Determination of Formaldehyde in Seawater Samples by High-performance Liquid Chromatography with UV Detection using an Acety lacetone Derivatization

Kenji YOSHIKAWA *, Tatsuya MORITA *, Taichi ISHIKAWA * and Akio SAKURAGAWA *

Abstract

High-performance liquid chromatography with UV detection using an acety laceton derivatization was developed in order to determine the presence of formaldehyde (HCHO) in seawater. Several operating and derivatization conditions were examined to analyze the low concentration of HCHO in seawater containing some coexisting species. When 40 (v/v)% methanol was used in the mobile phase, HCHO derivative was detected at nearly 5.4 min. The calibration curve obtained from the peak height for the HCHO derivative was linear, with a good correlation coefficient of 0.99. The relative standard deviation (n = 10) of the peak height was 3.4%. The detection and quantitation limits were 3 µg/L and 11 µg/L, respectively. Although inorganic species have little effect on this method, organic species show an opposite tendency. Using a 2.5 fold concentration of the derivatization reagents in the derivatization reaction, the interference due to the organic species was resolved. This developed method was applied to the determination of HCHO in seawater samples.

Key Words: Formaldehyde, High-performance liquid chromatography, UV detection, Acety lacetone derivatization, Seawater

1. Introduction

From the viewpoint of the influence on marine ecosystems, formaldehyde (HCHO) in seawater has received a lot of attention in recent years. For example, HCHO was used as a fungicide for young fish in aquafarms1. However, a concern about environmental risks in marine ecosystem is increasing whenever HCHO-containing effluents from aquafarms are released continuously into the ambient seawater without any dilution2,3. In fact, the ecosystem effect, such as mass deaths or feminization of male shellfish around the aquafarm, has been reported4. Because of these factors, one item to be monitored for the conservation of water creatures has been defined by the Ministry of the Environment. Formaldehyde in seawater is limited to 0.03 mg/L. Therefore, a correct qualitative or quantitative analysis of HCHO in seawater is very important from the viewpoint of marine ecosystem protection.

Formaldehyde is typically analyzed using gas chromatography (GC) including the mass detection technique5-8, high-performance liquid chromatography (HPLC)9-15, flow injection analysis (FIA)16, 17 and capillary electrophoresis (CE)18-21. For GC, the measurement of the nonvolatile species is difficult. In addition, FIA has to continue carrying away a reaction reagent during the measurement. Although CE has a powerful and flexible separation ability owing to its high resolution efficiency, the detection limit is unsatisfactory compared with the other method. In view of the analysis of the nonvolatile species, HPLC was used in this study.

Underivatized HCHO shows absorption in the ultraviolet region, but the molar absorbance coefficient of HCHO is small, and sensitive analysis is difficult. Therefore, it is necessary to derivatize HCHO18. The most widely used derivatization agent is 2,4-dinitrophenylhydrazine (DNPH), which reacts with the carbonyl group of the carbonyls to form hydrazones that can be separated using GC or HPLC9,11-15. Derivatization of carbonyls with o-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBOA)5, 7, dansylhydrazine (DNSH)20 and acety lacetone (AA) have also been reported10, 22-25.

At first, the authors tried to analyze the HCHO in seawater by means of the HPLC with DNPH derivatization method. This method is also applied to the determination of HCHO in the atmosphere, but tends to be affected by moisture. Actually, we applied it to the analysis of HCHO in seawater samples, but the stability of the HCHO derivative in seawater was poor. Similarly, analysis of the HCHO with PFBOA and ethyl 3-oxobutanoate20 derivatization method was also examined. Although the baseline stability was favorable, the analytical results showed a tendency to be affected by the coexisting species in seawater.

This study demonstrates the determination of HCHO

* Department of Materials and Applied Chemistry, College of Science and Technology, Nihon University, 1-8-14, Kanda-Surugadai, Chiyoda-ku, Tokyo 101-8308 Japan
in seawater by HPLC with UV detection using an acetylacetone derivatization method. First, optimal operating and derivatization conditions of HCHO (mobile phase, pH of the reaction and the amount of derivatization reagent etc.) were examined. Then, we confirmed the analytical precision of HCHO, and the effect of coexisting species in seawater. Finally, the developed method was applied to determining the presence of HCHO in two seawater samples.

