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Development of Novel Reagent for Hantzsch Reaction for the Determination of Formaldehyde by Spectrophotometry and Fluorometry

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A novel reagent, acetoacetanilide (AAA), was introduced to the determination of formaldehyde based on Hantzsch reaction. A simple and highly sensitive fluorometric method was achieved by using AAA. The main advantages in the use of this reagent are: the reaction is carried out at room temperature without any heating system, the cyclization product based on Hantzsch reaction is soluble in water, and the product can be detected by spectrophotometry and fluorometry. The maximum absorption wavelength of the product occurs at 368 nm, and the maximum excitation and emission wavelengths are found at 370 and 470 nm, respectively. Several important experimental variables of the procedures were examined; particularly, the reaction temperature, reaction time, concentrations of reagents, and pH of the reagent solution were optimized for improving the detecting sensitivity. The calibration graph was linear in the range of 1×10^{-7} – 1×10^{-6} M or much higher concentrations. The limit of detection (LOD), based on three times of the standard deviation of the reagent blank, was 2.0×10^{-8} M. The proposed method was applied to the determination of formaldehyde in environmental water samples. Many foreign species commonly existing in water samples did not interfere with the determination of formaldehyde in the proposed method.

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Introduction

Formaldehyde (HCHO) is the most common aldehyde in the environment. It is widely present in aquatic and air environments as one of environmental pollutants; it is known to be toxic for human beings at high concentrations and to be irritating the respiratory tract, eye and skin.[1] However, formaldehyde is one of the important chemicals used in industries for manufacturing building materials and numerous household products. Such usage has recently become a matter of concern as one cause of the sick house syndrome, which is sometimes a very serious problem in Japan.[2] Although formaldehyde is a gas at normal room temperature, it is readily soluble in water. In the natural environment, formaldehyde is present in rain water, cloud water and fog water. Owing to the influence of formaldehyde to nature and human bodies, a simple, rapid and highly sensitive method for the determination of formaldehyde in environmental water is urgently required.

Various methods for the determination of formaldehyde have been proposed. Of these, spectrophotometric methods have been widely used for the determination of formaldehyde. Most of these methods are based on the reaction of formaldehyde with organic reagents and/or inorganic reagents, such as Schiff's reagents,[3] pararosaniline,[4] *p*-phenylenediamine,[5] Chromotropic acid,[6] J acid,[6] phenyl J acid,[6] *p*-aminoazobenzene,[7] brilliant cresyl blue-bromate,[8] and malachite green-sulfite.[9] However, some of the spectrophotometric methods are not sensitive enough and can be subject to numerous interferences from coexisting substances. HPLC with 2,4-dinitrophenylhydrazine (DNPH) as a derivatization agent[10-13] is the most frequently used method. Recently, Burini and Coli[14] 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, require long analysis time, and are sometimes less sensitive.

There are many fluorescence methods used for the determination of formaldehyde. Among them, the fluorometric methods based on the Hantzsch reaction, which involve the cyclization of amine, aldehyde and β-diketone to form a dihydropyridine derivative, have been widely used for the detection of formaldehyde in aqueous solutions. Nash[15] introduced a colorimetric method into analytical chemistry for HCHO; it was based on the Hantzsch reaction of formaldehyde with acetylacetone or 2,4-pentanedione in the presence of ammonia to form a yellow product of 3,5-diactyl-1,4-dihydrolutidine (DDL). Later, Belman[16] found that, without any other changes, highly sensitive measurements could be made by fluorometry instead of spectrophotometry. Fluorescence detection allows the measurement of formaldehyde at the μg l^{-1} level. This reagent system results in less quantitative product for the determination of all of the aliphatic aldehydes except formaldehyde because of the mild reaction for analysis. The method however, is time-consuming and needs high temperature. In 1968, Sawicki and Carnes[17] proposed new reagents for the fluorometric detection of formaldehyde: 5,5-dimethyl-1,3-cyclohexanedione (dimedone) and 1,3-cyclohexanedione (CHD). Both of them offer excellent

