Fluorometric Flow Injection Method for the Determination of Ammonia Based on Hantzsch Reaction and Its Application to Ammonia Determination in the Atmosphere

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Abstract

A Hantzsch reaction was, for the first time, applied to the determination of ammonia, and a novel, simple and highly sensitive method based on the reaction among ammonia, acetylacetone and formaldehyde was developed. The yellow color product, 3,5-diacetyl-1,4-dihydrolutidine, was detected fluorometrically at an excitation wavelength (λ_{ex}) of 420 nm and an emission wavelength (λ_{em}) of 505 nm. The reaction conditions were optimized, and then the method was successfully applied to ammonia determination in the atmosphere. The optimal conditions were examined with respect of improving the sensitivity of ammonia determination under the moderate conditions. Under the optimized conditions, the proposed method was characterized by a linear calibration with a correlation coefficient of 0.9995 in the range of 1×10^{-6} M to 5×10^{-5} M. Sample throughput of 21 h^{-1}, the standard deviation (RSD) of 0.56% (n=8) for 1×10^{-5} M ammonia standard solution, and the detection limit of 10^{-7} M (S/N=3) were achieved. The interference from cations and anions was also investigated. The proposed method was applied to the determination of micro amounts of ammonia in indoor and outdoor air by coupling with a batchwise collection/concentration method.

Keywords Flow injection, Hantzsch reaction, ammonia, fluorometric detection

1. Introduction

Ammonia (NH_3) is a micronutrient in water systems, and is an important link to the nitrogen cycle of aquatic ecosystems [1]. It exists at relatively low concentrations in natural environment, such as in air, rain, river, lake and sea, and in soil. Ammonia content is also a basic parameter in food and pharmacological analysis, as well as in clinical and biological chemistry [2]. Nowadays, the ammonia generation from fertilizer and domestic animals excretion into environment is more and more increasing, and therefore a rapid and sensitive method for the determination of trace amounts of ammonia is urgently required. However, the determination of ammonia is still a delicate task: two main reasons for this can be put forward. First, it is highly susceptible to contamination, and second, classic indophenol-blue (IPB) methods do not appear to be well controlled in many laboratories [3]. For use in unpolluted natural media, most methods suffer either from the lack of sensitivity, low sample throughput or a more or less significant salt effect, and sometimes from a combination of several of these problems.

This work aims at developing an alternative method for the rapid, easy, low-cost and highly sensitive determination of ammonia, which could be applied to atmospheric air samples.

2. Experimental

2.1 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 ammonia 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 ammonia determination were measured at λ_{ex}=420 nm and λ_{em}=505 by a fluorescence detector (Jasco, 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).
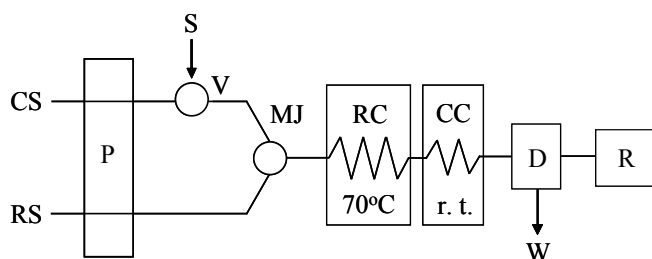

Fig. 1 Shemetic diagram of flow injection system for the determination of ammonia by fluorometric detection. CS: carrier solution (ultrapurified water); RS: 0.3 M acethylacetone, 0.13 M formaldehyde and 0.4 M phosphate buffer at pH 6.3; P: double-plunger pump (each flow rate: 0.3 ml min^{-1}); V: six-way valve; S: sample; MJ: mixing joint; RC: reaction coil (0.5 mm i.d.x 8 m; 70°C); CC: cooling coil (0.5 mm i.d.x 2 m; water bath, r. t. : room temperature); D: detector (fluorometer); R: recording system; W: waste.

