Flow-Injection Spectrofluorometric Determination of Trace Amounts of Formaldehyde in Water after Derivatization with Acetoacetanilide

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Abstract

A novel fluorophotometric method for formaldehyde determination in environmental waters was developed: the method does not require any enrichment procedures. A flow injection analysis method for the spectrofluorometric determination of formaldehyde in waters, which is based on the reaction of formaldehyde with acetoacetanilide and ammonia, is proposed. The proposed method shows a good linearity from 0.50 to 40 x 10^{-7} M, and the limit of detection (LOD) of 3 x 10^{-9} M (0.09 ppb) is achievable. The sample throughput is 15 h^{-1}. One of the main advantages in the proposed method is that the reaction can be carried out at room temperature without any heating system. The effect of various interferences possibly present in the real water samples was investigated. Most cations and anions, as well as organic compounds, do not interfere with the determination of formaldehyde in environmental water samples. The proposed method is very simple, rapid, less expensive, and highly sensitive, and can be applied to the environmental water samples, such as rain, tap water and river water, at low concentration levels without any enrichment procedure.

Keywords: Spectrofluorometric; Water samples; Flow-injection; Formaldehyde determination; Acetoacetanilide

1 Introduction

The organic compound, formaldehyde, is a gasous substance with a pungent smell, and it is the simplest aldehyde. Formaldehyde is unfavorable for our health because at low concentration levels, formaldehyde can cause irritation of eyes, nose, throat, and skin. Further, people with asthma may be more sensitive to the effects of inhaled formaldehyde. Therefore, formaldehyde is one of the analytically interesting substances in aquatic and air environment as an environmental pollutant. Although formaldehyde is a gas at room temperature, it is readily soluble in water. In an aqueous media, formaldehyde can polymerize, and formalin actually contains only a little formaldehyde in the form of H_2CO monomer. Oral administration of large amounts of formaldehyde can cause severe pain, vomiting, coma, and possible death. Formaldehyde can enter drinking water as a result of human activities, major sources being the discharge of industrial wastes and oxidative water treatment processes such as ozonation and chlorination [1].

Due to the influence of formaldehyde to nature and human bodies, a number of analytical methods have been proposed. Trace amounts of formaldehyde have been commonly determined by spectrophotometric methods [2-8]. However, some of them are not sensitive enough for the analysis of real water samples and are sometimes subject to numerous interferences, which are serious problems. HPLC with 2,4-dinitrophenylhydrazine (DNPH) as a derivatization agent [9-12] is one of the most frequently used methods. Recently, Burini and Coli [13] reported a HPLC system coupled with a diode array detector for formaldehyde determination after derivatization with ethyl 3-oxobutanoate: the limit of detection (LOD) was 0.024 μg mL^{-1}. HPLC procedures, however, are time-consuming and are less adaptable to water samples.

The fluorometric methods based on the Hantzsch reaction, which involved the cyclization of amine, aldehyde and *β*-diketone to form a dihydropyridine derivative, have often been used for the detection of formaldehyde in aqueous solutions. Nash [14] introduced a colorimetric method into analytical chemistry for HCHO, which was based on Hantzsch reaction of formaldehyde with acetylacetone (AA) or 2,4-pentanedione in the presence of ammonia to form a yellow product of 3,5-diactyl-1,4-dihydrolutidine (DDL). Later, Belman [15] found that without any other changes, highly sensitive measurement could be made by fluorometry, instead of spectrophotometry. This detection reaction with AA gave less product of lutidine with all aliphatic aldehydes except formaldehyde because of the mildness of the reaction conditions of analysis. However, this method is in general time-consuming and needs high temperature. Later, Sawicki and Carnes [16] proposed other reagents for the fluorometric detection of formaldehyde: 5,5-dimethyl-1,3-cyclohexnedione (dimedone) and 1,3-cyclohexanedione (CHD). Both of them can offer excellent sensitivity for the detection of formaldehyde, though such reactions require high temperature for the reaction, and furthermore, the CHD methods can suffer from positive interference from H_2O_2 [17]. Aiming at developing sensitive methods of analysis for formaldehyde, flow injection analysis (FIA) has been frequently used. Fluorometric FI methods have been studied with 4-amino-3-penten-2-one (Fluoral P) [18], 1,3-cyclohexanedione (CHD) [19], 5,5-dimethylcyclohexane-1,3-dione (dimedone) [20], acetylacetone [21] and acridine yellow-bromate [22]. In addition, Li et al. [23] developed an FI chemiluminescence method with bromate-rhodamine 6G system, which showed a detection limit of 0.3 $\mu g\ l^{-1}$ (0.3 ppb). However, they are simple but are not sensitive enough and are subject to interferences from other compounds.