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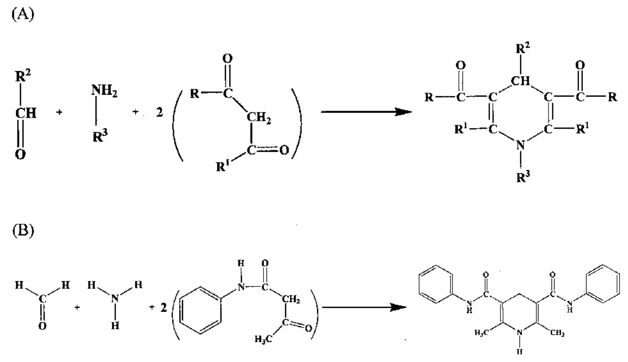

Scheme 1 (A) The Hantzsch reaction requires a β-diketone, an aldehyde and ammonia or an alkylamine. (B) Acetoacetanilide as a reagent for formaldehyde detection.

sensitivity at the detection of HCHO. However, their reactions require high temperature, and furthermore, CHD can suffer positive interference from H_2O_2.[18]

In this work, a novel detection reagent, acetoacetanilide (AAA), is proposed for the determination of formaldehyde. The detection reaction involves the cyclization between AAA and formaldehyde in the presence of ammonia. The proposed method can provide simplicity, rapidity and high sensitivity for formaldehyde detection, as well as low cost and commercial availability.

Principle of the detection of formaldehyde

The Hantzsch reaction has frequently been used for spectrophotometric and fluorometric detection of formaldehyde. In this work, AAA was proposed for formaldehyde detection based on the Hantzsch reaction. Two molecules AAA 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, as shown in Scheme 1. The maximum absorption wavelength of the product occurs at 368 nm, and the maximum excitation and emission wavelengths are found at 370 and 470 nm, respectively. Substitution of acetylacetone with acetoacetanilide allowed selectivity of the condensation reaction and did not involve any other aldehyde, such as acetaldehyde or benzaldehyde.

Experimental

Instrumentation

All fluorometric measurements were performed with a spectrofluorometer (Hitachi, 650-10 S, Japan) equipped with a xenon lamp, dual monochromators, a 1 × 1 cm quartz cell and a recorder (Toa Dempa, FBR-251A, Japan). The slit widths for both excitation and emission monochromators were set at 10 nm. Absorption measurements were performed on a UV-visible recording spectrophotometer (Shimadzu, UV-2400PC, Japan). A pH meter (Mettler Toledo, MP220, Switzerland) was used for adjusting the pH of the reagent solution. All measurements were performed in a temperature-controlled room (25.0 ± 0.1°C).

Reagents

All chemicals used in this work were of analytical reagent grade, and water purified with a Milli Q Labo (Millipore, Japan) was used 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 ethanol and diluting the mixture to 100 ml with purified water. An ammonium acetate stock solution was prepared by dissolving 77.10 g of ammonium acetate (Wako Pure Chemicals) in purified water and diluting it to 250 ml with purified water. Formaldehyde stock solution (0.1 M) was prepared by diluting an appropriate amount of 36 – 38% aqueous HCHO solution (Wako Pure Chemicals) to 100 ml with purified water, followed by an accurate concentration determination using the iodometric method. The working standard HCHO solutions were further diluted with purified water. A dilute solution of HCHO was freshly prepared before the experiments. Rain water samples were collected in Okayama University campus, during September 2005. Filtration was performed with pieces of filter paper (Advantec, No. 5B).

