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Application of Fenton reaction for nanomolar determination of hydrogen peroxide in seawater

*Emmanuel F. Olasehinde, Shinya Makino, Hiroaki Kondo, Kazuhiko Takeda, Hiroshi Sakugawa**

Graduate School of Biosphere Science, Department of Environmental Dynamics and Management, Hiroshima University, 1-7-1 Kagamiyama, Higashi Hiroshima 739-8521, Japan

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ABSTRACT

A simple and sensitive method for the determination of nanomolar levels of hydrogen peroxide (H_2O_2) in seawater has been developed and validated. This method is based on the reduction of H_2O_2 by ferrous iron in acid solution to yield hydroxyl radical (•OH) which reacts with benzene to produce phenol. Phenol is separated from the reaction mixture by reversed phase high performance liquid chromatography and its fluorescence intensity signals were measured at excitation and emission of 270 and 298 nm, respectively. Under optimum conditions, the calibration curve exhibited linearity in the range of (0–50) × 10^3 nmol L^{-1} H_2O_2. The relative standard deviations for five replicate measurements of 500 and 50 nmol L^{-1} H_2O_2 are 1.9 and 2.4%, respectively. The detection limit for H_2O_2, defined as three times the standard deviation of the lowest standard solution (5 nmol L^{-1} H_2O_2) in seawater is 4 nmol L^{-1}. Interference of nitrite ion (NO_2^-) on the fluorescence intensity of phenol was also investigated. The result indicated that the addition of 10 μmol L^{-1} NO_2^- to seawater samples showed no significant interference, although, the addition of 50 μmol L^{-1} NO_2^- to the seawater samples decreases the fluorescence intensity signals of phenol by almost 40%. Intercomparison of this method with well-accepted (*p*-hydroxyphenyl) acetic acid (POHPAA)-FIA method shows excellent agreement. The proposed method has been applied on-board analysis of H_2O_2 in Seto Inland seawater samples.

1. Introduction

Hydrogen peroxide (H_2O_2) is one of the reactive oxygen species found in seawater as a product formed photochemically from dissolved organic matter (DOM) [1]. This important chemical may also arise from other sources including wet deposition [2], dry deposition [3,4] and biological production [5]. Photodissociation of H_2O_2 produces hydroxyl radical (•OH), which is one of the most important oxidizing species in natural water [6]. Over the past three decades, there is a considerable interest in H_2O_2 in seawater because it is involved in metal redox chemistry [7] and as a potential toxicant to marine organism [8]. Techniques for the determination of H_2O_2 in seawater have been the subject of substantial research efforts, each attempting to attain higher sensitivity, selectivity and reproducibility. Analysis methods of H_2O_2 in seawater generally fall into three groups [9]. The first group utilizes fluorescence methods [10–14] which are based on the peroxidase-mediated oxidation of a reagent molecule by H_2O_2 or organic peroxides. This elegant method is highly sensitive and relatively free from interference, however, it suffers the disadvantages common to many enzyme assays notably

* *Corresponding author*. Tel.: +81 82 424 6504; fax: +81 82 424 6504.
E-mail address: hsakuga@hiroshima-u.ac.jp (H. Sakugawa).
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reagent instability and high cost [15]. The second group employs chemiluminescence techniques for H_2O_2 determination. The most common chemiluminescence technique is based on the metal-catalysed oxidation of luminal [16–19]. This method is known to be an attractive analytical method because of its higher sensitivity and low detection limit but it suffers the disadvantage of interference with ferrous ion (Fe^{2+}) through the reduction of oxygen in alkaline solutions producing H_2O_2 [16]. Colorimetric methods [20–22], which are employed by the final analysis group, rely on the enzyme-mediated oxidation of reagent molecules by peroxides to form stable chromophores. Miller et al. reported that this method lacks the sensitivity for peroxide analysis in oligotrophic waters. Thus, more selective as well as sensitive methods for the determination of H_2O_2 in seawater are still required. It has been reported that Fenton chemistry could be applied to the determination of H_2O_2 in atmospheric samples [15,23]. However, as far as we know, application of this chemistry to the determination of H_2O_2 in seawater has not yet been reported.

