benzaldehyde. However, benzaldehyde is a known byproduct of ozone (Richardson et al., 1999), and its structure needs to be specialized. Carboxy groups are easily ionized, enabling analysis with ion chromatography (IC) and contributing to higher sensitivity in liquid chromatography (LC)/MS analysis. Furthermore, carboxy groups improve hydrophilicity, reduce adsorption on experimental instruments and improve analytical precision. Therefore, the carboxyl group is considered a strong candidate for the specificity of vinylbenzene.

To make this study more comprehensive, vinylbenzenes other than *p*-VBA, *m*-vinylbenzoic acid (*m*-VBA), *o*-vinylbenzoic acid (*o*-VBA), *p*-chlorocinnamic acid, and *p*-bromocinnamic acid were examined for their suitability as reagents for ozone determination. The effect of persulfuric acid on the indigo and *p*-VBA methods was also examined (Zhou et al., 2019).

Although it is widely known that carbonyl is formed by the reaction of ozone with a C–C double bond, it has not been established as a quantitative method for ozone. This study demonstrates the robustness and advantages of the *p*-VBA method by various experiments and analyses.

2. Methodology

2.1 Environmental Water Samples

As a representative of river water, a sample from the Katsura River in Kyoto Prefecture, Japan was used. As a representative example of wastewater, the secondary effluent from a sewage treatment plant in Kyoto prefecture, Japan was used. As a representative example of seawater, Osaka Bay coastal water in Osaka prefecture, Japan was used. The samples were filtered through 1-µm pore size glass microfiber filters (GF/B from Cytiva, Japan). The basic water qualities of the samples are listed in Table S1.

2.2.Reagents

The purity of all reagents in this study was determined by the supplier. The following were acquired from different sources: *p*-VBA (purity: 99%), *m*-formylbenzoic acid (*m*-FBA, purity: 99.2%), *p*-chlorobenzaldehyde (purity: 99.8%), *p*-bromobenzaldehyde (purity: 99.2%) from FUJIFILM Wako Chemical Corporation, Japan; *p*-FBA (purity: 97%) from Apollo Scientific Ltd., UK; indigo potassium salt (purity: 70.2%), *o*-formylbenzoic acid (*o*-FBA; purity: 99.8%), and *p*-bromocinnamic acid (purity: 99.9%) from Tokyo Chemical Industry Co., Ltd., Japan; *m*-VBA (purity: 95% < regarded as 97.5%) and *o*-VBA (purity: 99.7%) from Thermo Fisher Scientific Inc., USA; *p*-Chlorocinnamic acid (purity: 99.8%) from Merck KGaA, Germany; the rest of the reagents were of analytical grade.

The hypobromous acid solution was adjusted by mixing the sodium bromide and hypochlorous acid solutions (Tokunaga et al., 2007). Ozone solution was prepared by blowing ozone gas into ice-cold or room temperature (20–25°C) ultrapure water or a 1 mM phosphate buffer solution (pH 3). Ozone gas from an ozone generator (SGA-01-PAS2, Sumitomo Precision Products Co., Ltd., Japan) was blown into water to remove NOx and dehumidified. The *p*-VBA solution was prepared by dissolving *p*-VBA in pure water or environmental water samples reacted with 50 µM ozone (initial concentration) for more than 1 h, post which, the ozone was removed by nitrogen aeration.

At this pre-ozone treatment, the environmental water samples were diluted 1.07 times. The concentrations of all *p*-VBA solutions were 0.10 g L^{-1}.

2.3.Chemical analysis

Absorbance was measured using a spectrophotometer (UV-1900i, SHIMADZU CORPORATION, Japan).

Dissolved oxygen was measured using a dissolved oxygen meter (Multi 3510 IDS, Xylem Analytics Germany Sales GmbH & Co. KG., Germany).

The dissolved ozone concentration was measured by direct absorption spectrophotometry (von Sonntag and von Gunten, 2012), for the indigo (Bader and Hoigné, 1981) and the *p*-VBA methods. For direct absorption spectrophotometry, a quartz cell with an optical path length of 1 cm was sealed immediately after syringe injection of ozone water three times the cell volume and measured at a wavelength of 260 nm (molar absorptivity coefficient: 3200 M^{-1} cm^{-1}) (von Sonntag and von Gunten, 2012). By performing the direct absorption spectrophotometry and the indigo method or a reaction with vinylbenzenes almost simultaneously, the molar absorption coefficient of indigo or the yield of benzaldehydes were determined based on the direct absorption spectrophotometry. The ozone water at this time was adjusted to a pH of 3 using phosphoric acid at room temperature, and the ozone in the syringe decreased at a rate of 0.07% per minute, which can be regarded as no decrease in the syringe over the timescale of the experiment. The volume of ozone water, before and after addition to volume, was determined by converting the difference in mass. The density used in the conversion was 1.02 kg L^{-1} for seawater and 1.00 kg L^{-1} for others.

The hypochlorite ion, hypobromite ion, and hydrogen peroxide concentrations were measured by absorption spectrophotometry at a wavelength of 292 nm (molar absorptivity: 350 M^{-1} cm^{-1}) (Soulard et al., 1981), 329 nm (molar absorptivity: 345 M^{-1} cm^{-1}) (Soulard et al., 1981) and 240 nm (molar absorptivity: 39.4 M^{-1} cm^{-1}) (Nelson and Kiesow, 1972).

The concentrations of benzaldehydes and vinylbenzenes were measured using UPLC/UV or UPLC/MS [ACQUITY UPLC system with PDA eλ detector (optical path length 1 cm) or Xevo TQ MS detector from Waters Corporation, United States]. The MS detector was used only for *m*/*z* measurements of precursor ions, and all quantitation was performed using a UV detector. Detection wavelengths and examples of detailed analysis conditions are shown in Tables S2, S4, S5 and S7.