2. Experimental

2.1 Apparatus

The equipment consists of a DG-1580-53 three line degasser, a PU-1580 intelligent HPLC pump, a CO-1565 intelligent column oven and an MD-1515 Multi wavelength detector (JASCO Corporation, Tokyo, Japan). Model 7725 (Rheodyne, Cotati, CA, USA) with a 100 μL sample loop was used as a manual injection valve. All separations with the HPLC system were performed using a Mightysil RP-18 GP (4.6 mm i.d. × 150 mm, Kanto Chemical, Tokyo, Japan). ChromNAV (JASCO Corporation, Tokyo, Japan) was used for data acquisition and data handling.

2.2 Reagents

Acetonitrile and methanol (Wako Pure Chemical, Osaka, Japan) were used in the mobile phase. As a derivatization reagent, acetylacetone and ammonium acetate were purchased from Wako Pure Chemical. Formaldehyde standard solution (1000 mg/L in methanol, for chemical analysis) was obtained from Kanto Chemical (Tokyo, Japan). Each standard solution was prepared by diluting this solution. Acetic acid (Wako Pure Chemical) was used as a pH adjuster. Except for a HCHO standard solution, chemicals used in this study were all of analytical grade purity. Water was purified with a Milli-Q purification system (Millipore, Bedford, MA, USA) with a specific resistance of 18.2 MΩ.

2.3 Derivatization method of HCHO

The reaction scheme according to the acetylacetone derivatization is shown in Fig.1. A derivatization reagent was prepared as follows: 5.0 (w/v)% of ammonium acetate and 0.2 (v/v)% of acetylacetone were added to less than 100 mL of water into a beaker. This solution pH was adjusted to 5.7 with 0.3 mL of the straight acetic acid. Resultant solution was prepared by use of a 100 mL of measuring flask. In a stopped test tube, 3.0 mL of derivatization reagent and 3.0 mL of either a standard solution or a seawater sample were mixed together. After stirring, it was heated to 40°C in a water bath for 30 min. Then, this solution was cooled to room temperature.

2.4 Operating conditions

In the mobile phase, 20 (v/v)% acetonitrile or 40 (v/v)% methanol was used. Each flow rate of the mobile phase was 1.0 mL/min. All separations were performed using a Mightysil RP-18 GP (4.6 mm i.d. × 150 mm). The column temperature was maintained at 30°C. The injection volume of each sample solution was 100 μL. Detection was carried out with a UV detector. The signal wavelength was set at 413 nm.

3. Results and discussion

3.1 Effect of the mobile phase on separation behavior

In the reversed-phase liquid chromatography, the organic solvent in the mobile phase has a profound effect on the separation behavior. At first, acetonitrile or methanol was used in the mobile phase and examined the most suitable condition for each. When the organic solvent concentration in the
The mobile phase is higher, the polarity is lower. Therefore, the retention time of the HCHO derivative decreased with an increase in the organic solvent concentration. The optimum mobile phase concentration was 20\(^{\text{v/v}}\)% acetonitrile or 40\(^{\text{v/v}}\)% methanol.

A typical chromatogram of the HCHO derivative under the optimum operating conditions is shown in Fig.2. Although the resolution of the residual derivatization reagent and the HCHO derivative was favorable, 40\(^{\text{v/v}}\)% methanol was selected in the optimal mobile phase on the basis of the detection sensitivity and the retention time.

### 3.2 Examination of the derivatization reagent

#### 3.2.1 Effect of the amount of ammonium acetate in the derivatization reagent

The effect of the amount of ammonium acetate in the derivatization reagent was examined within the range from 1.0 to 25.0\(^{\text{w/v}}\)%, with the amount of acetylacetone fixed at 0.2\(^{\text{v/v}}\)%.

The relationship between the amount of ammonium acetate and the peak height of the HCHO derivative is shown in Fig.3. Within the range from 1.0 to 3.0\(^{\text{w/v}}\)% of ammonium acetate, the peak height of the HCHO derivative increased. When the amount of ammonium acetate was greater than or equal to 3.0\(^{\text{w/v}}\)% of the derivatization reagent, there was little change in the peak height. The optimum amount of ammonium acetate in the derivatization reagent was determined to be 5.0\(^{\text{w/v}}\)%.