2.2 Reagents

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

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solutions, RS, was prepared by mixing adequate volume of a 0.96 M acetylacetone stock solution, a 0.65 M formaldehyde stock solution and a 2 M phosphate buffer (Na_2HPO_4-KH_2PO_4), followed by the addition of a dilute phosphoric acid solution for the purpose of adjusting concentrations and pH. An acetylacetone stock solution was prepared by diluting 5.0 ml of acetylacetone (Wako Pure Chemicals, Osaka, assay min.99%) with the purified water to give a 50 ml solution. A 0.65 M formaldehyde stock solution was prepared by diluting 4.8 ml of a commercially available formaldehyde solution (Wako Pure Chemicals, Osaka: 36.0% ~ 38.0%) to 100 ml with the purified water. A phosphate stock solution was prepared by dissolving 141.96 g of disodium hydrogenphosphate (Wako Pure Chemicals, Osaka) and 136.09 g of potassium dihydrogenphosphate (Wako Pure Chemicals, Osaka) in the purified water and diluting it to 1000 ml with the purified water.

A 0.100 M standard stock solution of ammonia was prepared by dissolving 0.535 g of ammonium chloride (Wako Pure Chemicals, Osaka) in the purified water and diluting it to 100 ml with the purified water. The stock solution was standardized by the titration method. The working solutions were daily prepared by appropriate dilution of the standard stock solution with water.

2.3 Principle of the detection of ammonia in aqueous solution

The determination of micro amounts of ammonia is of increasing importance in biochemical and environmental studies [4-8]. Several techniques for the trace determination, such as chemiluminescence [9], spectrophotometry [7-11], cathodic stripping voltammetry [3] and photoacoustic [5], have reported so far: they are sometimes lack in sensitivity and high detection limits, and require ultapure reagents.

The Hantzsch reaction [12, 13], which is based on the reaction between formaldehyde and acetylacetone in the presence of ammonia and the formation of a yellow product of 3,5-diacetyl-1,4-dihydrolutidine, was frequently used for spectrophotometric [14, 15] ($\lambda = 412$ nm) and fluorometric ($\lambda_{ex} = 420$ nm, $\lambda_{em} = 505$ nm) detection of formaldehyde [15, 16]. In this study, however, the Hantzsch reaction was modified to be applied to the determination of ammonia by using a flow injection analysis with a fluorometric detector. The condensation reaction of ammonia with formaldehyde and acetylacetone is shown in Scheme 1.
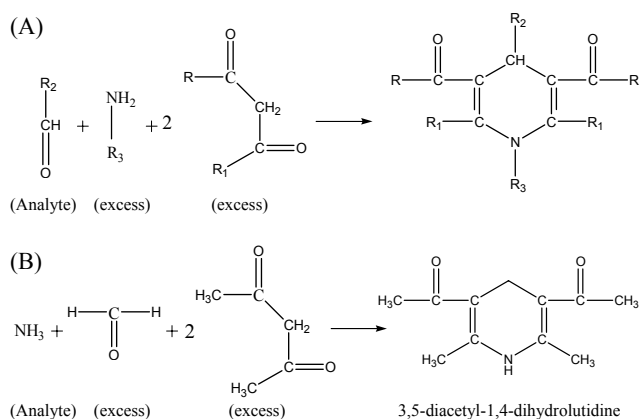
2.4 Flow injection procedure for the determination of ammonia in aqueous solutions

A diagram 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 to introduce the working standard solutions of ammonia into the carrier stream for the preparation of a calibration graph. The standard ammonia solution was mixed with the reagent solutions, and flowed into the reaction coil (RC), which was wound round an aluminum cylindrical block controlled at 70°C. At the downstream of the reaction coil, the solution was cooled down to a room temperature with a water bath. Fluorescence changes of the reaction product were measured with the fluorescence detector: an excitation and an emission wavelength were 420 nm and 505 nm, respectively. The flow signals were recorded with a Toa Dempa recorder.