2.4.Experimental Method

2.4.1. Reaction rate measurements

The rate constants of the reactions of *p*-VBA and *m*-VBA with ozone were determined by competition kinetics (von Sonntag and von Gunten, 2012) with CA and the pH was adjusted to 7.1 with phosphate buffer or 1.0 with hydrochloric acid. On the time scale, phosphoric acid and hydrochloric acid did not react with *p*-VBA, *m*-VBA, and CA. The reaction rate of ozone with *p*-VBA or *m*-VBA ($k_{\mathrm{O3,VBA}}$, $\mathrm{M^{-1}\ s^{-1}}$) was calculated using the reaction rate of ozone with CA ($k_{\mathrm{O3,CA}}$, $\mathrm{M^{-1}\ s^{-1}}$), as shown in Eq. (1):

$$k_{\mathrm{O3,VBA}} = \frac{\ln \frac{[\mathrm{VBA}]_\infty}{[\mathrm{VBA}]_0}}{\ln \frac{[\mathrm{CA}]_\infty}{[\mathrm{CA}]_0}} k_{\mathrm{O3},CA} \quad (1)$$

where the subscript "0" and "∞" represent the concentration before and after the reaction, and [VBA] represents the concentration of *p*-VBA or *m*-VBA (M). The reaction rate constants of ozone with CA are 1.2×10^6 or $3.8 \times 10^5\ \mathrm{M^{-1}\ s^{-1}}$ in the completely dissociated state and 1×10^5 or $5 \times 10^4\ \mathrm{M^{-1}\ s^{-1}}$ in the completely non-dissociated state (Leitzke et al., 2001; Lung, 1996). In addition, the pK_a values of *p*-VBA and CA were measured by potentiometric titration.

Reaction rate constants of *p*-FBA with ozone, hypochlorous acid, hypobromous acid and permanganate ion were measured. Details of the experimental method are described in Supporting Information.

2.4.2. False positive ozone measurements in p-VBA and indigo methods

Hypochlorite, hypobromite, potassium permanganate, hydrogen peroxide and persulfuric acid solutions were added to the stirred *p*-VBA solution and the amount of *p*-FBA formed after the reaction was measured. These oxidants were also added to the indigo solution and the amount of absorbance loss at a wavelength of 600 nm was measured. The molar ratio of each oxidant to *p*-VBA or indigo was 1:10. Indigo solutions containing malonic acid have also been used, as reports reveal that the use of malonic acid can mask false positives of ozone due to hypochlorous acid and hypobromous acid (Bader and Hoigné, 1981).

2.4.3. Absorbance measurements of benzaldehydes and vinylbenzenes

Molar absorption coefficients of *p*-FBA, *m*-FBA, and terephthalic acid (TA) were measured. The terephthalic acid is thought to be produced when *p*-FBA is oxidized. Absorbance was measured in a cell with 1 mm optical path length. In addition, the *p*-FBA solution containing 8 mg L^{-1} dissolved oxygen was divided into glass tubes and stored in a dark room at 4°C, and their absorbances were measured at several days interval.

2.4.4. Comparison of ozone concentrations in environmental water samples measured by the p-VBA and indigo methods

Changes in the dissolved ozone concentration in the environmental water were measured using the *p*-VBA and indigo methods. Ozone water was injected into the environmental water maintained at 20°C and initial ozone concentration of the mixture was 50 μM. Ozone water was made from pure water and cooled on ice, and the environmental water samples were diluted 1.07

times by injection of ozone water. The stability of the *p*-FBA generated during the application of the *p*-VBA method to environmental water was evaluated.

2.4.5. Measurement error comparison method

The formulae for calculating the ozone concentration ($\mathrm{q_{ind}}$ or $\mathrm{q_{p\text{-}VBA}}$, μM) using the indigo method [Eq. (2)] and the *p*-VBA method [Eq. (3)] is as follows: For simplicity, it does not consider errors due to the background noise at 600 nm wavelength for the indigo method,

$$\mathrm{q_{ind}} = \frac{\mathrm{A_{ind}V_{ind} - A_{mix}V_{mix}}}{\varepsilon(\mathrm{V_{mix} - V_{ind}})} \times 10^6 \qquad (2)$$

$$\mathrm{q}_{p\text{-}\mathrm{VBA}} = \frac{\mathrm{C}_{p\text{-}\mathrm{FBA}}\mathrm{PV_{mix}}}{\mathrm{Y}\left(\mathrm{V_{mix}} - \mathrm{V}_{p\mathrm{VBA}}\right)} \qquad (3)$$

where $\mathrm{A_{ind}}$ and $\mathrm{A_{mix}}$ are the absorbance (measured in a cell with a 1 cm optical path length) for the indigo solution and a mixture of indigo solution and water containing ozone (no unit). $\mathrm{V_{ind}}$, $\mathrm{V}_{p\text{-}\mathrm{VBA}}$, and $\mathrm{V_{mix}}$ are the volumes of the indigo solution, *p*-VBA solution, and a mixture of ozone water and indigo solution or *p*-VBA solution (mL), respectively. ε is the molar absorption coefficient ($\mathrm{M^{-1}\ cm^{-1}}$). For this error comparison, a common ε of 20000 $\mathrm{M^{-1}\ cm^{-1}}$ was set (Bridgewater et al., 2012). The absorbance of the indigo solution was set (Bridgewater et al., 2012) to 2 and 0.2. $\mathrm{C}_{p\text{-}\mathrm{FBA}}$ is the concentration of *p*-FBA (μM) in the mixture of ozone water and *p*-VBA solution when the purity of the *p*-FBA reagent is 1. P is the purity of the *p*-FBA reagent and Y is the yield of *p*-FBA to ozone in the reaction between ozone and *p*-VBA.

When each measurement error ($\Delta\mathrm{q_{ind}}$ or $\Delta\mathrm{q}_{p\text{-}\mathrm{VBA}}$, μM) is assumed to be random and independent of each other, the errors of the indigo and *p*-VBA methods are as shown in Eqs. (4) and (5) (Taylor, 1996); Δ represents the measurement error, $\Delta\mathrm{V_{ind}}$ and $\Delta\mathrm{V}_{p\text{-}\mathrm{VBA}}$, and were determined to be 0.08% using actual measurements of the pipettes (MPA-10000 from A&D COMPANY, LIMITED, Japan),