In Japan, the maximum concentration of formaldehyde, which is allowed in drinking water, is now 80 ppb (2.7 μM). In general, concentrations of formaldehyde in tap waters range from 0.15 to 15 ppb in Japan [1], and therefore, any enrichment procedures are always necessary for the preconcentration of formaldehyde before measurement [1, 24, 25]. Now, a simple and highly sensitive method for formaldehyde determination is required for the direct analysis of water samples without any preconcentration techniques.

Recently, the authors examined several detection reactions for formaldehyde based on Hantzsch reaction using novel reagents, which were benzoylacetone, N-methylacetoacetamide, n-acetoacetyl-o-toluidine and acetoacetanilide (AAA) [26]. Of these, acetoacetanilide was found to be most reactive, selective and sensitive by a batchwise /spectrofluorometry. Furthermore, acetoacetanilide is more sensitive than the ones reported so far.

In this work, a novel detection reagent, acetoacetanilide, for the determination of formaldehyde is proposed for the flow injection method coupled with spectrofluorometry: the detection is based on Hantzsch reaction, which involves the cyclization between acetoacetanilide and formaldehyde in the presence of ammonium acetate. The reaction conditions were optimized by a flow injection method, and the method was applied to the determination of trace amounts of formaldehyde in environmental waters.

2 Experimental

2.1 Reagents

All chemicals used in this work were of analytical reagent grade, and the water purified with a Milli Q Labo (Millipore, Japan) was used throughout the experiments for the preparation of all solutions.

A 0.2 M acetoacetanilide stock solution was prepared by dissolving 3.544 g of acetoacetanilide (Wako Pure chemicals, Osaka) in 50 ml of ethanol and diluting it to 100 ml with the purified water.

An ammonium acetate stock solution was prepared by dissolving 77.10 g of ammonium acetate (Wako Pure chemicals, Osaka) in the purified water and diluting it to 250 ml with the purified water.

A 0.10 M standard solution of formaldehyde was prepared by diluting 0.78 ml of 36.0-38.0% HCHO solution (Wako Pure chemicals, Osaka) to 100 ml with purified water, followed by an accurate concentration determination using the iodometric method. The working standard solutions were daily prepared by accurate dilution of the standard stock solution.

For interference testing, the following compounds were used: sodium chloride, sodium nitrate, sodium nitrite, sodium sulfate, sodium sulfite, sodium carbonate, copper (II) chloride, iron (III) nitrate, hydrogen peroxide, acetic acid, acetone, propionaldehyde and acetaldehyde.

2.2 Apparatus

A schematic diagram of a flow injection analysis (FIA) system is shown in Fig. 1. A double-plunger micro pump (Sanuki Kogyo, RX-703T, Japan), P, was used for propelling a carrier solution (CS) and a reagent solution (RS). A six-way valve (Sanuki Kogyo, Japan), V, was used for introducing standard formaldehyde solutions and samples into the carrier stream. A 0.5 mm i.d. PTFE tubing was used for flow lines. A thermostating dry bath (Iuchi, EB-303, Japan) was used throughout the whole experiment. Peaks for HCHO determination were measured at λ_{ex} = 370 nm and λ_{em} = 470 by a fluorescence detector (Shimadzu, RF-10A XL, Japan) with a micro flow-though cell (16 μl). Peak height was recorded with a chart strip recorder (TOA, FBR-251A, Japan). All measurements were performed in a temperature-controlled room (25.0 ± 0.1^{o}C).

2.3 Flow injection procedure for the determination of formaldehyde in aqueous solutions

The manifold of the flow injection system used in this work is shown in Fig. 1. In the proposed method, the flow rate of the carrier and the reagent solution was set up at 0.3 ml min^{-1}. A six-way valve with a sample loop (300 µl) was used for introducing the working standard solutions of formaldehyde into the carrier stream for the preparation of a calibration graph. The standard formaldehyde solution was mixed with the reagent solutions, and flowed into the reaction coil (RC). Fluorescence changes of the reaction product were measured with the fluorescence detector: an excitation and an emission wavelength were 370 and 470 nm, respectively. The flow signals were recorded with a chart strip recorder.