3. Results and discussion

3.1 Optimization of experimental variables for the flow injection method

The Hantzsch reaction with acetylacetone has sometimes been applied to flow-injection determination of aldehyde, since it has several possible advantages. However, it has never been applied to the flow injection determination of ammonia. In this work, the optimal conditions for ammonia determination were examined with respect of improving the sensitivity of ammonia determination under moderate conditions.

The optimization of experimental parameters was performed using the FIA manifold with fluorometric detection in Fig. 1. In the reaction of ammonia with the reagents, a reaction time is very important for improving the reaction efficiency of ammonia. The effect of the flow rate of the carrier and the reagent solution was investigated in the range of 0.3 to 0.6 ml min^{-1}. The results obtained (Fig. 2) indicate that when the flow rate was slowest, 0.3 ml min^{-1}, the highest peak height was obtained and the peak height decreased with increasing the flow rate because the reaction time was not sufficient. In order to obtain higher sensitivity, each flow rate of 0.3 ml min^{-1} was adopted for further studies.
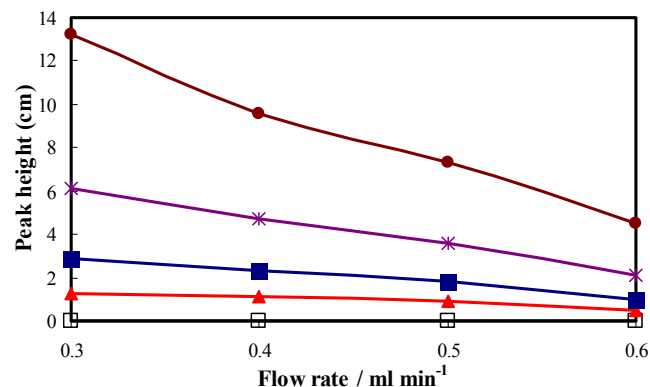

Scheme 1 Hantzsch reaction (A) aldehyde determination; (B) ammonia determination.

Fig. 2 Effect of flow rate on signal intensity of ammonia. Ammonia concentration: □ : blank; ▲ : 1x10^{-5} M; ■ : 2x10^{-5} M; ∗ : 4x10^{-5} M; ● : 8x10^{-5} M.

The sample injection volumes of 100, 150, 200, 250, 300, 350 and 400 µl were tested by changing the length of the sample loop of the injection valve. From the results obtained (Fig. 3), it can be seen that larger volumes were preferable to obtain higher peak, and the volumes above 300 µl gave almost identical peak height: a sample volume of 300 µl was adopted for the highest sensitivity.
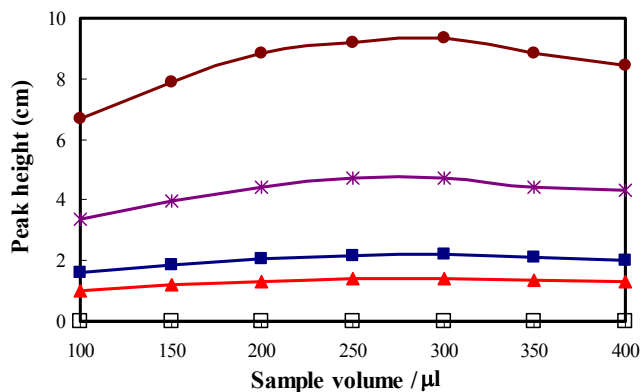

Fig. 3 Effect of sample volume on signal intensity of ammonia. Ammonia concentration: □ : blank; ▲ : 1×10^{-5} M; ■ : 2×10^{-5} M; ∗ : 4×10^{-5} M; ● : 8×10^{-5} M.