$$\Delta q_{\mathrm{ind}} = \sqrt{\left(\frac{\partial q_{\mathrm{ind}}}{\partial A_{\mathrm{ind}}}\Delta A_{\mathrm{ind}}\right)^2 + \left(\frac{\partial q_{\mathrm{ind}}}{\partial A_{\mathrm{mix}}}\Delta A_{\mathrm{mix}}\right)^2 + \left(\frac{\partial q_{\mathrm{ind}}}{\partial V_{\mathrm{ind}}}\Delta V_{\mathrm{ind}}\right)^2 + \left(\frac{\partial q_{\mathrm{ind}}}{\partial V_{\mathrm{mix}}}\Delta V_{\mathrm{mix}}\right)^2 + \left(\frac{\partial q_{\mathrm{ind}}}{\partial\ \varepsilon}\Delta \varepsilon\right)^2} \quad (4)$$

$$\Delta q_{p\text{-VBA}} = \sqrt{\left(\frac{\partial q_{p\text{-VBA}}}{\partial C_{p\text{-VBA}}}\Delta C_{p\mathrm{FBA}}\right)^2 + \left(\frac{\partial q_{p\text{-VBA}}}{\partial P}\Delta P\right)^2 + \left(\frac{\partial q_{p\text{-VBA}}}{\partial V_{p\text{-VBA}}}\Delta V_{p\mathrm{VBA}}\right)^2 + \left(\frac{\partial q_{p\text{-VBA}}}{\partial V_{\mathrm{mix}}}\Delta V_{\mathrm{mix}}\right)^2 + \left(\frac{\partial q_{p\text{-VBA}}}{\partial Y}\Delta\right.}$$

ΔV_{mix} was determined to be 1 μL using actual balance measurements (HR-100A from A&D COMPANY, LIMITED, Japan). ΔA_{ind} and ΔA_{mix} were set because the transmittance had an error of 0.02% due to stray light (Sooväli et al., 2006) and the actual errors appeared larger than this because of other factors. Δε was set (Gordon and Bubnis, 2002) to 5%. ΔP was set (Malz and Jancke, 2005) at 1.5%. $\Delta C_{p\mathrm{FBA}}$ and ΔY values were determined from the experimental results of this study.

The measurement error was determined for three mixing ratios used for both the indigo and *p*-VBA methods. Five analyses were performed for each mixing ratio. The volume of the indigo and *p*-VBA solutions was set to 3 mL. Indigo solution with an absorbance of 2.05 and about 1.4 mg L^{-1} (Maximum:1.66, Minimum:1.04) ozone water was used; ozone concentration within each mixing ratio is the same. Experiments using ozone water at lower concentrations were not performed because ozone is unstable. The errors of the indigo and *p*-VBA methods were determined using Eqs. (6) and (7);

$$\Delta q_{\mathrm{ind}} = \sqrt{\left(\frac{\partial q_{\mathrm{ind}}}{\partial E_{\mathrm{ind}}}\Delta E_{\mathrm{ind}}\right)^2 + \left(\frac{\partial q_{\mathrm{ind}}}{\partial \varepsilon}\Delta \varepsilon\right)^2}\text{, where } E_{\mathrm{ind}} = \frac{A_{\mathrm{ind}}V_{\mathrm{ind}} - A_{\mathrm{mix}}V_{\mathrm{mix}}}{V_{\mathrm{mix}} - V_{\mathrm{ind}}} \quad (6)$$

$$\Delta q_{p\text{-VBA}} = \sqrt{\left(\frac{\partial q_{p\text{-VBA}}}{\partial E_{p\text{-VBA}}}\Delta E_{p\text{-VBA}}\right)^2 + \left(\frac{\partial q_{p\text{-VBA}}}{\partial P}\Delta P\right)^2 + \left(\frac{\partial q_{p\text{-VBA}}}{\partial Y}\Delta Y\right)^2}\text{, where } E_{p\text{-VBA}} = \frac{C_{p\text{-FBA}}V_{\mathrm{mix}}}{V_{\mathrm{mix}} - V_{p\mathrm{VBA}}} \quad (7)$$

ΔE was set as half of the 95% confidence interval of the five repeated experiments divided by the mean, and the other error terms were the same as those described above. In the evaluation by calculation, the errors of the indigo and *p*-VBA methods were compared using three representative examples of ozone concentrations of 1.4, 0.5, and 0.005 mg L^{-1} with 1.4 mg L^{-1} as the experimental setting.

3. Results and discussion

3.1. Reaction rate of ozone with p-VBA and m-VBA

The pK_a values of *p*-VBA and CA were determined to be 4.2 and 4.9 at 19–21ºC. Thus, *p*-VBA and CA are regarded as completely dissociated at pH 7.1 and as completely non-dissociated at pH 1.0. The reaction rate constant of ozone with *p*-VBA was 1.42 (95% CI: 1.41–1.44) times greater than that of ozone with CA at pH 7.1. The reaction rate constant of ozone with *p*-VBA was 8.2 (95% CI: 7.8–8.7) times greater at pH 1.0. Therefore, the reaction rate constant for ozone and *p*-VBA were 1.7×10^6 or 5.4×10^5 M^{-1} s^{-1} in completely dissociated state and 8×10^5 or 4×10^5 M^{-1} s^{-1} in completely non-dissociated state (Leitzke et al., 2001; Lung, 1996). In the subsequent experiment, the reaction rate constant was set to an average value of 1.1×10^6 M^{-1} s^{-1} in the completely dissociated state. In addition, the following assumptions were made: the mixing ratio (volume ratio of *p*-VBA or indigo solution to ozone-containing water and *p*-VBA or indigo solution) of the *p*-VBA method is 0.3 (refer to discussion below), and the mixing is instantaneous. The ozone concentration drops to 1/10,000th within 40 ms.

To the best of our knowledge, the earliest concentration measurement after mixing ozone and sample water is after 115 ms in Buffle et al (Buffle et al., 2006). In this study, the sample water was the secondary treatment wastewater and had a relatively high DOC. In the study, the ozone concentration was approximately halved from 0 to 115 ms, so the average first-order reaction rate constant for ozone was 6 s^{-1} if the decrease of ozone in this period is expressed as a first-order reaction. Since the rate of ozone disappearance decreases after the reaction, the reaction rate was less than 6 s^{-1} at 115 ms sampling point. If the mixing ratio is 0.3, the ozone disappearance rate in the *p*-VBA method is 740 s^{-1}. Thus, the consumption of ozone by the sample-derived material is less than 0.8%, which is an acceptable value for most experiments. Therefore, even though the reaction rate of *p*-VBA with ozone is slower than that of indigo (9.4×10^7 M^{-1} s^{-1}) (Muñoz and von Sonntag, 2000), it is sufficiently fast. The reaction rate constant of ozone with *m*-VBA was 1.3 (95% CI: 1.2-1.4) times greater than ozone with CA at pH 7.1 and was sufficiently fast.

