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Rapid and Highly Sensitive Determination of Low-Molecular-Weight Carbonyl Compounds in Drinking Water and Natural Water by Preconcentration HPLC with 2,4-Dinitrophenylhydrazine

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The aim of this research was to develop a simple procedure for a highly sensitive determination of low-molecular-weight (LMW) carbonyl compounds in drinking water and natural water. We employed a preconcentration HPLC system with 2,4-dinitrophenylhydrazine (DNPH) for the determination of LMW carbonyl compounds. A C-18 reverse-phase preconcentration column was used instead of a sample loop at the sample injection valve. A 0.1 – 5.0 mL portion of the derivatized sample solution was injected with a gas-tight syringe, and a 15% acetonitrile aqueous solution was pushed through the preconcentration column to remove the unreacted excess DNPH, which caused serious interference in the determination of formaldehyde. The detection limits were 1 – 3 nM with a relative standard deviation of 2 – 5% for 20 nM standard solutions ($n = 5$). The calibration curves were essentially unaffected by coexisting sea salts. Applications to commercial mineral water, tap water, river water, pond water and seawater are presented.

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Introduction

Low-molecular-weight (LMW) carbonyl compounds (*i.e.* aldehydes (RCOH) and ketones (RCOR′)) in the troposphere are mainly formed by photochemical oxidation process of anthropogenic and biogenic hydrocarbon.[1-3] They are also emitted directly by the combustion of fossil fuels and biomass by motor vehicles and industrial processes.[1] LMW carbonyl compounds in the atmosphere are easily dissolved in rain, cloud water and dew.[2,4] They are active participants in atmospheric chemical reactions in both gas and liquid phases, and they are involved in the generation of acids in atmospheric water.

LMW carbonyl compounds are deposited on the ground and the sea surface as rain. For surface water, atmospheric deposition is one of the most important sources of formaldehyde, since the concentrations of formaldehyde in rain are higher than those in surface water such as seawater and river water, by three orders of magnitude, or more. LMW carbonyl compounds are exchanged directly between the surface water phase and the atmosphere.[3,4] In natural water, LMW carbonyl compounds are produced by the photochemical degradation of dissolved organic matter (DOM).[6-8] The LMW carbonyl compounds in natural water are biologically labile, because they are taken up quickly by microorganisms.[6,7]

Aldehyde formation in the ozonization treatment for drinking water has been reported. The migration of carbonyl compounds from polyethylene terephthalate (PET) bottles into commercial bottled mineral water has also been investigated.[9] In Japan, the concentration of formaldehyde in tap water is regulated at less than 80 μg/L (= 2.7 μM), and is monitored regularly.

Some analytical methods have been reported for LMW carbonyl compounds in water. The 2,4-dinitrophenylhydrazine (DNPH) derivatization method, in which aldehyde-DNPH derivatives are subsequently separated by high performance liquid chromatography (HPLC) and detected by ultraviolet absorption, has been applied to rain and dew samples.[3,4] A solid phase extraction (SPE) technique has been used in attempts to lower the detection limits for aqueous samples. Kieber and Mopper have reported on the determination of picomolar concentrations of carbonyl compounds in seawater by using a DNPH derivatization/C-18 SPE cartridge with large-volume injection and large-bore column HPLC.[6-8] A gas chromatography (GC) method based on the DNPH derivatization was also reported, although DNPH derivatives have low volatility and poor thermal stability.[10] HPLC-fluorescence using dansylacetamidooxyamine is also a sensitive method for the determination of carbonyl compounds.[11]

Gas chromatography/mass spectrometry (GC/MS) coupled with *o*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBOA) derivatization is one of the most sensitive methods for determining aldehydes in water.[9,12-16] The concentrations of 4 aldehydes in tap water and bottled mineral water, determined by using head space-GC/MS with PFBOA derivatization, have been reported.[13] In Japan, a method based on GC/MS with PFBOA derivatization is the recommended method for monitoring aldehydes in tap water. In PFBOA-GC/MS, however, samples are treated with PFBOA in a sealed vial for 2 h at room temperature, or for 1 h at 60°C, due to the low reactivity of PFBOA with aldehydes.