Table 1 Spectrophotometric data of some β-diketones for formaldehyde determination

Reagent	ε/l mol^{-1} cm^{-1}	λ_{max}/nm
Acetylacetone	1.2×10^4	412
Benzoylacetone	1.1×10^4	422
Acetoacetanilide	6.1×10^3	368
Benzoyltrifluoroacetone	—	—
2-Thenoyltrifluoroacetone	45	500

Table 2 Fluorometric data of some β-diketones for formaldehyde determination

Reagent	λ_{ex}/nm	λ_{em}/nm	Relative sensitivity
Acetylacetone	420	505	1.00
Benzoylacetone	—	—	—
Acetoacetanilide	370	470	1.92
Benzoyltrifluoroacetone	—		—
2-Thenoyltrifluoroacetone	—		—

Standard procedure for the optimization of experimental variables

To a 10 ml calibrated flask were added 5 ml of 4.0 M ammonium acetate (pH 7.5), 2.0 ml 0.2 M acetoacetanilide, 2.0 ml ethanol and a series of standard HCHO solutions, and then the mixtures were diluted to the mark with purified water. After standing for 10 min, the relative fluorescence intensities of the reagent blank and the sample solutions were measured at 470 nm with an excitation wavelength of 370 nm. Spectrophotometric measurement was performed using the same procedures as above, and the calibration graph was prepared in a usual manner.

Results and Discussion

Selection of cyclization reagents

In order to improve sensitivity and selectivity for trace determination of formaldehyde, we developed novel reagents and propose them in this paper. The reaction mechanism of the Hantzsch reaction was first explained by Nash.[15] In this work, several kinds of commercially available β-diketone analogues, such as benzoyltrifluoroacetone, benzoylacetone, 2-thenoyl-

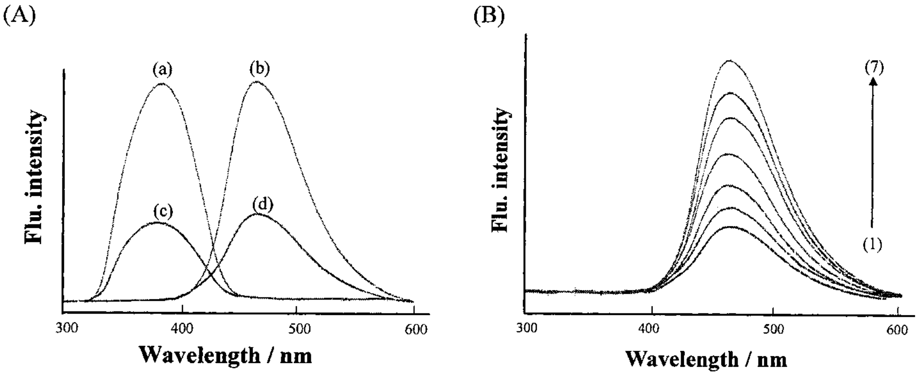

Fig. 1 Fluorescence spectra for HCHO determination by using acetoacetanilide (AAA) as a reagent. A: (a) and (c) are the excitation spectra of 10 × 10^{-7} M HCHO and the blank, respectively; (b) and (d) are the emission spectra of 10 × 10^{-7} M HCHO and the blank, respectively. B: Emission spectra of 0 - 10 × 10^{-7} M HCHO. (1) Reagent blank, 0 M, (2) 1 × 10^{-7}, (3) 2 × 10^{-7}, (4) 4 × 10^{-7}, (5) 6 × 10^{-7}, (6) 8 × 10^{-7}, (7) 10 × 10^{-7} M.

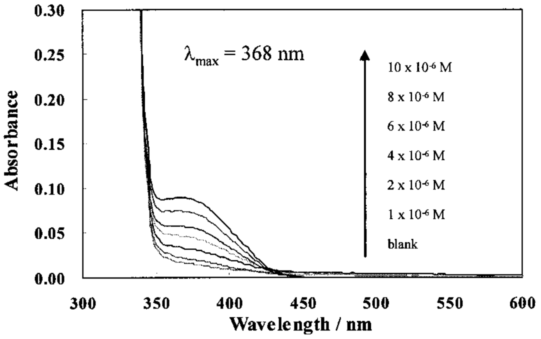

Fig. 2 Absorption spectra and calibration graph for HCHO determination in the concentration range of 1 - 10 × 10^{-6} M.