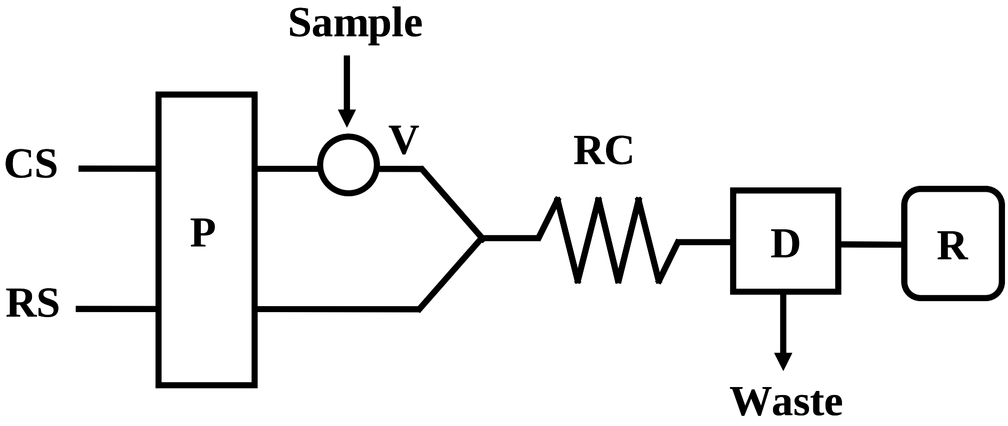

Fig. 1 FIA system for the determination of formaldehyde using acetoacetanilide as a reagent.

CS: carrier solution (distilled water)**;** RS: 0.05 M acetoacetanilide and 2.0 M ammonia acetate solution in 40% ethanol solution (pH 7.5); P: pump RX-703T; V: six ways valves with 300 µl loop; RC: reaction coil (10 m x 0.5 mm i.d.); D: fluorometric detector FP-920; R: recorder; temperature: 25 ^{o}C.

3 Results and discussion

3.1 Optimization manifold parameters for spectrofluorometric determination of formaldehyde

The optimization of manifold parameters was performed using the FIA manifold with fluorometric detector in Fig. 1. The effect of reaction temperature on signal intensity was examined by varying the temperature from 25 to 80 ^{o}C using the dry heating bath. The results obtained are shown in Fig. 2. The results showed that at temperature of 50 ^{o}C gave the strongest intensity, while above 50 ^{o}C, the baseline is not stable and some air bubbles occurred. When higher sensitivity is required, heating system can be used. However, for convenient operation, 25 ^{o}C (room temperature) was selected as a compromise between the sensitivity and the convenience of the flow system in this work.

At room temperature, reaction time is very important for improving the reaction efficiency of the detection reaction. The effect of the flow rate of the carrier and the reagent solution was investigated in the range of 0.2 to 0.6 mL min^{-1}. The results obtained (Fig. 3) indicate that with increasing flow rate from 0.2 to 0.6 mL min^{-1}, the sensitivity of the detection of formaldehyde is lowered though the sampling frequency is higher. Considering the sensitivity and the sample throughput, 0.3 mL min^{-1} of the flow rate was chosen in the further experiments

The effect of mixing coil length was examined by varying the length from 4 m to 14 m. As shown in Fig. 4, the signal intensity increased with increasing the mixing coil length up to 10 m, and above 10 m, signal intensity was almost identical. A reaction coil length of 10 m was chosen as a compromise with respect of the sensitivity and the sample throughput.

The sample injection volumes of 100, 200, 300, 400 and 500 µl were examined by changing the length of the sample loop of the injection valve. From the results obtained (Fig. 5), it can be seen that larger volumes were preferable to obtain higher peak, and the volumes above 300 µl gave only a small increase in peak height. The sample volume of 300 µl was selected as a compromise of the sensitivity, the sample throughput and the sample size.

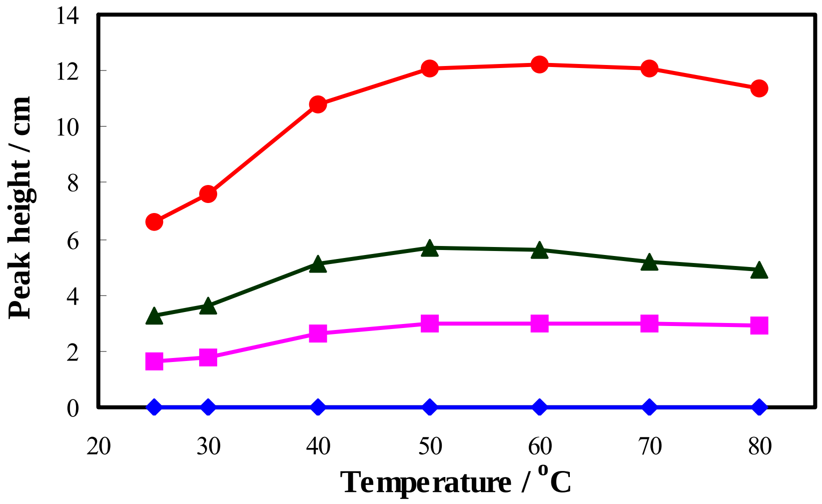

Fig. 2 Effect of reaction temperature.