In this paper, we present a simple, rapid and highly sensitive method for the determination of LMW carbonyl compounds, such as formaldehyde, acetaldehyde, propionaldehyde and

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glyoxal in natural waters, based on DNPH derivatization and HPLC. The method presented here is unique in being a highly sensitive determination using a simplified preconcentration and analytical procedure without cartridge extraction or solvent extraction. The simplified procedure employed in this study results in lower detection limits and better precision by reducing the chance of contaminations of the samples and reagents.

Experimental

Reagents

Acetonitrile, methanol and tetrahydrofuran (THF), used as mobile phases for HPLC, were of HPLC grade and were used without further purification. Deionized water for the HPLC mobile phases was obtained from a Milli-Q Plus water purification system, to which predeionized water treated with Amberlite EG-5 ion-exchange resin (Organo Co.) was supplied. DNPH was recrystallized from acetonitrile and stored in the dark in air-tight vials. The derivatization reagent was prepared in a 25 mL glass vial by dissolving 20 mg of recrystallized DNPH in 15 mL of a solution containing conc. HCl, acetonitrile and water (2 + 1 + 5). The DNPH derivatization reagent was purified by a method described by Kieber and Mopper.[8]

A stock solution of formaldehyde (10 mM) was made by diluting paraformaldehyde in commercial HPLC-grade distilled water purchased from Sigma-Aldrich Japan Co. Ltd. Stock solutions of acetaldehyde, propionaldehyde and glyoxal (10 mM) were also prepared in commercial HPLC-grade distilled water. These stock solutions were kept at 4˚C in the dark, and were stable for several weeks. Diluted and mixed serial standard solutions were prepared in commercial HPLC-grade distilled water just prior to use, and were derivatized with DNPH.

Commercial synthetic compounds of DNPH derivatives of formaldehyde, acetaldehyde, acetone, and propionaldehyde purchased from Sigma-Aldrich Japan (Tokyo, Japan) were used to determine the derivatization efficiency.

Sample collection

River water samples were collected from the Ohta River on May 25, 2005 and June 14, 2005. Ohta River is one of the biggest rivers in the Hiroshima Prefecture of Japan, and flows through Hiroshima City. Pond water samples were collected from Budo Ike located in the Higashi-Hiroshima Campus of Hiroshima University on August 3, 2005. Seawater samples were collected at a depth of 25 m in the center of Hiroshima Bay (34˚11.0′ N, 132˚21.0′ E, depth of water: 33 m) on October 1, 2005 by R/V Toyoshio Maru, which belongs to Hiroshima University. Various brands of bottled mineral water were purchased from local stores. Tap water was collected in our laboratory in Hiroshima University located in Higashi-Hiroshima City, Japan.

Derivatization procedure

The samples were not filtered, since filtration often causes carbonyl compound contamination; in particular, filtered samples were found to be highly contaminated with formaldehyde and acetone. Samples stored in laboratories have also been found to become contaminated. To avoid contamination, a natural water sample was derivatized within 10 min after sampling on the sampling site. A 10 mL water sample was immediately transferred to 12 mL glass vials with 0.5 mL of DNPH derivatization reagent, and was sealed tightly until HPLC analysis. The vial was capped, shaken briefly and allowed to stand for at least 1 h at room temperature (20 – 30˚C). The derivatized samples were transferred to the laboratory and were analyzed within 8 h by using a HPLC system (mentioned in the next section). Seawater samples were analyzed on a research vessel, Toyoshio Maru.