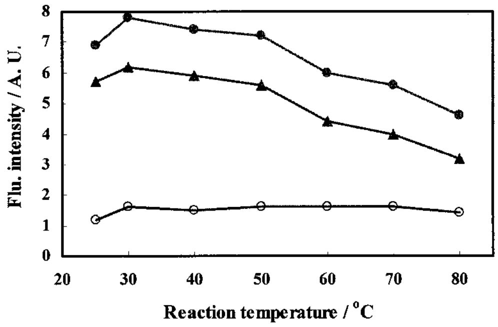

Fig. 3 Effect of reaction temperature on the fluorescence intensity. Acetoacetanilide concentration, 0.01 M; ethanol concentration, 20%; ammonium acetate concentration, 2.0 M; pH, 7.0; reaction time, 20 min. HCHO concentration: ○, 0 (blank); ●, 2 × 10^{-6} M; ▲, difference in fluorescence.

trifluoroacetone and acetoacetanilide, were examined by using spectrophotometric and fluorometric methods; the results are compared with the results obtained using acetylacetone. All results are summarized in term of molar absorptivity (ε) and maximum absorption wavelength from spectrophotometry (Table 1), and excitation and emission wavelength from fluorometry (Table 2), respectively. The product obtained using benzoylacetone with formaldehyde is not fluorescent. Of the reagents examined, acetoacetanilide (AAA) gave the largest molar absorptivity, except for acetylacetone, and the fluorescence intensity of the product obtained with AAA was the largest. Because of these results, AAA was used for further experiments.

Spectral characteristics

The cyclization reaction between AAA and formaldehyde in the presence of ammonia is shown in Scheme 1. The excitation and emission spectra obtained with AAA in the presence of and in the absence of formaldehyde were measured. The fluorescence intensity was enhanced greatly in the presence of formaldehyde. This result indicates that a new fluorescence compound was formed. The maximum wavelength of the excitation and the emission were 370 and 470 nm, respectively (Fig. 1). By using the same conditions as those for fluorometric measurements, the absorption spectra were also studied. The results (Fig. 2) show the maximum absorption wavelength of the product in the presence of formaldehyde is 368 nm.

Optimization of experimental conditions

Effect of reaction temperature. In general, organic reactions in aqueous solution are slow compared with inorganic reactions. The Hantzsch reaction with such diketones as acetylacetone and its analogues are usually slow, and therefore detection reactions must be carried out at higher temperature than room temperature. So, effect of the reaction temperature is very important for the reaction of HCHO. The effect of temperature was investigated by varying it from 25 to 80°C. The results (Fig. 3) indicate that the product obtained at 30°C gave strongest intensity, which is a little larger than that obtained at room temperature. Above 30°C, the intensity gradually decreased with increasing temperature. In this work, the temperature, 25°C, was chosen for convenient operation at room temperature.

Effect of reaction time on fluorescence intensity. The influence of the reaction time on the fluorescence intensity was studied. The results indicated that the suitable reaction time was about 10 to 30 min. Thus, the reaction time of 10 min was selected for the further experiments.

Effect of organic solvent, ethanol, on fluorescence intensity. The effect of ethanol concentration on fluorescence intensity was examined in the ranges of 10 - 50%. Figure 4 shows that with increasing concentration of ethanol, the peak height increased up to 30%. Above 30%, the intensity becomes almost

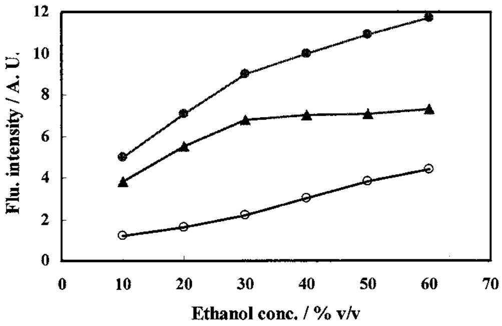

Fig. 4 Effect of ethanol concentration. Acetoacetanilide concentration, 0.01 M; ammonium acetate concentration, 2.0 M; pH, 7.0; reaction time, 20 min; reaction temperature, 25°C. HCHO concentrations are the same as in Fig. 3.