The second order reaction rate constants of *p*-FBA with ozone, hypochlorite ion, hypobromite ion and permanganate were less than 7, 0.2, 0.2 and 0.7 M^{-1} s^{-1} at pH 7. Therefore, *p*-FBA is stable against these oxidants.

3.2. Yields of benzaldehydes to ozone

The ratio of benzaldehyde produced to consumed ozone is shown in Figure 1. The yield of *p*-FBA is 1 whether *p*-VBA is fully dissociated (pH 7) or fully non-dissociated (pH 1.7). The yield of *p*-FBA was maintained at 1, even in the presence of coexisting substances in environmental water samples. Within experiments using *p*-VBA solution at pH 7 with phosphate buffer, it has been confirmed that 1 mol of ozone reacts with 1.01 (95% CI: 0.99–1.03) mol of *p*-VBA in the stoichiometry. The data presented in Figure S1 show that the photodiode array-detected peak which increased after the reaction was the only *p*-FBA peak observed. Different researchers have reported different values (2900–3600 M^{-1} cm^{-1} at 258–260 nm) for the molar absorption coefficient of ozone in direct spectroscopy (Bader, 1982; Hart et al., 1983; Kilpatrick et al., 1956; Taube, 1957). Conversely, based on the assumption that 1 mol of ozone reacts with *p*-VBA to produce 1 mol of *p*-FBA, this study supports the molar absorption coefficient of 3200 M^{-1} cm^{-1} used here. Therefore, ΔY in Eq. (5) was set to 0. Unlike phosphoric acid, ammonium carbonate is a volatile buffer solution suitable for LC/MS analysis. However, ammonia reacted with ozone at a moderate rate (Garland et al., 1980; Hoigné and Bader, 1983) (reaction rate constant: 20 or 44 M^{-1} s^{-1}). If the reaction rate between ammonia and ozone is 44 M^{-1} s^{-1}, and with the use of 0.1 g L^{-1}

p-VBA solution, the maximum concentration of ammonium carbonate is 9 mM when the amount of ammonia that reacts with ozone is less than 1/1000th of the amount of *p*-VBA. Unlike the indigo method, the *p*-VBA method is independent of pH. The recommended ammonium carbonate concentration is 9 mM or less in the *p*-VBA method.

The data presented in Figure S1 show no new peaks other than benzaldehyde from solutions in which vinylbenzenes other than *o*-VBA reacted with ozone and the yield of benzaldehydes from vinylbenzenes other than *o*-VBA is considered 1. In addition, Figure S1 depicts a new peak different from *o*-FBA in the solution in which *o*-VBA and ozone reacted. Thus, *o*-FBA formation competes with at least one other substance and is likely to be sensitive to reaction conditions (temperature, pH, etc.). Therefore, in terms of benzaldehydes yield, vinylbenzenes other than *o*-VBA are suitable reagents for ozone determination.

3.3. False positive ozone in p-VBA and indigo methods

The false-positive ozone (mol) to hypochlorous acid, hypobromous acid, hydrogen peroxide and persulfuric acid (mol) in the indigo method and hypochlorous acid in the *p*-VBA method are shown in Figure 2. False-positive ozone (mol) to hydrogen peroxide in the *p*-VBA method was below the lower limit of quantification and less than 10^{-4} mol in 4 h.

Formation of *p*-FBA by hypobromous acid and potassium permanganate in *p*-VBA method were completed within minutes after the reaction. When the *p*-VBA solution at pH 7 contained 50 mM phosphoric acid and 7 mM ammonium carbonate, false-positive ozone (mol) to hypobromous acid and potassium permanganate was 0.013 (95%CI: 0.006-0.014) and 0.017 (95%CI: 0.016-0.018) mol. When the *p*-VBA solution at pH 7 only contained 50 mM phosphoric acid, false-positive ozone (mol) to hypobromous acid and potassium permanganate was 0.0007 (95%CI: 0.0005–0.0008) and 0.012 (95%CI: 0.011–0.013) mol. The false-positive ozone was higher in the presence of ammonium carbonate. Therefore, the use of ammonium carbonate is undesirable when hypobromous acid or permanganate ions are present.

The false positive ozone (mol) to permanganate ion in the indigo method was 1.5 mol and decolorization of indigo was completed within minutes after the reaction. In a previous study, 1 mol of permanganate ion decolorized 1.8 mol indigo (Rakness et al., 2010), which is different from the findings of our study. This difference may be attributed to the quality and quantity of the impurities in the indigo reagent. The stoichiometry of permanganate ions, as well as other oxidants and indigo, may vary depending on the source and date of acquisition of the indigo reagent. However, the fact that the *p*-VBA method is less likely to falsely detect ozone than the indigo for all oxidants is considered irrefutable.

The false positive ozone (mol) to persulfuric acid in the indigo method was below the lower limit of quantitation. For persulfuric acid, the indigo method had the fewer false positive than the *p*-VBA method. However, the amount of false positives in the *p*-VBA method is not large if analyzed immediately. The method for measuring ozone concentration when persulfuric acid is coexisting should be determined by comprehensively considering the other advantages of the *p*-VBA method.

The mass scan (range: 30–300 *m*/*z*) of a solution of *p*-VBA reacting with the permanganate ion presented a main peak of 179 *m*/*z*, which is two OH added to the C–C double bond of *p*-VBA. The mass scan (range: 30–300 *m*/*z*) of a solution of *p*-VBA reacted with hypobromous acid and exhibited a main peak of 163, 243, and 245 *m*/*z*, which is OH and Br added to the C–C double bond of *p*-VBA. The chromatogram data are shown in Figure S2. The product of reaction between *p*-VBA and hypochlorous acid was also not identified.