In the present study, we investigated the applicability of Fenton reaction to the determination of nanomolar levels of H_2O_2 in seawater. This method is based on the reduction of H_2O_2 by Fe^{2+} in acid solution to yield $^{\bullet}OH$ which is scavenged by benzene to produce phenol as shown below:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \quad (1)$$

$$^{\bullet}OH + C_6H_6 \rightarrow C_6H_5OH \quad (2)$$

Phenol produced by Fenton reaction was analyzed by high performance liquid chromatography (HPLC) with fluorescence detector. The amount of phenol produced is directly proportional to the original amount of H_2O_2 present in the sample. The validity of this method was established by simultaneous analysis of H_2O_2-spiked seawater samples and compared to the well-known (*p*-hydroxyphenyl) acetic acid-FIA method.

2. Experimental

2.1. Reagents and chemicals

All reagents were reagent grade and used as received unless otherwise stated. All solutions were prepared with ultrapure water obtained from a Milli-Q Plus system (Millipore; $\geq 18.2\,M\Omega\,cm$). Acetonitrile and benzene (HPLC grade) were purchased from Nacalai Tesque (HPLC grade). Nitrite standard solution ($1000\,mg\,L^{-1}$) was obtained from Kanto Chemical Co. Inc., and sulphuric acid was obtained from Sigma–Aldrich, Japan. Iron(II) sulphate pentahydrate was purchased from Nacalai Tesque (Guaranteed Reagent). A $0.1\,mol\,L^{-1}$ Fe(II) solution was prepared by dissolving 1.39 g iron(II) sulphate pentahydrate into 50 mL of $0.07\,mol\,L^{-1}$ H_2SO_4. A $3.0\,mol\,L^{-1}$ sulphuric acid stock solution was prepared by diluting 16.3 mL of 98% H_2SO_4 to 100 mL with water. H_2O_2 solution (ca. 30%) was obtained from Wako Pure Chemical Industries. The stock standard solution of H_2O_2 ($1.0 \times 10^{-2}\,mol\,L^{-1}$) was prepared by diluting 1.0 mL of 30% H_2O_2 to 100 mL with water and the concentration was determined based on the molar extinction coefficient at 240 nm ($\varepsilon = 38.1\,L\,mol^{-1}\,cm^{-1}$ [14]). The working standard solutions for H_2O_2 were prepared daily by accurate dilution of the stock standard solution just before use. Benzene stock solution ($2 \times 10^{-2}\,mol\,L^{-1}$) was prepared by diluting 88.8 μL of 99.7% benzene in 50 mL with water.

2.2. Seawater

The sampling stations were located in Hiroshima Bay, the western Seto Inland Sea, Hiroshima city, Japan as shown in Fig. 1. The Seto Inland Sea is one of the typical closed seas which connect to open ocean by only four channels. The apparent residence times of fresh water, which are calculated as the fresh water volumes (estimated with the salinity distribution) divided by the total discharges are around 100 d for the entire bay [11,24]. Seawater samples from various depths and locations were collected by Niskin sampling bottles with CTD carousel multi-sampling system (General Oceanic Inc., U.S.A.) during the cruise of the R/V Toyoshio Maru belonging to Hiroshima University on 7–11 May 2007 and immediately transferred to clean amber 1 L glass bottles. The samples were filtered immediately through a pre-cleaned glass fiber filter (Advantec, 0.45 μm nominal rating) and analysis was performed within 1 h of sample collection.

2.3. Apparatus

An HPLC system consisting of a PU-2089 plus pump (Jasco, Japan), a Rheodyne injection valve (Cotati, CA, USA) with a 50 μL sample loop and a FP-2020 plus intelligent fluorescence detector (Jasco, Japan) interfaced with a C-R6A Chromatopac integrator (Shimadzu, Japan) was used. The separations were carried out on a RP-18 GP column (150 mm × 4.6 mm i.d., 5 μm) from Kanto Kagaku (Japan) with acetonitrile–water mixture (40/60, v/v) as eluent at a flow rate of $1\,mL\,min^{-1}$. The detector was operated at 270 and 298 nm for excitation and emission, respectively. The flow injection system used for H_2O_2 analysis by the POHPAA method consists of a pump (model 2P2H, Sanuki Kogyo, Japan), autosampler (TOSOH, model AS 8020), fluorescence detector (Rf-10AXL, Shimadzu, Japan) and C-R6A Chromatopac integrator (Shimadzu, Japan).