HCHO concentration, ◆: 0 (blank); ■: 1×10^{-6}M; ▲: 2×10^{-6} M; ● : 4×10^{-6} M.

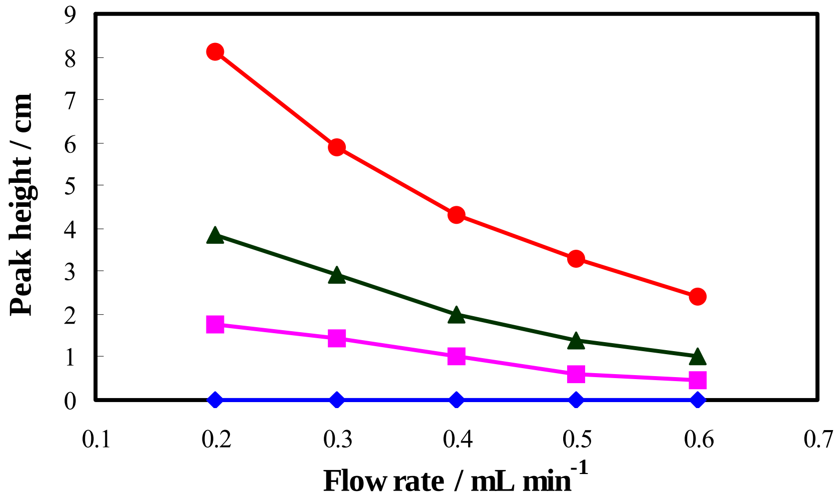

Fig. 3 Effect of flow rate.

HCHO concentration, ◆: 0 (blank); ■: 1×10^{-6}M; ▲: 2×10^{-6} M; ● : 4×10^{-6} M.

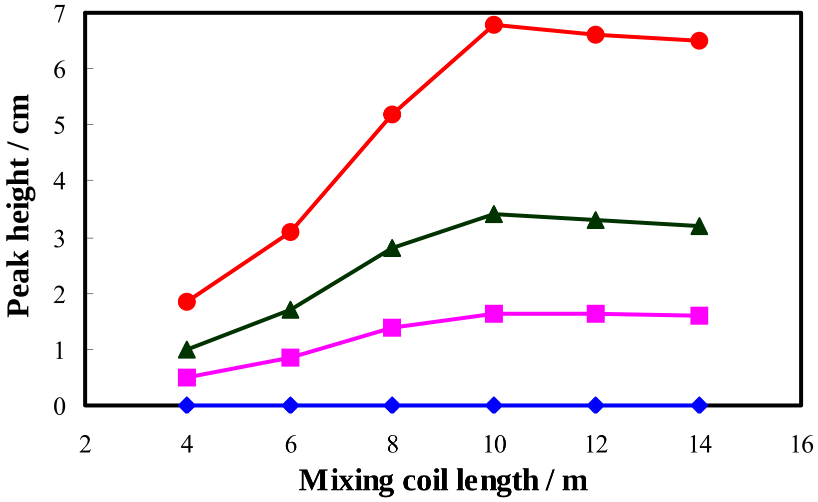

Fig. 4 Effect of mixing coil length.

HCHO concentration, ◆: 0 (blank); ■: $1x10^{-6}$M; ▲: $2x10^{-6}$ M; ● : $4x10^{-6}$ M.