3.4. Molar absorption coefficients

The molar absorption coefficients of *p*-FBA, *p*-VBA, *m*-FBA, *m*-VBA, and TA are shown in Figure S3 and listed in Table S6. Ozone dissolved in ultrapure water reacted with the *p*-VBA solution at pH 7, and the absorbance of the solution after the reaction at wavelengths of 233 and 279 nm was measured by direct absorption spectrophotometry. Further, the concentration of *p*-FBA (M) was calculated according to Eqs. (8) and (9), and the concentration of ozone was determined; the ratio of the value measured by this method to that measured by direct absorption spectrophotometry was 99% (95% CI: 95–104),

$$\varepsilon_{p\text{-FBA},233}\,[p\text{- FBA}] + \varepsilon_{p\text{-VBA},233}\,[p\text{- VBA}] = \frac{\mathrm{Abs}_{233}}{c}, \tag{8}$$

$$\varepsilon_{p\text{-FBA},279}\,[p\text{- FBA}] + \varepsilon_{p\text{-VBA},279}\,[p\text{- VBA}] = \frac{\mathrm{Abs}_{279}}{c}, \tag{9}$$

Where ε is the molar absorption coefficient ($\mathrm{M}^{-1}\ \mathrm{cm}^{-1}$) at wavelength of *p*-VBA or *p*-FBA, Abs is the absorbance measured in a 1 cm cell at a wavelength (no unit), and c is the optical path length (cm). The wavelength of 233 nm is the wavelength at which the ratio of the molar absorption coefficients of *p*-FBA to those of *p*-VBA are large and wavelength of 279 nm is the opposite. Cooled highly-concentration ozone water adjusted with pure water or a phosphoric acid solution is often used in ozone treatment research. The ozone concentration in such water can be measured easily with a spectrophotometer using this method.

The largest difference ($1.39 \times 10^4\ \mathrm{M}^{-1}\ \mathrm{cm}^{-1}$) between the molar absorption coefficients of *p*-FBA and TA was observed at a wavelength of 262 nm in the non-dissociated state. The change in absorbance at 262 nm of the *p*-FBA solution stored in a dark room at 4°C was within a narrow range of 0.5767–0.5788 over 29-d (Figure S3); the absorbance measurements were conducted at a temperature of 25°C and pH of 1. If 1% *p*-FBA was changed to TA, the absorbance decreased by 0.0048. In addition, no change in the absorbance was observed at other wavelengths (205–315 nm), suggesting that *p*-FBA did not change to terephthalic acid or other compounds within a month, even when the *p*-FBA solution contained 8 mg L^{-1} of dissolved oxygen. Therefore, *p*-FBA is an excellent quantitative reagent that is stable over a long period of time. On the contrary, *p*-FBA of about 5 µg L^{-1} was detected in the *p*-VBA solution about a week after adjustment. Therefore, it is needed to measure the *p*-FBA concentration in the *p*-VBA solution each time and correct the amount of *p*-FBA produced, particularly when measuring low ozone concentrations.

The maximum absorption wavelengths of *p*-VBA and *m*-VBA are very different. When UV detectors are used for analyses, it will be possible to improve the measurement sensitivity by proper use of *p*-VBA and *m*-VBA.

3.5. Comparison of ozone concentrations in environmental water samples measured by the p-VBA and indigo methods

The molar absorption coefficient of indigo at 600 nm was determined to be 2.4×10^4 (95% CI: $2.24–2.55 \times 10^4$) M^{-1} cm^{-1}. Figure 3 shows the dissolved ozone concentration measured by the indigo and *p*-VBA methods in environmental water. In the river water, the measurements of the *p*-VBA method tended to fall below those of the indigo method over time. In treated wastewater, the measurements of the *p*-VBA and indigo methods were almost the same. In seawater, there was a significant difference between the measurements obtained using the *p*-VBA and indigo methods. The reaction rate constant between bromide ions and ozone at 20°C has been reported (Haag and Hoigne, 1983) to be 160 M^{-1} s^{-1}. Thus, even considering ozone consumption by only bromide ions, the ozone concentration dropped to 1.4 µM after 30 seconds, the first measurement point. This condition was satisfied by the measurements obtained by the *p*-VBA method but not by the indigo method. *p*-FBA above 1 µg L^{-1} was not detected in solutions of river water, treated wastewater, or seawater after ozonation. If the ozonated water sample contains *p*-FBA, the ozone concentration measurements should be corrected.

Measurements of the *p*-FBA concentration produced when the *p*-VBA solution was mixed with environmental water containing ozone over an 18-h period showed no change in p-FBA concentration (Figure S4). Thus, *p*-FBA concentrations were shown to be stable in environmental water after ozone treatment. Therefore, the *p*-VBA method is also appropriate for situations in which the analysis cannot be performed immediately.

The calibration curve for *p*-FBA had good linearity ($R^2 = 0.998$, 1/x weighting) in the range of 1 μg L^{-1} to 5 mg L^{-1}, with each concentration analyzed eight times. The details of the analytical conditions are shown (Table S7). Half of the 95% CI divided by the mean was 5.9% for 1.020 μg L^{-1}, 2.9% for 5.262 μg L^{-1}, 0.75% for 51.71 μg L^{-1}, 0.43% for 509.6 μg L^{-1}, and 0.61% for 4.974 mg L^{-1}. These results were plotted on a graph with the *p*-FBA concentration on the horizontal axis and the errors on the vertical axis, with a straight line connecting each plot. $\Delta \mathrm{C}_{p\mathrm{VBA}}$ is defined using these straight lines. The linearity of the calibration curve was lost at about 5 μg L^{-1} when methanol was not added to the sample for determination of chlorobenzaldehyde and bromobenzaldehyde. However, the use of methanol is undesirable because it is cumbersome and costly. As there is linearity in the calibration curve of *p*-VBA up to 1.020 μg L^{-1} in an aqueous solution, the enhancement of water solubility by the carboxyl group plays an important role in improving the accuracy and convenience of the analysis. *p*-VBA is a better reagent for ozone concentration determination than *p*-chlorocinnamic acid or *p*-bromocinnamic acid.

3.6. Comparison of measurement error between p-VBA and indigo methods

The molar absorption coefficient of indigo determined in this study was 20% larger than what was considered common (20000 M^{-1} cm^{-1}), confirming that the indigo method needed calibration. In addition, the relative error of the measurement was 7%, which was larger than the setting value of 5% for comparison of measurement error. This result demonstrates that the error by the indigo method is underestimated for the following comparison.