2.4. Analytical procedure

Two hundred microlitres of $2 \times 10^{-2}\,mol\,L^{-1}$ benzene stock solution was added to 3.0 mL of seawater samples or H_2O_2 standard solution in a 5 mL amber vial giving a concentration of $1.2 \times 10^{-3}\,mol\,L^{-1}$ benzene and mixed by shaking. Fifty microlitres of $0.1\,mol\,L^{-1}$ Fe^{2+} in $0.07\,mol\,L^{-1}$ H_2SO_4 solution was added to the solution and was allowed to stand for 5 min at room temperature. The final pH of the solution was adjusted to be ca. 4 from the sulphuric acid added. An aliquot of the solution was injected into the HPLC system for analysis. Phenol and benzene were separated by reversed phase high performance liquid chromatography. The retention time of phenol and benzene was 4.0 and 6.9 min, respectively. H_2O_2 standard solutions were prepared in seawater by appropriate dilution of the stock solution. Calibration was then achieved by plotting the peak areas of phenol produced in each standard solution against the H_2O_2 concentration. Seawater samples that were filtered in a similar manner and stored in the dark at room

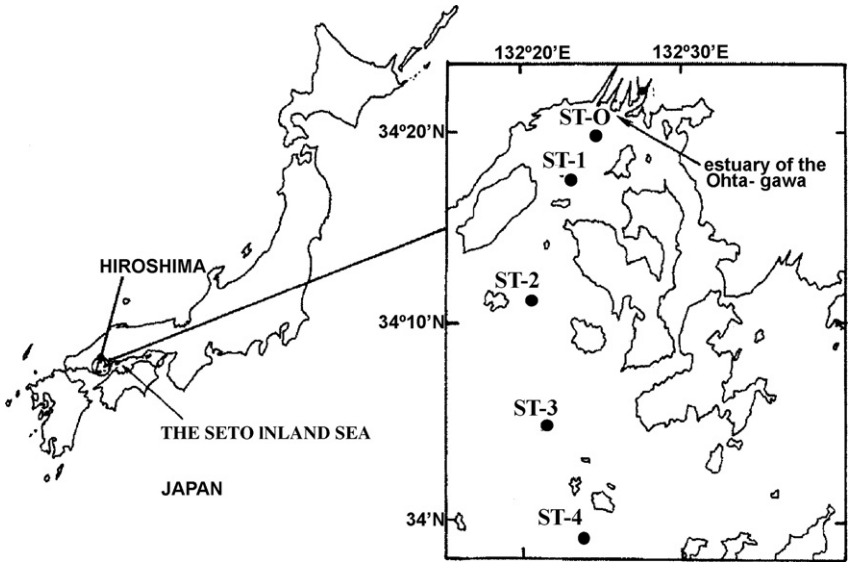

Fig. 1 – Sampling locations.

temperature were used as the blank. All procedures were carried out in dark condition to minimize H_2O_2 decomposition and formation.

3. Results and discussion

3.1. *Optimization of reaction conditions*

3.1.1. *Effect of reaction time*

The influence of reaction time on the fluorescence (FL) intensity of phenol was investigated. The result (Fig. 2) indicated that the fluorescence intensity increased with increase in reaction time and maximum fluorescence intensity was attained after a reaction time of 5 min. Instead of a further increase, the fluorescence intensity slightly decreased with increase in the reaction time. Burbano et al. reported that a large fraction of Fe^{2+} was rapidly oxidized to ferric ion (Fe^{3+}) in the first 3–5 min of reaction [25]. Meanwhile, it is expected that