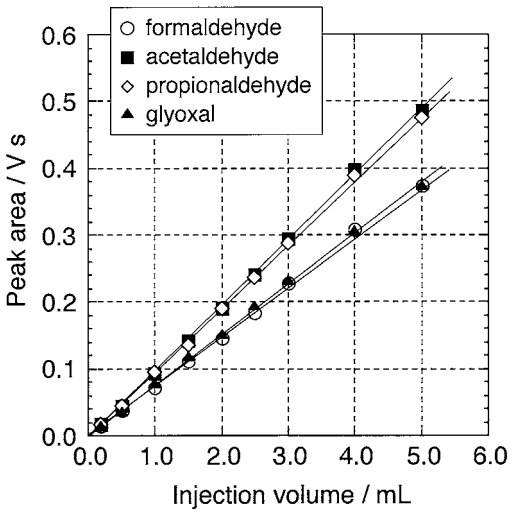

Fig. 1 Relationship between the injection volumes of derivatized 100 nM standard solutions and peak heights of formaldehyde, acetaldehyde, propionaldehyde and glyoxal.

Standard solutions were also derivatized by the same procedure with water samples. The derivatization efficiency was evaluated by comparisons with commercial synthetic DNPH derivatives of carbonyl compounds. The derivatization efficiencies at 100 nM were 0.97 ± 0.02 for formaldehyde, 0.87 ± 0.01 for acetaldehyde and 0.88 ± 0.01 for propionaldehyde, respectively. The reaction time of DNPH derivatization, in the range from 1 to 8 h, had no significant effect on the derivatization efficiency at room temperature, although the derivatization efficiencies at 30 min were 0.5 – 0.8. However, the derivatization efficiency for acetone was lower, 0.40 ± 0.01.

HPLC system

The HPLC system used was a Jasco LC-1500 gradient HPLC system equipped with a Jasco UV-1575 UV/Vis absorption detector and a C-18 reverse-phase column (Kanto Kagaku, RP-18GP 5 μm 4.6 mm i.d. × 150 mm length) for separation. The UV/Vis absorption detector was operated at 365 nm. Two mobile phases were used: (A) a Milli-Q water, THF and methanol mixture (Milli-Q water:THF:methanol = 7:2:1) and (B) 90% acetonitrile in Milli-Q water. The gradient program was as follows: isocratic at 90% A for 6 min, 90% A to 60% A in 24 min, 60% A to 0% A in 10 min and isocratic at 100% B for 10 min (total: 50 min/sample). The flow rate of the mobile phase was 1.0 mL min^{-1} and the analytical column was kept at 40˚C. A C-18 reverse-phase preconcentration column (Kanto Kagaku, RP-18GP 5 μm 4.6 mm i.d. × 5 mm length) was installed instead of a sample loop at the sample injection valve. A 0.1 – 5.0 mL portion of the derivatized sample solution was injected with a gas-tight syringe *via* a sample injection valve.

Results and Discussion

Sample injection volume and removal of unreacted excess DNPH

The peak areas for each carbonyl compound increased linearly

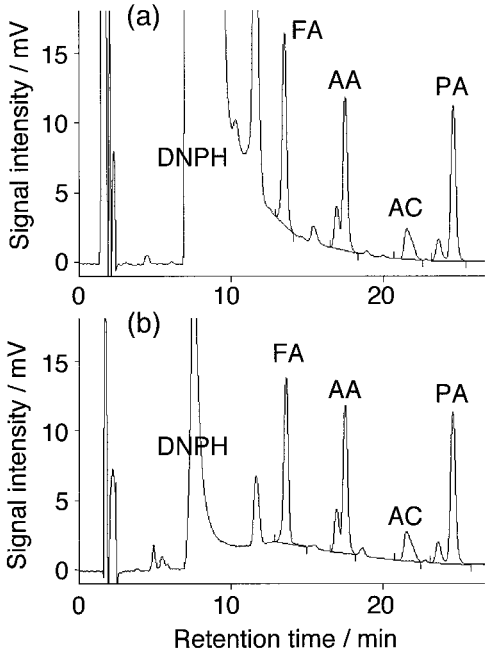

Fig. 2 (a) HPLC chromatogram of 2.5 mL injection of derivatized 100 nM standard solution of carbonyl compounds, and (b) chromatogram of 2.5 mL injection of derivatized standard solution with 2.5 mL of 15% acetonitrile aqueous solution after injection of derivatized standard solution. Peaks: DNPH, unreacted excess DNPH; FA, formaldehyde; AA, acetaldehyde; AC, acetone; PA, propionaldehyde.