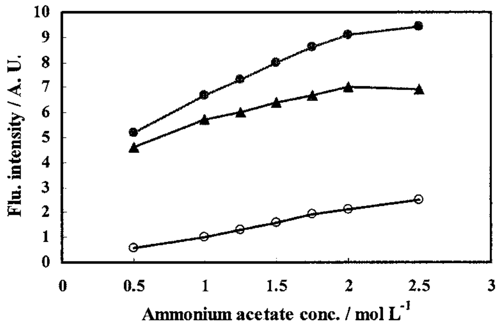

Fig. 5 Effect of ammonium acetate concentration. Acetoacetanilde concentration, 0.04 M; ethanol concentration, 30%; pH, 7.0; reaction time, 20 min; reaction temperature, 25°C. HCHO concentrations are the same as in Fig. 3.

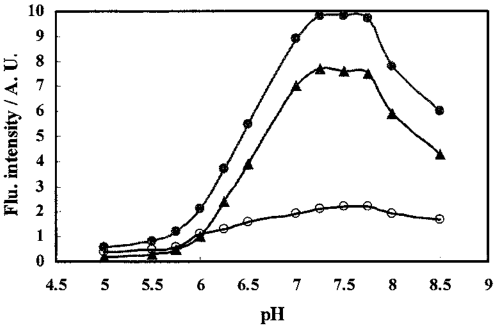

Fig. 6 Effect of pH. Acetoacetanilde concentration, 0.04 M; ethanol concentration, 30%; ammonium acetate concentration, 2.0 M; reaction time, 20 min; reaction temperature, 25°C. HCHO concentrations are the same as in Fig. 3.

Table 3 Tolerance limit of foreign ions in the determination of 10^{-6} M HCHO

Foreign ion	Added as	Tolerance limit[a] ([ion]/[HCHO])
Na^+	NaCl	10000
K^+	KCl	10000
NO_3^-	$NaNO_3$	10000
SO_4^{2-}	Na_2SO_4	10000
CO_3^{2-}	Na_2CO_3	10000
HAc	HAc	1000
Mg^{2+}	$MgCl_2$	1000
Acetone	Acetone	1000
Benzaldehyde	Benzaldehyde	1000
Propionaldehyde	Propionaldehyde	500
Acetaldehyde	Acetaldehyde	500
Methanol	Methanol	500
ClO^-	NaClO	100
Ca^{2+}	$CaCl_2$	100
Ni^{2+}	$NiCl_2$	100
Cu^{2+}	$CuCl_2$	100
Fe^{2+}	$FeSO_4$	10
Fe^{3+}	$Fe(NO_3)_3$	10
SO_3^{2-}	Na_2SO_3	2

a. Defined as ±5% relative error. Maximum concentrations of foreign ions tested.

identical. Therefore, aqueous ethanol (30%, v/v) was chosen in the further experiments.

Effect of acetoacetanilide (AAA) concentration. The effect of acetoacetanilide concentration on fluorescence intensity in the ranges of 0.005 - 0.07 M was varied. The results obtained indicate that higher AAA concentration favored the complete reaction, resulting in higher fluorescence values. However, this can also cause, to some extent, an increase in the reagent blank absorption. As a result, the difference in fluorescence intensity (ΔF) slightly increased with increasing AAA concentrations up to 0.04 M. In the further experiments, 0.04 M AAA was selected.

Effect of ammonium acetate concentration. The preliminary examination showed that the fluorescence intensity was much higher from acetate buffer than from phosphate buffer. Replacement of ammonia by ammonium acetate allowed the efficient synthesis of Hantzsch's compounds under mild conditions. Hence, in this work, ammonium acetate was chosen as a buffer and a component of the reagent solution. The effect of ammonium acetate concentration was examined in the ranges of 0.5 - 2.5 M. The results obtained are shown in Fig. 5, which indicates that the difference in fluorescence intensity (ΔF) increases with increasing ammonium acetate concentrations. As a compromise among the experimental conditions, buffer capacity and reaction sensitivity, the ammonium acetate concentration, 2 M, was selected in the further experiments.