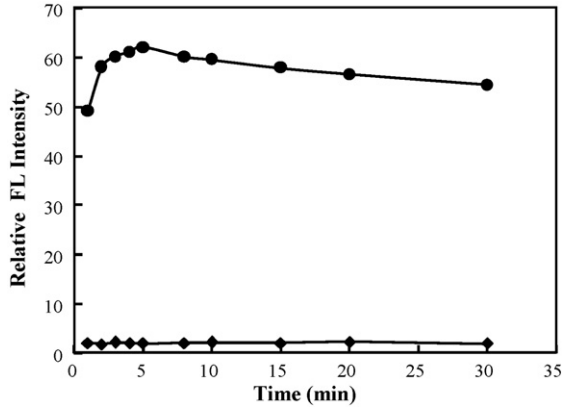

Fig. 2 – Effect of reaction time on fluorescence intensity of phenol (♦) blank and (●) sample. Experimental conditions—[H_2O_2]: 300 nmol L^{-1}; pH 4.0; [Fe^{2+}]: 1.5 mmol L^{-1}.

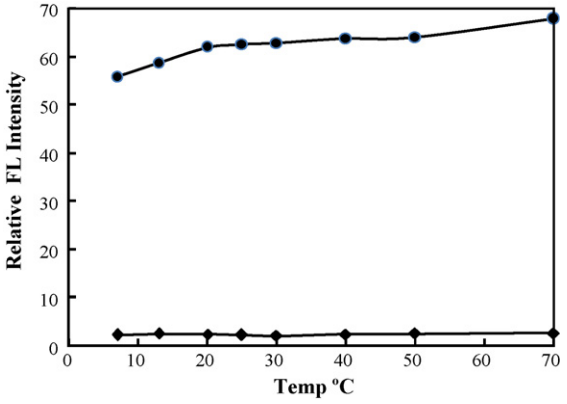

Fig. 3 – Effect of temperature on fluorescence intensity of phenol (♦) blank and (●) sample. Experimental conditions—[H_2O_2]: 300 nmol L^{-1}; reaction time: 5 min; [Fe^{2+}]: 1.5 mmol L^{-1}; pH 4.0.

the fluorescence intensity should remain fairly constant after a reaction time of 5 min. However, a more probable explanation to the slight decrease in the fluorescence intensity may be attributed to the subsequent reactions of •OH with already formed phenol, with simultaneous formation of higher oxidized products [26]. Therefore, a reaction time of 5 min was recommended in our experiment.

3.1.2. *Effect of temperature on fluorescence intensity*

The effect of temperature on the reaction was investigated by varying the temperature from 7 to 70 °C in a thermostated water bath. As shown in Fig. 3, the fluorescence intensity increased with temperature and remained fairly constant over the temperature range of 20–50 °C, though the fluorescence intensity at 70 °C was 10% higher than that of the room temperature (20 °C). Room temperature was chosen as the operational temperature for convenience.

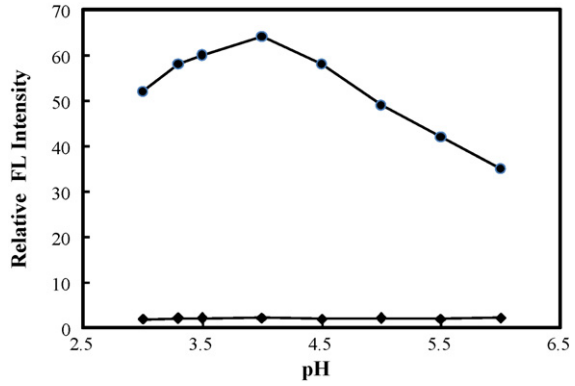

Fig. 4 – Influence of pH on fluorescence intensity of (♦) phenol blank and (●) sample. Experimental conditions—[H_2O_2]: 300 nmol L^{-1}; [Fe^{2+}]: 1.5 mmol L^{-1}; reaction time: 5 min.