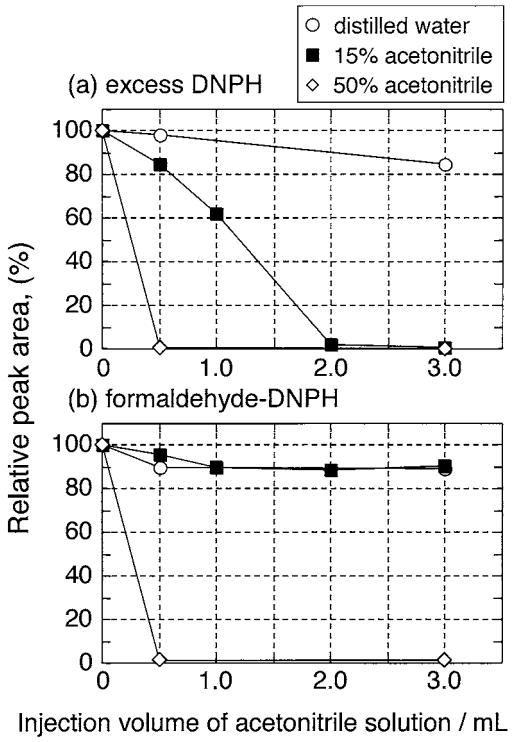

Fig. 3 Dependence of the relative peak areas of unreacted excess DNPH and formaldehyde-DNPH derivative on the injection volume of an aqueous acetonitrile solution that was pushed through a preconcentration column after the injection of a derivatized standard solution. The concentrations of carbonyl compounds were 100 nM and the injection volume of derivatized standard solution was 2.5 mL.

with an increase in the injection volume of the sample up to 5.0 mL, as shown in Fig. 1. A typical chromatogram for carbonyl compounds at 100 nM is presented in Fig. 2(a). An unacceptably large peak of unreacted excess DNPH was present. The unreacted excess DNPH peak overlapped with the peak of the formaldehyde-DNPH derivative and caused a serious interference in the determination of formaldehyde. To remove the unreacted excess DNPH peak, an aqueous acetonitrile solution or distilled water was pushed through the preconcentration column by a gas-tight syringe *via* the sample injection valve after injection of the derivatized sample solution. The dependences of the relative peak areas of excess DNPH and the formaldehyde-DNPH derivative on the injection volume of aqueous acetonitrile, which was injected after injection of the derivatized sample, is shown in Fig. 3. The injection of 3 mL of distilled water did not remove the excess DNPH from the preconcentration column. On the other hand, a 50% acetonitrile solution completely washed both the excess DNPH and the formaldehyde-DNPH derivative off the preconcentration column. The excess DNPH peak area was decreased by 2 mL of an aqueous 15% acetonitrile solution, and the formaldehyde-DNPH derivative peak intensity was essentially unchanged up to 3 mL of 15% acetonitrile. A typical chromatogram of a 2.5 mL injection of derivatized standard solution, where 2.5 mL of an aqueous 15% acetonitrile solution was pushed through the preconcentration column after injection of the derivatized sample solution, is shown in Fig. 2(b). The injection of 2.5 mL of an aqueous 15% acetonitrile did not have a significant effect on the peaks of the carbonyl compounds-DNPH, although the excess DNPH peak area was decreased.

The small peaks in front of the main peaks of acetaldehyde and propionaldehyde in Fig. 2 are those of the respective isomers of 2,4-dinitrophenylhydrazone formed from DNPH and unsymmetrical carbonyl compounds.[17] The *E*-/*Z*-isomers of 2,4-dinitrophenylhydrazone often cause an analytical error to determine the concentration of unsymmetrical carbonyl compounds. In this study, the peak ratios of isomers for the sample and the standard were the same and unchanged. The peak areas of acetaldehyde-DNPH derivative and propionaldehyde-DNPH derivative were calculated by adding the peak areas of both isomers. A peak appearing in front of the formaldehyde-DNPH derivative is attributable to 2,4-dinitrophenylazide formed from DNPH and nitrite. Kieber and Seaton were reported in a determination of the subnanomolar concentration of nitrate by using 2,4-dinitrophenylazide.[18] The peak of 2,4-dinitrophenylazide was decreased by the injection of 2 mL of an aqueous 15% acetonitrile solution.