Effect of pH of solution. The pH of the reagent solution dramatically affects the observed analytical signal. The effect of pH on the fluorescence intensity was investigated in the ranges of 5.0 - 8.5. The pH of the reagent was adjusted with acetic acid or sodium hydroxide solution. The results obtained in Fig. 6 show that, in the pH range of 7.3 - 7.7, the fluorescence intensity was almost identical, while below pH 7.3 and above pH 7.7, the fluorescence intensity decreased drastically. Based on these results, the pH of 7.5 was chosen for the future experiments.

Interferences from foreign substances

The influences of foreign species were examined by adding a certain amount of interfering species in 1×10^{-6} M HCHO solution. The tolerable concentrations, defined as the concentration of foreign species causing less than ±5% relative error, were examined. The tolerance limits of the interfering ions in the determination of 1×10^{-6} M formaldehyde are summarized in Table 3. No interference was observed from sodium chloride, sodium nitrate, sodium sulfate, sodium carbonate and potassium chloride, even when they are present in

Table 4 Analytical results for the determination of formaldehyde in rain water samples

Sample[a]	HCHO found[b]/ μg l^{-1}	HCHO added/ μg l^{-1}	Recovered[b]/ μg l^{-1}	% Recovery
1	13.6 ± 0.2	15.0	28.2 ± 0.1	97.1
2	13.5 ± 0.1	15.0	28.2 ± 0.1	97.8
3	14.3 ± 0.1	15.0	29.8 ± 0.2	103.5
4	14.5 ± 0.1	15.0	30.0 ± 0.1	103.4
5	14.6 ± 0.2	15.0	29.8 ± 0.1	101.4

a. Rain water samples were sampled in Okayama University campus, in September 2005.
b. All values are means (n = 5).

10000-fold excess over formaldehyde. Acetone, benzaldehyde, acetic acid and magnesium chloride did not interfere up to 1000-fold excess over formaldehyde. Propionaldehyde, acetaldehyde and methanol did not affect till 500-fold excess over formaldehyde. Sodium hypochlorite, calcium chloride, nickel chloride and copper chloride interfered at concentrations higher than 10^{-4} M. Iron(II) sulfate and iron(III) chloride up to 10-fold excess over formaldehyde did not interfere. Sodium sulfite interfered with the determination of formaldehyde at the concentration ratio of more than 2.

Analytical parameters

The calibration curves for the determination of formaldehyde were constructed under the optimum conditions (Fig. 1). A series of HCHO standard solutions were measured to allow the preparation of a calibration graph. The fluorescence response was linear in the HCHO concentration range of $0 - 10 \times 10^{-7}$ M. The equation of the linear regression line was, for example, $y = 0.32x + 1.47$, where y and x are fluorescence intensity and HCHO concentration in 10^{-7} M, respectively, with a correlation coefficient of 0.9967.

The limit of detection (LOD) of formaldehyde, based on three times of standard deviation of the blank, was 2×10^{-8} M.

Application of the proposed method to real samples

Rain water is known to be a significant source of formaldehyde for ground and seawater. In order to evaluate the analytical applicability of the proposed method, the procedure established was applied to the determination of formaldehyde in rain water samples. All samples were filtered using pieces of filter paper. The recovery of formaldehyde in the samples was checked by the addition of 15.0 μg L^{-1} standard formaldehyde solution to each rain water sample. The results are shown in Table 4. The recoveries from 97.1 to 103.5% were obtained, which indicates that the proposed method is reliable and accurate.

Conclusions

A new reagent, acetoacetanilide, was proposed in the spectrophotometric and fluorophotometric determination of formaldehyde. The advantages of the proposed method are: the reaction of acetoacetanilide with formaldehyde can be carried out at room temperature without any heating procedure and the detection can be done by spectrophotometry and fluorophotometry. The proposed method is recommended when a simple and sensitive method is necessary for the determination of HCHO under relatively moderate conditions.

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