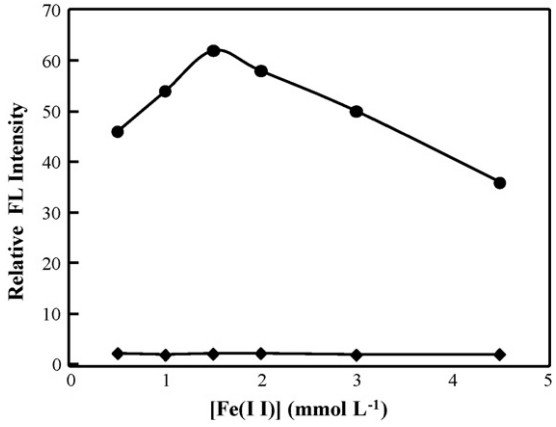

Fig. 5 – Effect of Fe^{2+} concentration on fluorescence intensity of phenol (♦) blank and (●) sample. Experimental conditions—[H_2O_2]: 300 nmol L^{-1}; pH 4.0; reaction time: 5 min.

3.1.3. *Influence of pH on fluorescence intensity*

Burbano et al. reported that catalytic decomposition of H_2O_2 carried out by a transition metal such as Fe^{2+} depends on the pH of the reaction media [25]. Hence, the effect of the pH on the fluorescence intensity of phenol was studied. The result (Fig. 4) showed that the fluorescence intensity increased with increase in pH. However, at pH above 4, the fluorescence intensity decreased because of the decrease of the soluble Fe(II) ion in the solution, probably due to the precipitation of Fe^{2+}. It has been reported that the precipitation of Fe^{2+} is strictly dependent on pH of the solutions. Below pH 5, the amount of the precipitate formed decreased with decreasing pH. The precipitation became more pronounced when pH exceeded 5, then reached a plateau at pH 8 when 100% of Fe^{2+} precipitated [27]. This phenomenon was experimentally confirmed by the presence of turbidity in the samples of our experiment carried out above pH 5, although the turbidness was not clear at pH 4–5. Kavitha and Palanivelu in their study also stated that the drop in fluorescence intensity above pH 4 may be attributed to precipitation of $Fe(OH)_3$, which lowers the concentration of free soluble iron species available for reacting with peroxide [28]. Hence, lesser concentration of •OH is generated which reacts with benzene to produce phenol and consequently decreases the fluorescence intensity. In this study, experiments were carried out at pH 4 for optimization.

3.1.4. *Effect of the concentration of Fe^{2+} ion*

The effect of the concentration of Fe^{2+} ion on the fluorescence intensity of phenol was investigated in a series of experiments. In these experiments, the concentration of Fe^{2+} ion varied from 0.5 to 4.5 mmol L^{-1} for fixed 300 nmol L^{-1} H_2O_2 standard solution at pH 4.0 and a reaction time of 5 min. The result (Fig. 5) shows that the fluorescence intensity increased with increase in Fe^{2+} ion concentration and reached the peak at 1.5 mmol L^{-1} of Fe^{2+} ion. However, further increase in Fe^{2+} ion concentration decreased the fluorescence intensity. Joseph et al. reported that •OH is reduced in the presence of high concentration of metals ions as presented in Eq. (3) [29]:

$$Fe^{2+} + {}^{\bullet}OH \rightarrow Fe^{3+} + OH^{-}, \quad k_1 = 4.3 \times 10^8\ M^{-1}\ s^{-1} \tag{3}$$

The reduction of the fluorescence intensity may be attributed to the scavenging of •OH by excess Fe^{2+} ion, hence lowers the concentration of •OH available to produce phenol. Therefore, 1.5 mM of Fe^{2+} ion was used as the optimum concentration in our experiments.

3.2. *Analytical performance*

Under the conditions optimized for the determination of H_2O_2, the analytical characteristics of the proposed method were evaluated by examining linear range, precision and detection limit. The calibration curves for H_2O_2 were linear from 0 to 500 nmol L^{-1} in the high-sensitivity region of the detector, and from 50×10^1 to 50×10^3 nmol L^{-1} in the low-sensitivity region of the detector. The regression equations and correlation coefficients were listed in Table 1. The detection limit

Table 1 – Analytical performance of the proposed method

Range of H_2O_2 concentration (mol L^{-1})	Regression equation	Correlation coefficient	*n*
$(0–5) \times 10^{-7}$	$y = 1.446 + 0.218c$[a]	0.9995	5
5×10^{-7} to 5×10^{-5}	$y = 18.50 + 0.219c$[b]	0.9999	7

y represents the relative fluorescence intensity.
[a] *c*: concentration in nmol L^{-1}.
[b] *c*: concentration in μmol L^{-1}.