#### 3.2.2 Effect of the amount of acetylacetone in the derivatization reagent

Similarly, the effect of the amount of acetylacetone in the derivatization reagent was examined within the range from 0.05 to 0.8\(^{\text{v/v}}\)% of acetylacetone, fixed at 5.0\(^{\text{w/v}}\)% of ammonium acetate. The relationship between the amount of acetylacetone and the peak height of the HCHO derivative is shown in Fig.4. Within the range from 0.05 to 0.1\(^{\text{v/v}}\)% of acetylacetone, the peak height of the HCHO derivative increased. When the amount of acetylacetone was greater than or equal to 0.1\(^{\text{v/v}}\)% of the derivatization reagent, there was little change in the peak height. The optimum amount of acetylacetone in the derivatization reagent was determined to be 0.2\(^{\text{v/v}}\)%.

### 3.3 Examination of the derivatization reaction

#### 3.3.1 Effect of pH in the derivatization reaction

In seawater, there may be HCHO as a polyacetal\(^{26}\). The polyacetal does not cause a derivatization reaction and generate the HCHO derivative. An acidic condition is necessary to inhibit the generation of the polyacetal, but it is not appropriate for a derivatization reaction. Therefore, the pH

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**Fig.2** Chromatogram of the HCHO derivative

<table>
<thead>
<tr>
<th>Analytes:</th>
<th>1, Residual derivatization reagent; 2, HCHO derivative (0.5 mg/L).</th>
</tr>
</thead>
</table>
| Operating conditions: Mobile phase, | a) 20\(^{\text{v/v}}\)% CH\(_3\)CN, b) 40\(^{\text{v/v}}\)% CH\(_3\)OH; Flow rate of the mobile phase, 1.0 mL/min; Injection volume, 100 \(\mu\)L; Separation column, Mightysil RP-18 GP (4.6 mm i.d. \(\times\) 150 mm); Column temperature, 30 °C. Detection, diode-array detection; Detection wavelength, 413 nm.

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**Fig.3** Effect of the ammonium acetate amount in the derivatization reagent

- HCHO derivative (0.5 mg/L). Derivatization reagent: Ammonium acetate, 1-25\(^{\text{w/v}}\)%; Acetylacetone, 0.2\(^{\text{v/v}}\)%.
- Derivatization reaction: Reaction pH, 5.7; Reaction temperature, 40 °C; Reaction time, 30 min.

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**Fig.4** Effect of the amount of acetylacetone in the derivatization reagent

- Derivatization reagent: Ammonium acetate, 5.0\(^{\text{w/v}}\)%; Acetylacetone, 0.2\(^{\text{v/v}}\)%.
- Derivatization reaction: Reaction pH, 5.7; Reaction temperature, 40 °C; Reaction time, 30 min.
The effect of the pH on the derivatization reaction was examined within the range from 3.0 to 6.7, with the amount of ammonium acetate and acetylacetone fixed at 5.0 \(\text{(w/v)}\%\) and 0.2 \(\text{(v/v)}\%\), respectively. The relationship between the pH of the derivatization reaction and the peak height of the HCHO derivative is shown in Fig. 5BCMF. The pH of the derivatization reagent that does not contain acetic acid is 6.7. As shown in Table 1, the peak height was greatest in pH 6.7, but the relative standard deviation of the peak height was the highest, too. As a result, the optimum pH of the derivatization reaction was determined to be 5.7, based on the detection sensitivity and the peak height reproducibility of the HCHO derivative.

Generally, an acetylacetone derivatization reaction is promoted by warming more than 60°C. But volatilization loss and destabilization of the HCHO derivative may occur at a higher temperature. Therefore, the effect of the reaction temperature and required time on the derivatization reaction were examined within the range from 20 to 50°C and the range from 10 and 50 min, respectively. The result is shown in Fig. 5. As shown in Fig. 5, the peak height of the HCHO derivative increased with an increase in the reaction temperature and the period of the derivatization reaction. However, the peak height of the HCHO derivative decreased when the temperature and required period of the derivatization reaction were 50°C and 50 min, respectively. Generally, the major factor is thought to be the volatilization loss of HCHO and acetylacetone. In addition, it was possible for the stability of the HCHO derivative to decrease. The optimum temperature and required time for the derivatization reaction were determined to be 40°C and 30 min, respectively.