The comparison of the relative errors between the indigo and *p*-VBA methods is shown in Figure 4 (a). The *p*-VBA method presented a smaller relative error than the indigo method for any mixing ratio for both the calculated and measured values. When the measurement error of indigo's molar absorption coefficient is larger than 5% or when there is background noise at 600 nm wavelength, the error of the indigo method is bigger. In the indigo method, when the absorbance and mixing ratio were not properly adjusted according to the ozone concentration, the error was large. Hence, reduction of error in the indigo method involves determination of the mixing ratio and absorbance of the indigo solution in the pre-measurement, which is complicated. On the contrary, *p*-VBA method is convenient because a small measurement error was achieved with one *p*-VBA solution (for example, 0.1 g L^{-1}) and one mixing ratio (for example, 0.3), regardless of the ozone concentration to be measured. In addition, when measuring substances (e.g. pollutants) in a mixture of indigo or *p*-VBA solution and ozone-containing environmental water by LC or IC, the *p*-VBA method, which can fix the reagent concentration and mixing ratio, provides a more consistent baseline among different samples.

The error in volume measurement (ΔV_{ind}, $\Delta V_{\mathrm{p\text{-}VBA}}$ and ΔV_{mix}) in this study was very small, and such a small error may not be achievable in more complex experiments or outdoor surveys. The results of the relative error, for the error in the volume 10 times larger (ΔV_{ind} and $\Delta V_{\mathrm{p\text{-}VBA}}$ = 0.8% and ΔV_{mix} = 1 μL), are shown in Figure 4 (b). The *p*-VBA method is always more accurate than the indigo method. Regarding the *p*-VBA method, the tendency of the error to increase with increasing mixing ratio becomes more pronounced. The relative error is almost the minimum below a mixing ratio of about 0.3 at any ozone concentration. However, a mixing ratio that is too low may result in *p*-VBA not being distributed throughout the mixture, especially with insufficient agitation. Therefore, it is recommended that the *p*-VBA method be performed with a mixing ratio of about 0.3. When the mixing ratio is fixed at 0.3, the change in relative error with respect to changes in dissolved ozone concentration is shown in Figure S5.

In the *p*-VBA method, the measurements impacting the error in the measured ozone concentration at a mixing ratio below 0.75 consisted of purity of the *p*-FBA reagent or the concentration of *p*-FBA. Uncertainty of the purity is dominant when ozone concentration is high and uncertainty of the *p*-FBA concentration becomes dominant as ozone concentration decreases, and under the study conditions, the magnitude of $\frac{\partial \mathrm{q}_{p\text{-VBA}}}{\partial \mathrm{P}} \Delta \mathrm{P}$ and $\frac{\partial \mathrm{q}_{p\text{-VBA}}}{\partial \mathrm{C}_{p\text{-VBA}}} \Delta \mathrm{C}_{p\mathrm{FBA}}$ in Eq. (5) matched at 0.02 mg L^{-1}. Therefore, when ozone concentrations are low, multiple repetitions of the *p*-FBA concentration analysis are effective in reducing measurement errors. In the indigo method, the measurements mainly impacting the error are not volumetric measurements of mixtures of indigo solution and ozone water. Detailed figure of the error factors and their magnitude comparisons are shown in Figure S6. Assuming a relative error of 10% as the lower limit, the lower limit of quantitation for ozone in this calculation was 4 μg L^{-1} for the indigo method (using indigo solution with absorbance of 0.2) and 0.4 μg L^{-1} for the *p*-VBA method, with the *p*-VBA method being smaller.

4. Conclusion

The yield of *p*-FBA from the reaction of *p*-VBA and ozone was maintained at 1, even in the presence of coexisting substances in environmental water, and the *p*-VBA method can be used for the determination of dissolved ozone concentration.

The *p*-VBA method is more selective than the indigo method, and is more resistant to hypochlorous acid, hypobromous acid, permanganate and hydrogen peroxide.

To reduce the error, the indigo method requires adjustment of the concentration of the indigo solution and the mixing ratio according to the ozone concentration. On the contrary, the *p*-VBA method can measure a wide range of ozone concentrations with one concentration of the *p*-VBA solution and mixing ratio with less error than the indigo method.

As a reagent for ozone concentration determination, although fewer evaluations have been performed on *m*-VBA than on *p*-VBA in this study*,* *m*-VBA is considered a possible substitute for *p*-VBA. The most significant difference between *p*-VBA and *m*-VBA is the UV spectrum. It will be possible to improve the measurement sensitivity by proper use of *p*-VBA and *m*-VBA when UV detectors are used for analyses. *o*-VBA is inferior to *p*-VBA, because the yield of *o*-FBA to ozone is not 1. *p*-chlorocinnamic acid and *p*-bromocinnamic acid are inferior to *p*-VBA because the calibration curves for *p*-chlorobenzaldehyde and *p*-bromobenzaldehyde produced in the reaction with ozone show poor linearity.

Compared to the indigo method, the *p*-VBA method is more selective, more accurate, and can be performed simultaneously with measurement of other analytes and does not require cumbersome calibration. *p*-VBA can contribute widely to ozone treatment research and management.

Author Contributions

Kohei Kawaguchi conceived the study, analyzed the data, and wrote the manuscript. Taira Hidaka made important revisions to the manuscript. Taku Fujiwara supervised the study and made important revisions to the manuscript. The manuscript is contributed by all the authors. All the authors approved the final version of the manuscript.

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ABBREVIATIONS

p-FBA, *p*-formylbenzoic acid; *p*-VBA, *p*-vinylbenzoic acid; *m*-FBA, *m*-formylbenzoic acid; *m*-VBA, *m*-vinylbenzoic acid; *o*-FBA, *o*-formylbenzoic acid; *o*-VBA, *o*-vinylbenzoic acid; TA, terephthalic acid; CA, cinnamic acid; indigo, indigotrisulfonic acid; mixing ratio, volume ratio of *p*-VBA or indigo solution to the sum of ozone-containing water and *p*-VBA or indigo solution; UV, ultraviolet; MS, mass spectrometry; IC, ion chromatography; LC, liquid chromatography.