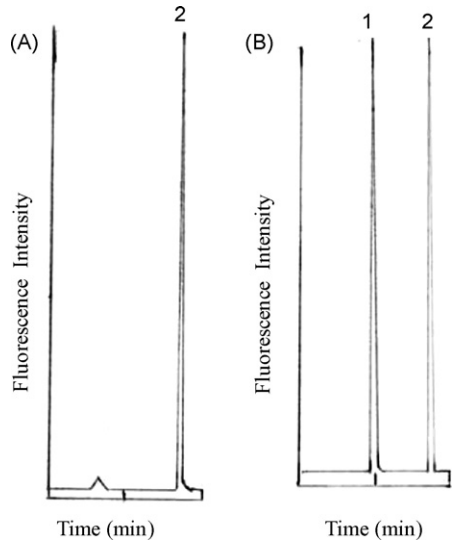

Fig. 6 – HPLC chromatograms for the determination of H_2O_2 in seawater sample using Fenton reaction: (A) before Fenton reaction and (B) after Fenton reaction. Peak no. 1: phenol and peak no. 2: benzene.

for H_2O_2, defined as three times the standard deviation of measured lowest standard solution (5 nmol L^{-1} H_2O_2) in seawater is 4 nmol L^{-1}. In order to evaluate the precision of the method, five injections of the standard solutions containing 500 and 50 nmol L^{-1} H_2O_2 were performed. The relative standard deviations of 1.9 and 2.4% were obtained, respectively. Typical HPLC chromatograms for the determination of H_2O_2 in seawater were shown in Fig. 6.

3.3. *Interference of NO_2^- ion on fluorescence intensity*

In order to assess the possible analytical applications of the above-described method, the interference of nitrite ion (NO_2^-) on fluorescence intensity of phenol was investigated. The result (Fig. 7) shows that the addition of 50 μmol L^{-1} NO_2^- ion to the seawater samples decreased the fluorescence intensity

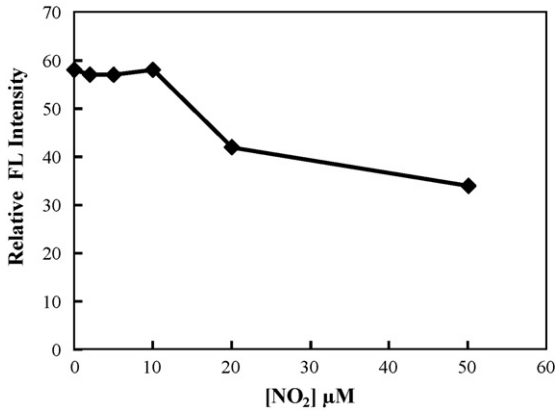

Fig. 7 – Effect of NO_2^- concentration on fluorescence intensity of phenol. Experimental conditions—[H_2O_2]: 300 nmol L^{-1}; pH 4.0; [Fe^{2+}]: 1.5 mmol L^{-1}; reaction time: 5 min.

Table 2 – Comparison of the results obtained by the proposed method and POHPAA-FIA method in H_2O_2-spiked seawater samples

Sample	H_2O_2 concentration (nmol L^{-1})[a]		R.D. (%)[b]
	Proposed method (A)	POHPAA-FIA (B)	
1	10.3	11.5	10.4
2	19.7	21.2	7.1
3	46.1	43.8	−5.3
4	82.4	85.7	3.9
5	105.1	100.3	−4.8
6	202.4	210.8	4.8
7	310.8	305.4	−0.9
8	340.3	350.7	1.8
9	459.4	450.8	−3.0
10	492.3	490.6	−0.6

[a] Mean value of H_2O_2 concentration.
[b] $\{(B - A)/B\} \times 100\%$.