Blank water

In the trace analysis of carbonyl compounds, the blank signal is strongly affected by the blank water employed. Sugaya *et al.* selected commercial bottled water as blank water for the analysis of aldehydes in drinking water by head space-GC/MS coupled with PFBOA derivatization.[13] Kieber and Mopper obtained a reagent blank by immediate injection of the derivatization reagent and a deep seawater mixture without any derivatization time.[8] In this study, deionized water, homemade distilled water, commercial bottled drinking water and commercial HPLC-grade distilled water were tested for use as blank water. A blank equivalent concentration (BEC), which was defined by dividing the blank signal intensity (peak area in chromatogram for derivatized blank water) by the slope of the calibration curve, was employed for evaluating blank water.

The deionized water purified by Amberlite MB-2 ion-

Table 1 BECs of the commercial HPLC-grade distilled water and precisions of 20 nM standard solutions prepared in the commercial HPLC-grade distilled water

	Formaldehyde	Acetaldehyde	Propionaldehyde	Glyoxal
BEC[a]/nM	10	2.8	1.3	1.6
Precision at 20 nM (n = 5), %	2.6	2.4	2.0	5.4

a. BEC was defined by dividing the blank signal intensity by the slope of the calibration curve.

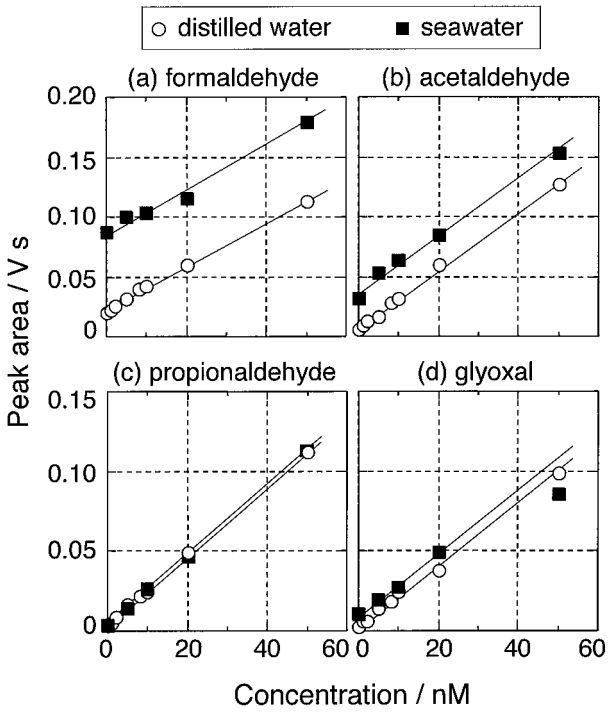

Fig. 4 Calibration curves of (a) formaldehyde, (b) acetaldehyde, (c) propionaldehyde and (d) glyoxal prepared in commercial HPLC-grade distilled water and seawater collected from the center of Hiroshima Bay.

exchange resin (Organo Co.) contained high acetaldehyde levels (~2000 nM). The anion exchange resin of Amberlite MB-2 has a dimethylethanol amine group as the ion-exchange group. This dimethylethanol amine group in the resin might be eliminated from the resin, and might change to ethanol. The resulting ethanol is easily oxidized to acetaldehyde. Thus, the acetaldehyde in the deionized water purified by Amberlite MB-2 might come from the anion exchange resin of Amberlite MB-2. Amberlite EG-5, which does not contained dimethylethanol amine as the ion-exchange group, is better than MB-2, although deionized water purified by Amberlite EG-5 still contained ~200 nM of acetaldehyde. Various brands of commercial bottled drinking water were tested as blank waters. The BECs of commercial bottled waters were 6 – 900 nM for formaldehyde, 3 – 4500 nM for acetaldehyde, and 1 – 7 nM for propionaldehyde and glyoxal. A European glass-bottled drinking water showed the lowest BECs in the various brands of water tested in this study. However, some unidentified peaks appeared in the chromatograms of the derivatized drinking water.