### 3.4 Analytical precision of the HCHO derivative

The linearity of the calibration curve, as well as the reproducibility and detection limit of the HCHO derivative were obtained. The calibration curve obtained from the peak height was plotted with three replicates for each level of concentration \(0.1-0.5 \text{mg/L}\). It was linear, and the correlation coefficient was 0.9976. The relative standard deviation (R.S.D.) of peak height with ten replicates for 0.3 mg/L was 3.4\%. The detection and quantitation limits were calculated using a standard deviation of the blank solution and the slope of the calibration curve. The detection and quantitation limits of the HCHO derivative were 3 and 11 \(\mu\text{g/L}\), respectively.
3.5 Study of degradation of HCHO and its derivative during storage

3.5.1 Study of degradation of HCHO derivative during storage

The HCHO derivative is volatile and photodegradable. Therefore, it is necessary to confirm the degradation of the HCHO derivative and examine the preservation method for the sample solution. The HCHO derivative was left for a certain time in the stopped test tube. And then we confirmed the effect of standing time on the HCHO derivative. The relationship between the standing time and the peak height of the HCHO derivative is shown in Fig. 6. Although the data in the range of 2-10 h are not shown in Fig.6, the peak height of the HCHO derivative was approximately constant until 10 h. But the peak height of the HCHO derivative decreased with an increase in the standing time. To prevent the degradation of the HCHO derivative, the preservation method was examined with a light resistant, refrigerated and combined method. The decrease in the peak height relaxed, but did not prevent a decrease in the peak height. Consequently, the stability of the HCHO derivative is low. Therefore, it is necessary to examine an effective preservation method.

3.5.2 Study of degradation of underivatized HCHO during storage

As previously described, the stability of the HCHO derivative is low. Therefore, the stability of the underivatized HCHO was confirmed. The stock solutions were preserved in a light resistant container under room temperature and refrigerated conditions. The result is shown in Table 2. In the former case, the peak height of the underivatized HCHO decreased with an increase in the standing time. The cause is thought to be related to the volatilization and photodecomposition of the HCHO. In contrast, the peak height would show no significant change in the latter case. Without the derivatization, it is preferable that the HCHO solution be preserved in a light resistant container under refrigerated conditions.

3.6 Effect of the coexisting species in seawater sample

3.6.1 Effect of the inorganic species

With the use of artificial sea water instead of the ultra pure water for the dilution of the sample solution, the effect of inorganic species in seawater was confirmed. There is really not much difference between the two slopes of the calibration curves [HCHO standard solution: \( y = (5.68 \times 10^4)x \), HCHO solution diluted with artificial seawater: \( y = (5.68 \times 10^4)x \)]. Moreover, the detection sensitivity was not largely affected. Therefore, the inorganic species contained in seawater, such as a cation and anion, have little effect on the analysis of the HCHO. Moreover, to confirm the reliability of the method, a known concentration of the HCHO is added to the sea water (Zushi coast, Oct. 15, 2009), and the slope of the calibration curve was compared. There is a difference compared to the slopes of the calibration curves of the previous solution [HCHO solution diluted with the real seawater: \( y = (5.42 \times 10^4)x \)].

3.6.2 Effect of the organic species

Under the optimized derivative conditions described in section 2.3, favorable analytical results were not obtained. Because the inorganic species has little effect as previously
discussed, organic species contained in seawater affect the analysis of the HCHO\(^2\). The examples of the organic species contained in seawater include protein, fatty acid, and so on. Derivatization reaction was interfered with because these organic species reacted with the derivatization reagent preferentially. In this study, we tried to reduce the interference of the derivatization reaction using excess derivatization reagents. The peak height of the HCHO derivative diluted by ultra pure water or real seawater was confirmed. The result is shown in Table 3. Using 12.5 (w/v)% ammonium acetate and 0.5 (v/v)% acetylacetone as the derivatization reagents in the derivatization reaction, both peak heights showed nearly equal value. Therefore, the interference of the derivatization reaction by the organic species was relaxed.