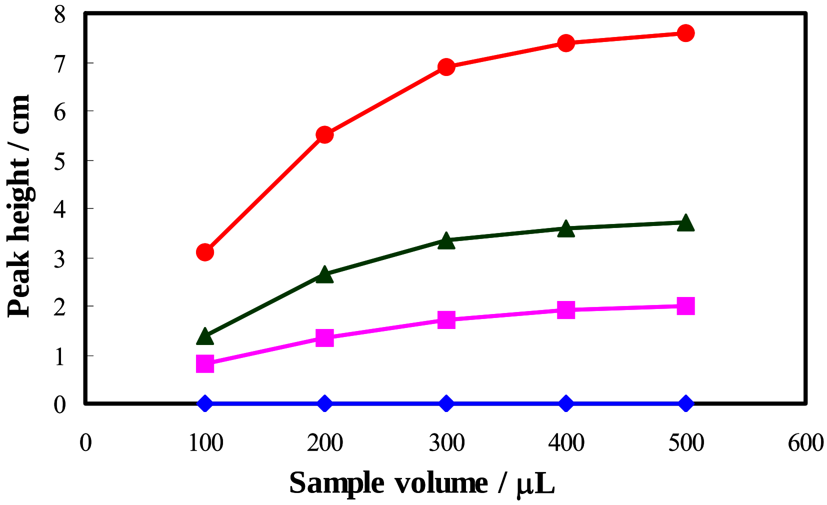

Fig. 5 Effect of sample volume.

HCHO concentration, ◆: 0 (blank); ■: $1x10^{-6}$M; ▲: $2x10^{-6}$ M; ● : $4x10^{-6}$ M.

3.2 Optimization of reagent concentrations for spectrofluorometric determination of formaldehyde

In this work, acetoacetanilide was proposed for formaldehyde detection based on Hantzsch reaction. Two molecules of an acetoacetanilide were initially involved in the unprecedented transformation: one reacts with formaldehyde and the other one reacts with ammonia to form an enamine-type intermediate, followed by a cyclodehydration to afford the dihydropyridine derivative [26]. Therefore, the effect of acetoacetanilide concentrations in the range of 0.02 ~ 0.08 M on the fluorescence intensity was studied. The results in Fig. 6 indicate that the peak height increased with increasing acetoacetanilde concentration up to 0.05 M, above which the signal intensity was almost identical. In this study, 0.05 M acetoacetanilde was selected.

Experimental results demonstrated that the reaction could proceed better in some organic solvents. A comparison of ethanol, methanol and acetone was examined. The results implies that higher and more constant sensitivity could be obtained in ethanol solution medium. Therefore, the effect of ethanol concentration in the range of 10 ~ 50% on fluorescence intensity was studied. Figure 7 shows that with increasing concentration of ethanol, the peak height increased. However, high reagent blank and noisy background was obtained when ethanol concentration was more than 40%. Therefore, 40% ethanol solution was chosen for the further experiments.

Ammonium acetate works as both buffer and one of the components of the reagents in the proposed method. The effect of ammonium acetate concentration was examined in the range of 0.5 ~ 2.5 M. The results obtained are shown in Fig. 8. It was found that the peak height increased with increasing ammonium acetate concentration. In the proposed method, 2.0 M ammonium acetate concentration was selected because of stronger capacity, higher sensitivity and better baseline.

In the reaction of formaldehyde with the proposed reagents, pH of the reagent solution is very important for the reaction efficiency. The effect of pH on the sensitivity was investigated in the range of pH 5.0 ~ 8.0 using ammonium acetate as buffers: the pH was adjusted with acetic acid and NaOH solution. The results obtained are shown in Fig. 9, which indicates that in the pH range over 6.5 ~ 7.5, the peak height is almost identical, and below pH 6.5 and above pH 7.5, the peak height becomes shorter. From these results, the pH of 7.5 was chosen for further experiments.

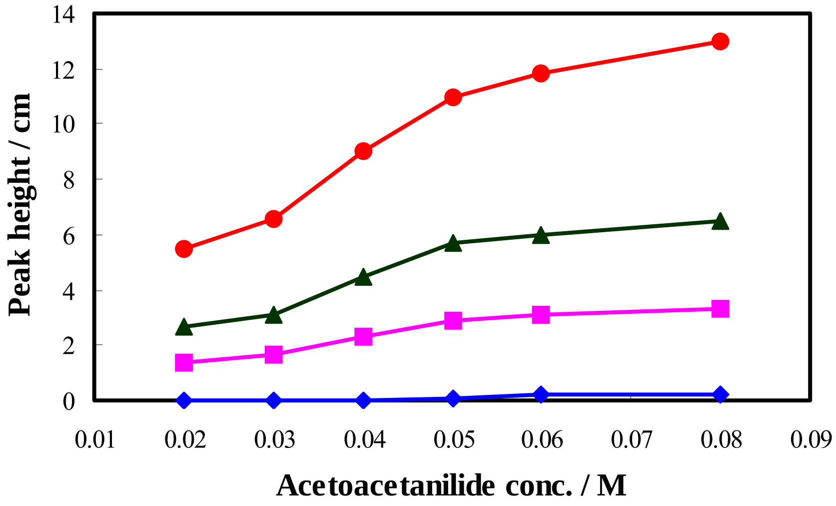

Fig. 6 Effect of concentration of acetoacetanilide.