The effect of mixing coil length was examined by varying the length from 3 m to 12 m. As shown in Fig. 4, the signal intensity increased with increasing the mixing coil length to 10 m, and above it signal intensity was almost identical. A reaction coil length of 8 m was chosen as a compromise with respect of the sensitivity and the sample throughput.
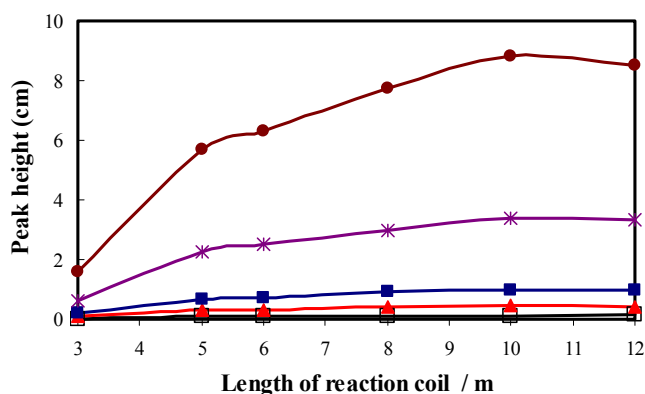

Fig. 4 Effect of reaction coil length on signal intensity of ammonia. Ammonia concentration: □ : blank; ▲ : 1×10^{-5} M; ■ : 2×10^{-5} M; ∗ : 4×10^{-5} M; ● : 8×10^{-5} M.

The effect of reaction temperature on signal intensity was examined by varying the temperature from 25 to 80 ºC using the dry heating bath. The results obtained are shown in Fig. 5. The peak height increased with increasing the temperature at the reaction coil up to 70 ºC, beyond which signal intensity remained constant. Thus, the temperature of 70 ºC was adopted for further studies.
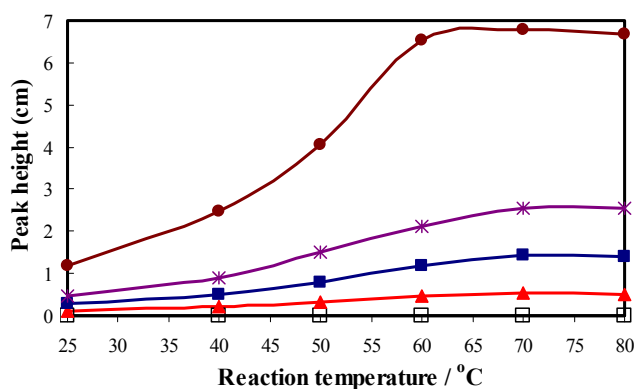

Fig. 5 Effect of reaction temperature on signal intensity of ammonia. Ammonia concentration: □ : blank; ▲ : 1×10^{-5} M; ■ : 2×10^{-5} M; ∗ : 4×10^{-5} M; ● : 8×10^{-5} M.

The acetylacetone concentration was examined in the range of 0.1 to 0.48 M. The results obtained (Fig. 6) showed that the signal intensity increased with increasing the acetylacetone concentration up to 0.3 M, beyond which signal intensity was identical. Thus, acetylacetone concentration of 0.3 M was chosen.
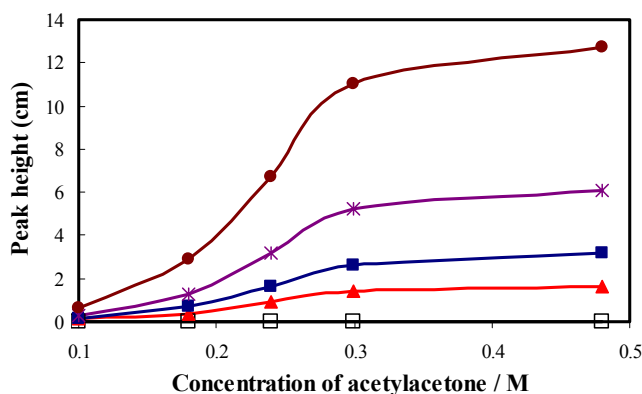

Fig. 6 Effect of acetylacetone concentration on signal intensity. Ammonia concentration: □ : blank; ▲ : 1×10^{-5} M; ■ : 2×10^{-5} M; ∗ : 4×10^{-5} M; ● : 8×10^{-5} M.