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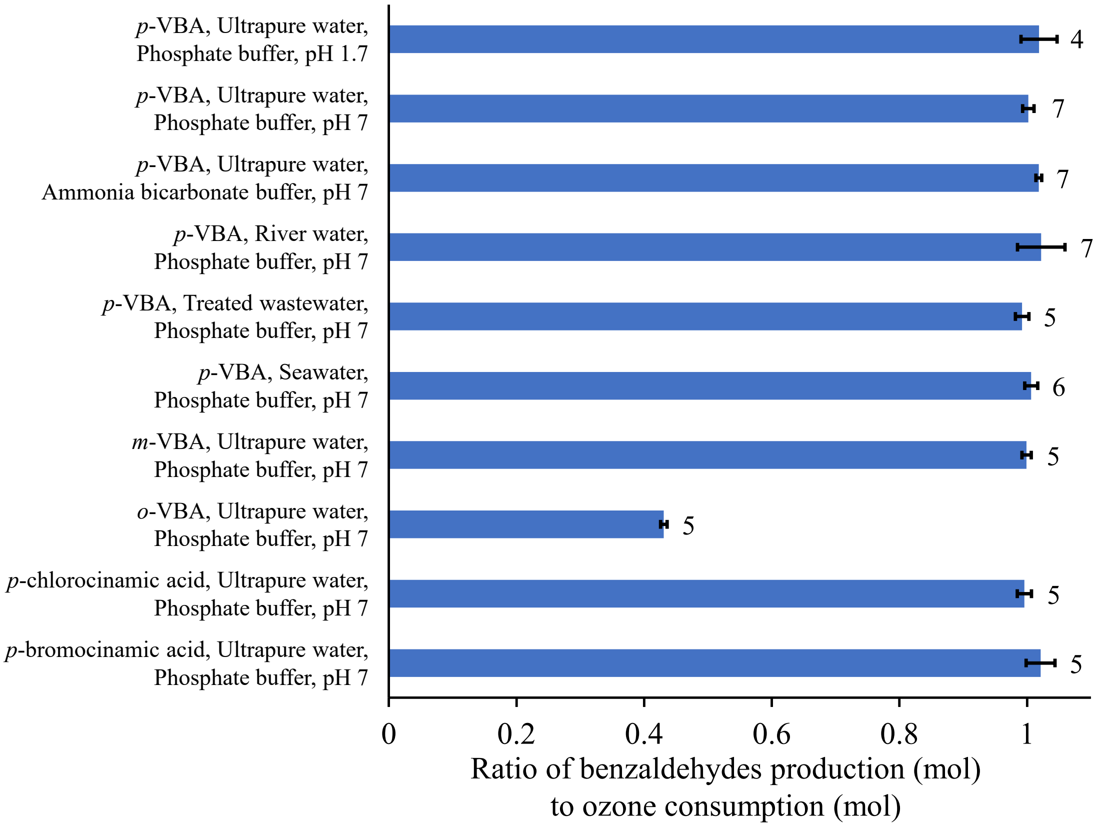

Figure 1. Yields of benzaldehydes from the reaction of ozone with vinylbenzenes solutions. The legend is written in the order of vinylbenzenes, solvent, buffer, and pH. Error bars indicate 95% confidence intervals. The number to the right of the error bars is the number of repetitions of the experiment

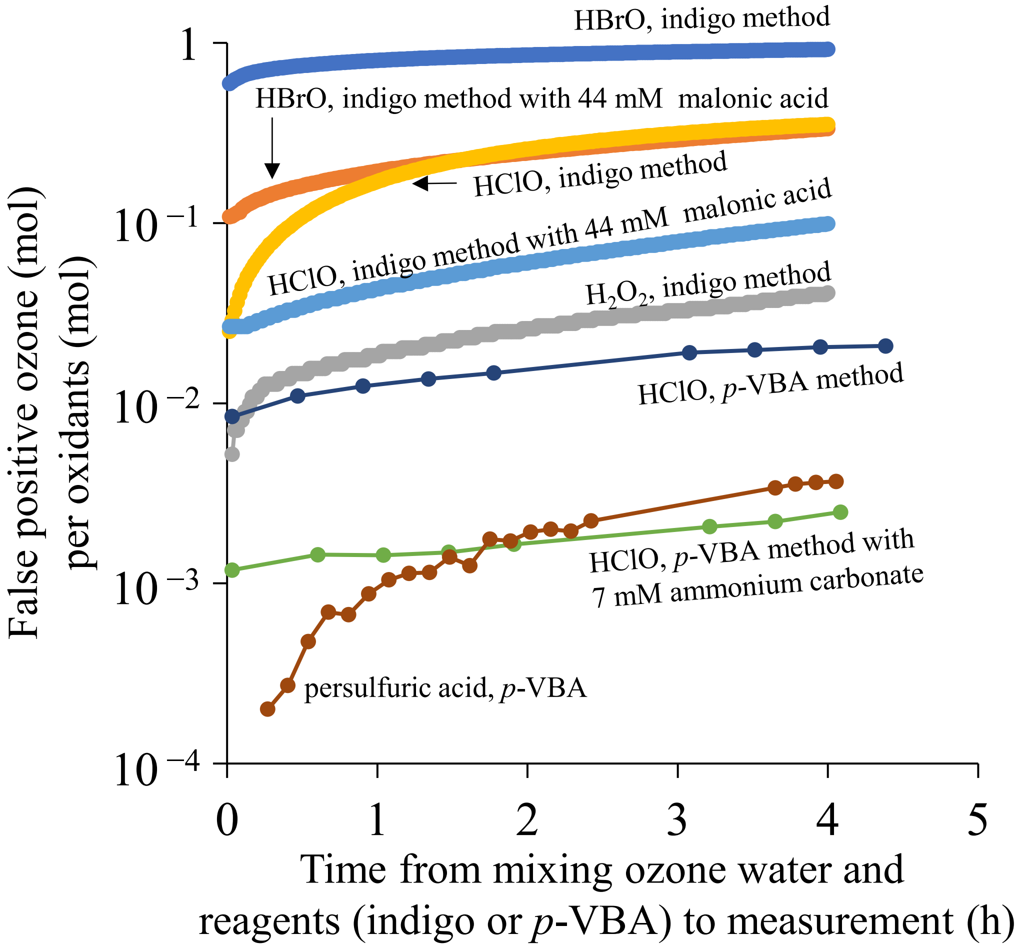

Figure 2. False positive ozone (mol) in *p*-VBA and indigo methods for hypochlorous acid, hypobromous acid, hydrogen peroxide and persulfuric acid (mol). All *p*-VBA solutions contained 50 mM phosphate buffer and were adjusted to pH 7.