HCHO concentration, ◆: 0 (blank); ■: 1x10^{-6}M; ▲: 2x10^{-6} M; ● : 4x10^{-6} M.

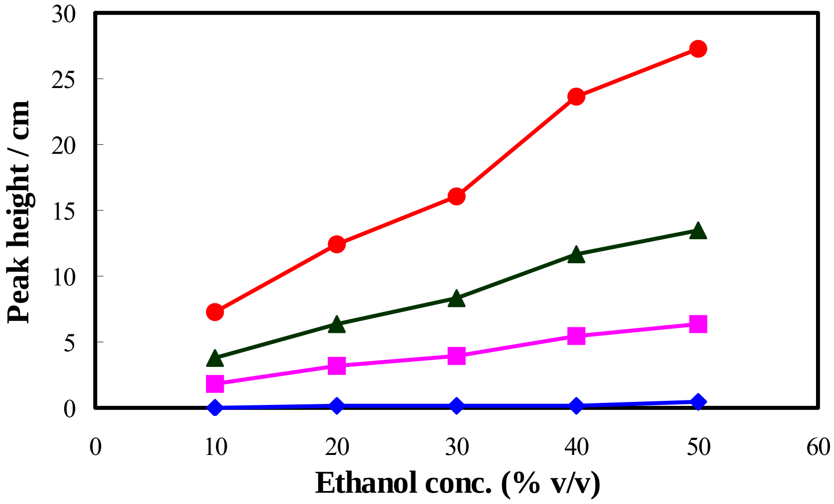

Fig. 7 Effect of concentration of ethanol.

HCHO concentration, ◆: 0 (blank); ■: 1x10^{-6}M; ▲: 2x10^{-6} M; ● : 4x10^{-6} M.

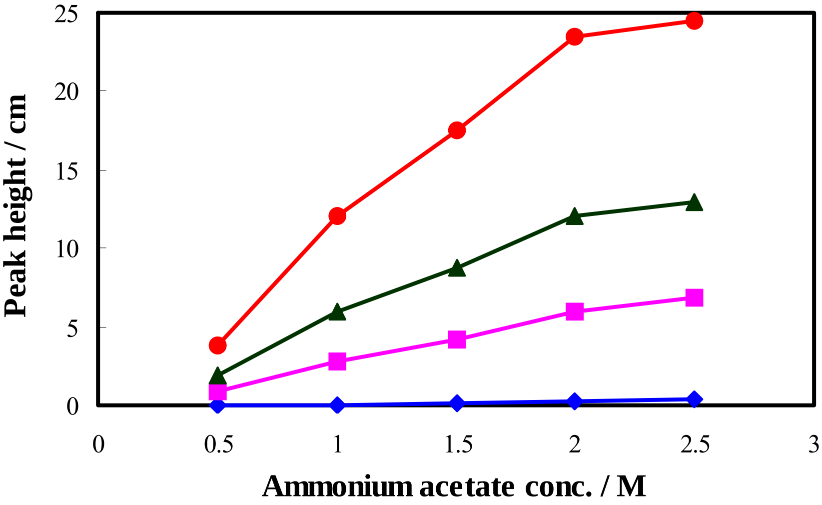

Fig. 8 Effect of concentration of ammonium acetate.

HCHO concentration, ◆: 0 (blank); ■: 1x10^{-6}M; ▲: 2x10^{-6} M; ● : 4x10^{-6} M.

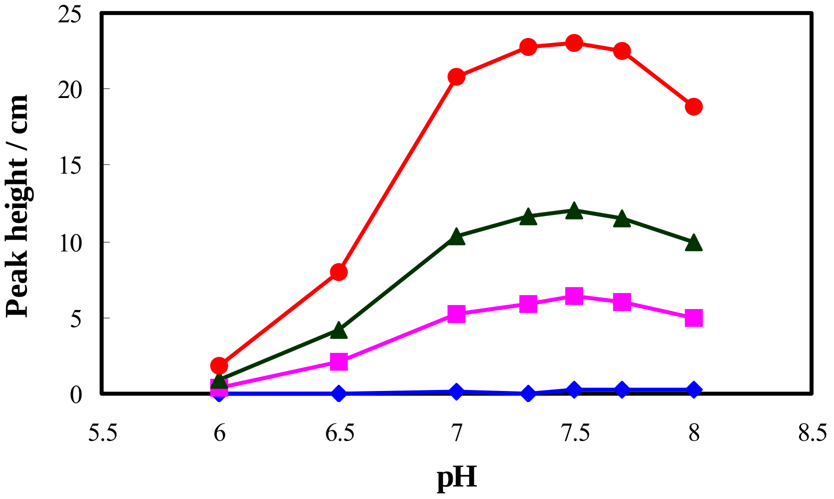

Fig. 9 Effect of pH.