by almost 40%. However, up to 10 μmol L^{-1} NO_2^- ion, there is no significant interference in the fluorescence intensity of phenol compared to samples without NO_2^- ion. Ardakani et al. reported that Fe^{2+} is converted to Fe^{3+} in the presence of NO_2^- ion [30]. Unlike Fe^{2+}–H_2O_2 reaction which generates $OH^\bullet$, Fe^{3+}–H_2O_2 does not give rise to $OH^\bullet$ and is therefore not expected to produce phenol:

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^\bullet + H^+ \quad (4)$$

Hence, the decrease in the fluorescence intensity may be due to the oxidation of Fe^{2+} to Fe^{3+} by NO_2^- ion. Amini et al. pointed out that the average concentration of NO_2^- in well, rain, river, snow and lake water samples are 113 nmol L^{-1}, 763 nmol L^{-1}, 1.65 μmol L^{-1}, 4.56 μmol L^{-1} and 1.53 μmol L^{-1}, respectively [31]. Fukushi et al., Burakham et al., and Ivanov et al. in their studies also reported that NO_2^- concentration in sea, rain and river water samples are 0.783–2.39, 1–4.5 and 0.7–1.17 μmol L^{-1}, respectively [32–34]. Therefore, it is evident that the proposed method can be applied to determine H_2O_2 in seawater and also other natural water samples containing less than 10 μmol L^{-1} NO_2^- ion.

3.4. *Fenton-HPLC and POHPAA-FIA intercomparison*

The method presented in this study was compared to POHPAA-FIA method for the determination of H_2O_2 in seawater. H_2O_2-spiked seawater samples were analyzed by the proposed method and POHPAA-FIA method simultaneously. The analytical procedure employed for the determination of H_2O_2 by the POHPAA method has been described elsewhere [11]. The POHPAA method is based on the enzyme-mediated reaction between H_2O_2 and (*p*-hydroxyphenyl) acetic acid to form a fluorescent POHPAA dimer [11]. This independent method is chosen because, in addition to its common use, it has been shown in two intercomparison studies to produce accurate results [9,35]. The result (Table 2) shows that the concentrations of H_2O_2 determined by the two methods are remarkably comparable. This good agreement indicates the successful applicability of the proposed method for the determination of H_2O_2 in seawater.

Table 3 – Concentration ranges of H_2O_2 measured in Seto Inland Sea

Sampling date	H_2O_2 (nmol L^{-1})	Depth (m)	Analytical method	Reference
May 2007	143–348	0–10	Fenton-HPLC	This study
May 2002	85–297	0–20	POHPAA-FIA	[36]
May 2001	79–183	0–20	POHPAA-FIA	[36]
June 1998	187–448	0–20	POHPAA-FIA	[36]
May 1997	97–496	0–20	POHPAA-FIA	[36]
May 1996	90–257	0–20	POHPAA-FIA	[36]
May 1996	90–257	0–20	POHPAA-FIA	[36]
August 1991	<400	0–20	POHPAA-FIA	[11]

3.5. *On-board analysis*

The method was employed for on-board analysis of H_2O_2 in Seto Inland Sea. The result (Table 3) shows that the concentration of H_2O_2 in the water depth of 0–10 m was 143–348 nmol L^{-1} ($n = 30$) which is in agreement with previous studies in similar regions [11,36].

4. Conclusions

In the present paper, we have demonstrated the applicability of Fenton reaction for the quantitative determination of nanomolar level of H_2O_2 in seawater. The proposed method is simple, sensitive, selective and convenient for the analysis of H_2O_2 in seawater samples. The result obtained by the determination of H_2O_2 in seawater samples spiked with H_2O_2 standard solutions is consistent with the well-accepted POHPAA method. Although, POHPAA method is highly sensitive and selective but it suffers the disadvantage of reagent instability and high cost. However, our proposed method uses inexpensive, stable and easily available chemical reagents that do not need refrigeration. Due to the wide dynamic linear range, relatively free from interference and low detection limit; the proposed method can be applied for the measurement of H_2O_2 in other natural water samples.

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