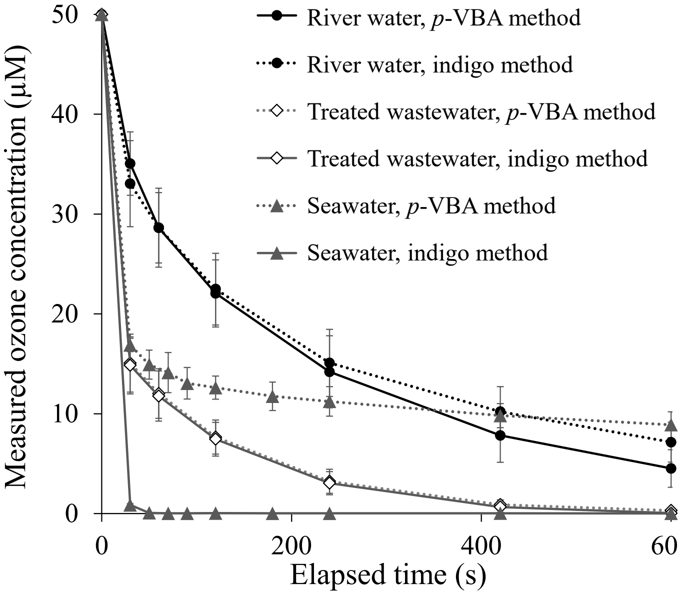

Figure 3. Measurements of the dissolved ozone concentration measured by the indigo and *p*-VBA methods in environmental water. The initial value is not an actual measured value, but a set value determined by the dilution rate and the concentration of ozone water to be injected. Measurements were taken twice, and the average value was plotted. Error bars indicate maximum and minimum values for two measurements

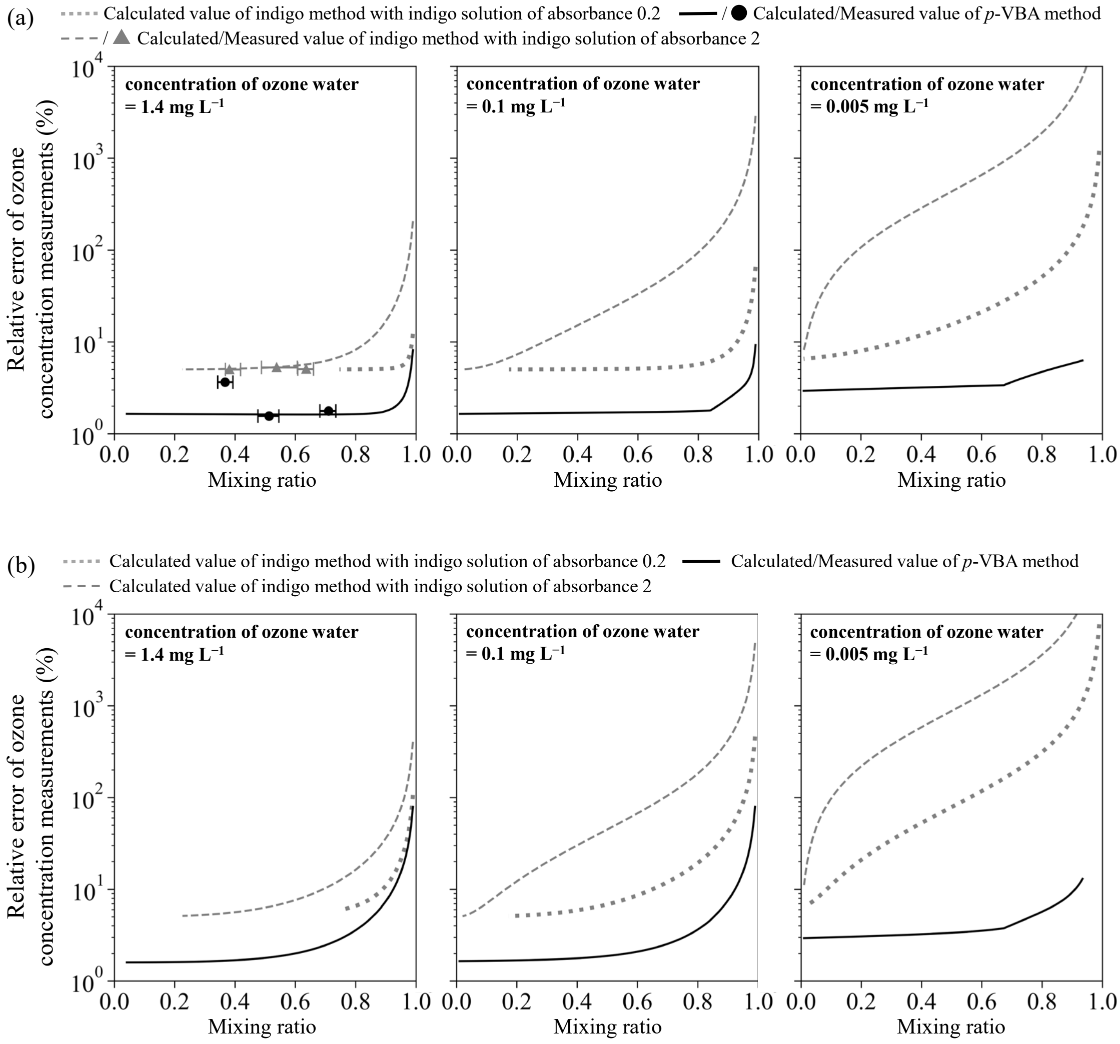

Figure 4. Comparison of the relative errors between the indigo method and the *p*-VBA method. Relative errors were calculated by Eq. (4)–(7). For calculated values, the following were excluded: Amount of substance in indigo or *p*-VBA is less than the amount of substance in ozone or the concentration of *p*-FBA exceeds the error-evaluated range (1.020 μg L^{-1} to 4.974 mg L^{-1}). The absorbance of indigo was the value when measured in a 1 cm cell. (a) Result of analysis to reproduce experimental conditions. Error bars indicate maximum and minimum values in the measurement. (b) Result of analysis when the measurement error of the volume is 10 times larger than (a)