BECs of the commercial HPLC-grade distilled water purchased from Sigma-Aldrich Japan Co. Ltd. are summarized in Table 1. The BECs were small enough for use as a blank water, except for acetone (40 nM for acetone). The BECs were stable and the standard deviation of BECs was ~3%. The chromatograms were very clear, and there were no unidentified peaks. However, the water, which was unsealed and stored in the laboratory, was contaminated by formaldehyde and acetone. The source of the carbonyl compounds contained in the water may be the ambient air. Homemade distilled water contained formaldehyde due to the dissolving of formaldehyde and acetone from ambient air. In this study, freshly opened HPLC-grade commercial distilled water purchased from Sigma-Aldrich Japan Co. Ltd. was employed as the blank water, and standard solutions were prepared in commercial distilled water.

Calibration curves, detection limits and precision

In Fig. 4, calibration curves for 4 carbonyl compounds diluted with the commercial HPLC-grade distilled water and seawater collected in the center of Hiroshima Bay are shown. The calibration curves for formaldehyde, acetaldehyde and propionaldehyde are linear up to 1000 nM, although the linear range for glyoxal is 0 – 100 nM. The slopes of the calibration curves in seawater are the same as those in distilled water, indicating that the calibration curves are essentially unaffected by coexisting sea salts. The intercepts of calibration curves for formaldehyde and acetaldehyde in seawater are higher than those in the commercial HPLC-grade distilled water. The differences correspond to the concentration of formaldehyde and acetaldehyde contained in seawater.

Precisions of 20 nM standard solutions (the relative standard deviation for 20 nM standard solutions that were independently derivatized) are also listed in Table 1. The detection limits, defined as the equivalent concentration of three times the standard deviation on the five measurements of 20 nM standard solutions prepared in the commercial HPLC-grade distilled water, are 1 – 3 nM. The detection limits calculated from three times the standard deviation of the BECs are 0.5 – 1 nM.

Kieber and Mopper reported that the relative standard deviation at the 30 nM level for LMW carbonyl compounds was ~7% (n = 8) and the detection limits were approximately 0.5 – 5.0 nM (signal-to-noise ratio of 3) when using the DNPH derivatization and a C-18 SPE cartridge preconcentration method.[8] Detection limits obtained using head space GC/MS with PFBOA derivatization were 0.5 μg/L for formaldehyde (~20 nM), 0.5 μg/L for acetaldehyde (~10 nM) and 0.3 μg/L for propionaldehyde (~3 nM).[13] Our detection limits and precision were superior compared to the values reported, in addition to better precisions.

Application to the analysis of mineral water, tap water and natural water

Table 2 gives the concentrations of LMW carbonyl compounds in commercial mineral water, tap water and natural water. The concentrations in mineral water are N.D. – 534 nM for formaldehyde and N.D. – 1890 nM for acetaldehyde, respectively. The differences between the maximum and minimum concentrations of formaldehyde and acetaldehyde were three orders of magnitude or more. Acetaldehyde levels in mineral waters packed in glass bottles were below the detection limits

Table 2 Concentrations of LMW carbonyl compounds in commercial mineral water, tap water, river water, pond water and seawater