### 3.7 Application to seawater samples

Under the above mentioned derivatization condition, the proposed method was applied to analyze the HCHO in seawater again. The slope of the calibration curve of HCHO diluted by real seawater samples basically corresponded [Zushi coast: \( y = (5.71 \times 10^{-7})x \), Lake Hamana: \( y = (5.72 \times 10^{-7})x \)]. Formaldehyde was not detected in the seawater of Zushi coast and Lake Hamana. In addition, to confirm the reliability of the method, a known concentration of HCHO shown in Table 4 is added to the seawater sample. Because the recovery showed nearly 100%, this method can be applied to the determination of HCHO in seawater, while avoiding the matrix effect.

### Table 3 Effect of the organic species in seawater

<table>
<thead>
<tr>
<th>Additive amount of the derivatization reagent</th>
<th>Peak height of the 0.5 mg/L HCHO [mAU]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium acetate (w/v)%</td>
<td>Acetylacetone (v/v)%</td>
</tr>
<tr>
<td>5.0 *1</td>
<td>0.2 *1</td>
</tr>
<tr>
<td>7.5</td>
<td>0.3</td>
</tr>
<tr>
<td>10.0</td>
<td>0.4</td>
</tr>
<tr>
<td>12.5</td>
<td>0.5</td>
</tr>
<tr>
<td>15.0</td>
<td>0.6</td>
</tr>
</tbody>
</table>

* 1 Additive amount of the derivatization reagent described in section 2.3.
* 2 Seawater of the Zushi coast (Oct. 15. 2009)

### Analytical results for HCHO in seawater

<table>
<thead>
<tr>
<th>Sample (Oct. 2009)</th>
<th>Found [mg/L]</th>
<th>Added [mg/L]</th>
<th>Found [mg/L]</th>
<th>Recovery [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zushi coast</td>
<td>0.10</td>
<td>0.11</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>Lake Hamana</td>
<td>0.30</td>
<td>0.30</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>0.50</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.10</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>0.29</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>0.51</td>
<td>102</td>
<td></td>
</tr>
</tbody>
</table>

n.d.: not detected

### Table 4 Analytical results for HCHO in seawater

### Conclusion

High-performance liquid chromatography using UV detection with acetylacetone derivatization was developed for the determination of HCHO in seawater samples. When 40 (v/v)% methanol was used in a mobile phase, HCHO derivative was detected for nearly 5.4 min. The linearity of the calibration curve, reproducibility and detection limit of the HCHO derivative are satisfactory. It has been proven that the underivatized HCHO standard solution was preserved in a light resistant container under refrigerated conditions. Although the inorganic species have little effect on this method, organic species show an opposite tendency. Using 12.5 (w/v)% ammonium acetate and 0.5 (v/v)% acetylacetone as the derivatization reagents in the derivatization reaction, the inhibition due to the organic species was resolved. This method can be applied to the determination of presence of HCHO in seawater samples.

### References


アセチルアセトン誘導体化法を用いた高速液体クロマトグラフィーによる海水中的ホルムアルデヒドの定量

吉川 賢治*, 森田 達弥*, 石川 大地*, 櫻川 昭雄*

要 旨
紫外吸収検出を組み合わせた高速液体クロマトグラフィーにより、海水中のホルムアルデヒド（HCHO）の定量を行った。多くの共存成分を含む海水中の低濃度 HCHO の分析を行うために、いくつかの分析条件及び誘導体化条件の検討を行った。移動相に 40 (v/v)% メタノールを用いることで HCHO 誘導体は約 5.4 分で検出された。ピーク高さから得られた検量線の相関係数は 0.99 以上、10 回繰り返し測定によるピーク高さの相対標準偏差は 3.4% と良好な結果を示した。また、検出限界は 3.0 μg/L、定量限界は 11.0 μg/L であった。本法にて、無機成分の影響は殆どないが、有機成分に関しては逆の傾向を示した。そのため、誘導体化反応において 2.5 倍濃度の誘導体化試薬を用いたところ、共存有機物による誘導体化反応の阻害は緩和された。以上より得られた本法を幾つかの海水中の HCHO の分析に適用した。

キーワード：ホルムアルデヒド、高速液体クロマトグラフィー、アセチルアセトン誘導体化法、UV 検出法、海水

* 日本大学理工学部物質応用化学科（〒101-8308 東京都千代田区神田駿河台1-8-14）