HCHO concentration, ◆: 0 (blank); ■: 1x10^{-6}M; ▲: 2x10^{-6} M; ● : 4x10^{-6} M.

3.3 Interference from foreign substances

The investigation of possible interferences was conducted with regard to possible chemical interferences and the problem of selectivity. The interference from low-molecular-weight aldehydes and other substances was investigated. It was found that their interferences with the determination of formaldehyde in water samples were negligible even when interfering substances were added at higher concentrations than commonly existing ones. However, the formaldehyde signal decreased seriously with the addition of more than two fold of sulfite ion. This interference is due to the reaction of formaldehyde with sulfite. Since sulfite is easily oxidized to sulfate, only a low concentrations of sulfite can remain in natural waters. Therefore, the proposed method is free from interference with the determination of formaldehyde in environmental waters. In Table 1, the tolerable concentration is defined as the concentrations of foreign substances causing less than ±5% relative error.

Table 1 Tolerable concentrations of foreign species for the determination of 1x10^{-6} M formaldehyde

Foreign substances	Tolerable conc. (M)	Tolerable limit ([species] / [HCHO])	Relative error (%)
Na^+	1 x 10^{-2}	10000	+ 4.2%
Cl^-	1 x 10^{-2}	10000	+ 4.2%
H_2O_2	1 x 10^{-2}	10000	- 4.2%
NO_3^-	1 x 10^{-2}	10000	+ 4.7%
HAc	1 x 10^{-2}	10000	- 3.7%
NO_2^-	5 x 10^{-3}	5000	- 4.4%
SO_4^{2-}	2 x 10^{-3}	2000	- 3.8%
Acetone	2 x 10^{-3}	2000	+ 3.7%
Propionaldehyde	1 x 10^{-3}	1000	+ 4.2%
CO_3^{2-}	1 x 10^{-3}	1000	+ 4.7%
Acetaldehyde	5 x 10^{-4}	500	+ 3.0%
Cu^{2+}	2.5 x 10^{-5}	25	+ 2.7%
Fe^{3+}	1 x 10^{-5}	10	+ 4.3%
SO_3^{2-}	2 x 10^{-6}	2	- 3.8%

3.4 Calibration graph and analytical features

Under the optimal conditions summarized in Table 2, the calibration graph was prepared over the range of 0.50 ~ 40 x 10^{-7} M formaldehyde with a correlation coefficient of 0.9999. The peak profile of HCHO for the calibration graph obtained are shown in Fig. 10. The equation of the calibration graph was expressed as $Y = 0.57X + 0.19$, where Y was peak height and X was formaldehyde concentration in 10^{-7} M. The standard deviation and the relative standard deviation of 12 replicate injections of 5.0 x 10^{-7} M were 0.052 and 1.7%, respectively.

The limit of detection, calculated as the concentration corresponding to three times of the baseline noise (S/N = 3), was 3 x 10^{-9} M (0.09 ppb). The proposed method is superior in terms of sensitivity compared with other flow injection spectrofluorometric methods as shown in Table 3.

Table 2 Summary of the optimal conditions for the proposed method

Parameter	Range examined	Optimal conditions
Reaction temperature (°C)	25 - 80	25
Flow rate (mL min^{-1})	0.2 – 0.6	0.3
Mixing coil length (m)	4 - 14	10
Sample volume (µL)	100 - 500	300
Acetoacetanilide conc. (M)	0.02 – 0.08	0.05
Ethanol conc. (% v/v)	10 - 50	40
Ammonium acetate conc. (M)	0.5 – 2.5	2.0
pH	6.0 – 8.0	7.5

Table 3 Comparison of flow-injection spectrofluorometric method for the determination of formaldehyde