Source		Bottle material	Concentration/nM			
			Formaldehyde	Acetaldehyde	Propionaldehyde	Glyoxal
Bottled drinking water						
A	Japan	PET	534	1890	3	N.D.
B	Japan	PET	136	418	N.D.	N.D.
C	USA	PET	301	1030	1	67
D	France	PET	71	22	N.D.	N.D.
E	France	PET	N.D.	11	N.D.	N.D.
F	Italy	Glass	5	N.D.	2	8
G-1	UK	Glass	N.D.	N.D.	N.D.	N.D.
G-2	UK	PET	8	44	N.D.	N.D.
H-1	UK	Glass	3	N.D.	2	8
H-2	UK	PET	N.D.	69	N.D.	N.D.
Tap water	Japan		40	13	16	54
River water						
Ohta River	Upstream A		N.D.	2	N.D.	19
	Upstream B		9	7	N.D.	11
	Midstream C		79	12	N.D.	17
	Downstream D		173	9	N.D.	20
	Downstream E		175	12	N.D.	36
Pond water						
Budo Ike	Noontime (12:00, Aug. 3, 2005)		199	11	N.D.	32
	Midnight (02:00, Aug. 4, 2005)		41	7	N.D.	22
Seawater						
ST-2	Midnight (01:00, Oct. 1, 2005)		31	7	N.D.	10
	Noontime (11:00, Oct. 1, 2005)		98	9	N.D.	5

(1 nM). Formaldehyde levels in bottled water from Japan and USA were higher than those from European countries. Concentrations of formaldehyde in bottled water from the United Kingdom were essentially unaffected by the bottle material. Our results indicate that the acetaldehyde in commercial mineral water may be associated with PET bottles, but formaldehyde may be related to other factors, such as the source of water, the pretreatment method, the bottling process, time since the water was bottled, the storage conditions and how tight the screw cap was closed. Concentrations of LMW carbonyl compounds in tap water were similar to those reported by Sugaya *et al.*[12]

The concentrations of LMW carbonyl compounds in river water were higher at downstream, urban areas of Hiroshima City. In the pond water and seawater, the concentrations of LMW carbonyl compounds were higher at noontime than at midnight, suggesting the photochemical formation of LMW carbonyl compounds in natural water. Propionaldehyde was not detected in natural water, such as river water, pond water and seawater. The concentrations of glyoxal were the same or higher than those of acetaldehyde. LMW carbonyl compounds were not detected in well water.

Conclusions

The concentrations of LMW carbonyl compounds in natural water and drinking water were on the order of nano mole L^{-1} or less. In addition, the samples, reagents and blank waters were easily contaminated by LMW carbonyl compounds. Formaldehyde is a well-known pollutant in indoor air, and many household products, such as wallpaper, building materials and furniture, are considered to be major sources of aldehydes in indoor air. Formaldehyde is easily dissolved in the water phase from ambient air due to its low Henry's coefficient. Ambient air must be one of the important sources for the contamination of samples, reagents and blank water by formaldehyde. Acetaldehyde is formed by the oxidation of ethanol, suggesting that ethanol use in laboratories can lead to acetaldehyde contamination. Many possibilities for contamination by LMW carbonyl compounds, which is one of the limiting factors of the detection limit and precision, are lurking in laboratories. The risk for contaminations by LMW carbonyl compounds increases with the number of analytical steps in the preconcentration and analysis. In the DNPH derivatization method with a C-18 SPE cartridge, many analytical steps, such as derivatization, preconditioning of SPE cartridge, preconcentration, washing and dryness of SPE cartridge, extraction and injection to HPLC are required. One of the greatest utilities of the LMW carbonyl compounds analysis mentioned in this study is its ease of operation. Our simplified preconcentration procedure and analytical system proposed in this study can reduce the chance of the contaminations of samples and reagents, and result in a lower blank and, consequently, in the lower detection limits and better precision. The simplified analytical procedure can lighten the burden on analytical operators in routine analysis, and data quality may be independent of the ability of the operator.

The method presented in this report has sufficient sensitivity for the monitoring of formaldehyde in tap water (regulation value of formaldehyde in Japan is 80 μg/L (= 2.7 μM)). Our method must be suitable for routine analysis and monitoring of aldehydes in tap water. The HPLC system used in this study can be outfitted for the analysis on a research vessel. The method with GC/MS or LC/MS requires more elaborate hardware, a gas tank and a vacuum pump, and the mass spectrometer is more cumbersome in its operation on the research vessel.

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