Reagent	Reaction temperature (°C)	Working range (M)	Detection limit (M)	Sample throughput (h^{-1})	Ref.
4-Amino-3-penten-2-one (Fluoral P)	60	$0.5 \sim 100 \times 10^{-6}$	-	12	[18]
1,3-Cyclohexanedione (CHD)	95	$0.14 \sim 1.4 \times 10^{-7}$	6×10^{-9}	-	[19]
5,5-Dimethycyclohexane-1,3-dione (Dimedone)	130	$1.7 \sim 3.3 \times 10^{-7}$ and $0.83 \sim 3.3 \times 10^{-6}$	3×10^{-8}	20	[20]
Acetylacetone (AA)	60	$1.5 \sim 15 \times 10^{-7}$	8×10^{-9}	20	[21]
Acetoacetanilide (AAA)	25	$0.50 \sim 40 \times 10^{-7}$	3×10^{-9}	15	This work

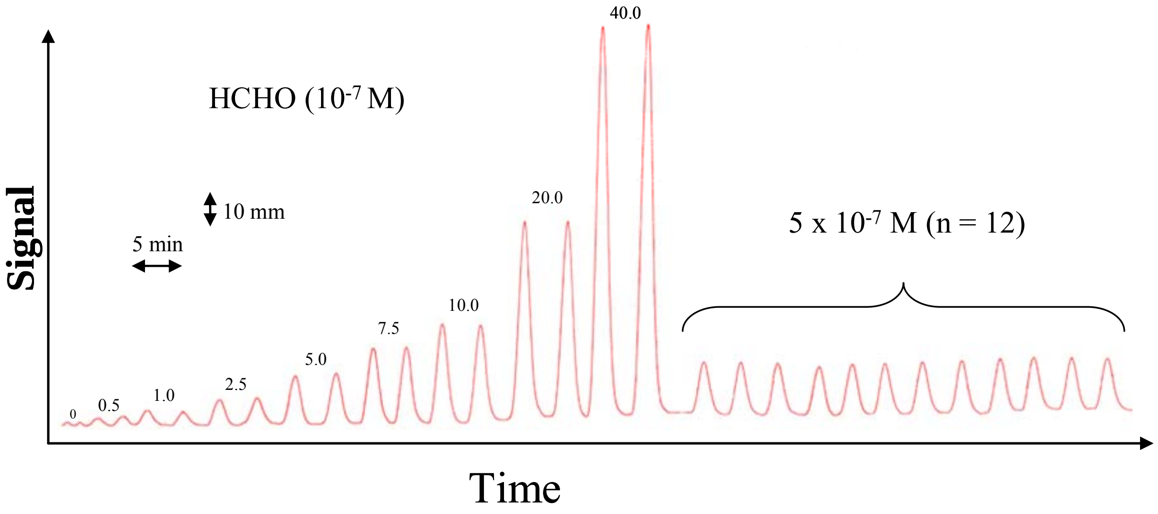

Fig. 10 Flow signals for formaldehyde determination.

HCHO concentration: 0-40x10^{-7} M; 0.05 M acetoacetanilide; 40% ethanol solution ; 2.0 M ammonium acetate; pH 7.5; flow rate: 0.3 mL min^{-1}; reaction coil length: 10 m; sample injection volume: 300 µL; reaction temperature: 25 °C.

3.5 Application of the proposed method to environmental water samples

The proposed method was applied to the determination of formaldehyde in rain, river and tap waters. All samples were filtered using filter papers before introducing to the flow injection system. In order to evaluate the validity of the proposed method for the determination of formaldehyde, the recovery test was performed. The samples were spiked with known amounts of formaldehyde solutions from 1.5 to 30.0 ppb, which covers the usual concentration ranges of formaldehyde in environmental water samples. Significantly high recoveries from 96.2 to 110.2% were obtained from the determination of formaldehyde in water samples (Table 4).

Table 4 Recovery tests of formaldehyde in environmental waters

Sample	**Concentration added (ppb)**	**Concentration recovered (ppb)**	**Recovery (%)**
Rain	0	15.7	-
	7.5	22.6	96.2
	15.0	31.0	101.9
	30.0	45.2	96.8
River water	0	3.53	-
	1.5	5.25	106.2
	3.0	6.83	108.4
	6.0	9.67	104.0
Tap water ^{1}	0	1.13	-
	1.5	2.9	110.2
	3.0	4.2	106.2
Tap water ^{2}	0	1.29	-
	1.5	2.91	107.6
	3.0	4.36	105.4

All values are means (n=4)

4 Conclusion

A new reagent, acetoacetanilide, is first introduced for the sensitive determination of trace amounts of formaldehyde.

The proposed method has several advantages over the previously reported methods: (1) simpler analysis system, (2) higher sensitivity, (3) less toxicity of the reagents used.

The proposed method can be directly applied to the determination of formaldehyde in the environmental water samples (rain, tap water and river waters) at low concentration levels without any enrichment procedure.

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