The effect of formaldehyde concentration was also examined by varying the concentrations from 0.065 to 0.26 M. The results obtained (Fig. 7) showed that the peak height increased with increasing formaldehyde concentrations up to 0.13 M, and above 0.16 M the peak height dramatically decreased: this is probably because the side reaction of formaldehyde with acetylacetone increases. In the present experiment, a formaldehyde concentration, 0.13 M, was selected.
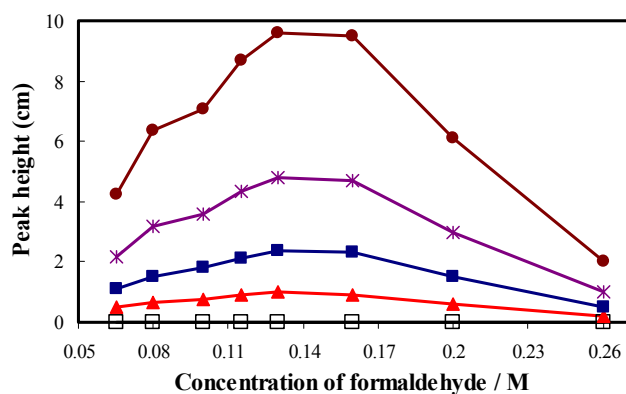
Fig. 7 Effect of formaldehyde concentration on signal intensity of ammonia. Ammonia concentration: □ : blank; ▲: 1×10^{-5} M; ■ : 2×10^{-5} M; ∗ : 4×10^{-5} M; ● : 8×10^{-5} M.

Fig. 9 Effect of the concentration of buffer component on signal intensity of ammonia. Ammonia concentration: □ : blank; ▲: 1×10^{-5} M; ■ : 2×10^{-5} M; ∗ : 4×10^{-5} M; ● : 8×10^{-5} M.

Since pH of the solution can affect the acceleration of the Hanzsch reaction, several kinds of buffer solutions with various pHs were examined: they are an acetate buffer, a phosphate buffer and a Good's buffer. The results obtained (Fig. 8) showed that the pH range of 6.0 to 6.4 gave the highest and identical peak height. The phosphate buffer was found to give the lowest blank peak. Thus the phosphate buffer was chosen. The pH of 6.3 was chosen in the present studies.
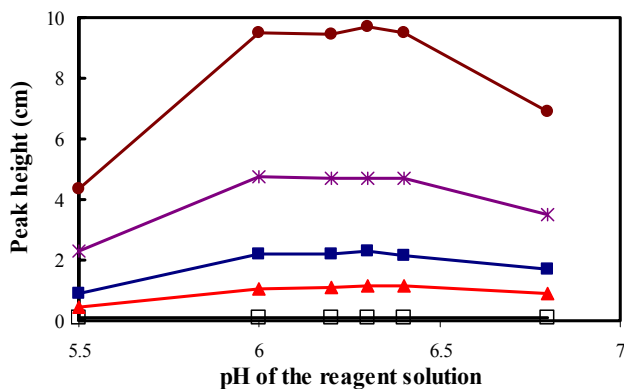
3.2 Interference of coexisting substances

The investigation of possible interferences was conducted with regard to possible chemical interferences and the problems of selectivity. The results obtained are summarized in Table 1, where the tolerable concentrations were defined as the concentration of a foreign species causing less than ±5% relative error. The results revealed that there is no serious interference from most of cations and anions, except for Cu^{2+}, Fe^{2+} and Fe^{3+}, which could be masked with EDTA.

Table 1 Tolerable concentrations of foreign ions in the determination of 1×10^{-5} M ammonia

Foreign ions	Added as	*Tolerable conc. limit (M)
Na^+	NaCl	2×10^{-2}
K^+	KCl	2×10^{-2}
Mg^{2+}	$MgCl_2$	4×10^{-3}
Ca^{2+}	$CaCl_2$	6×10^{-4}
Ni^{2+}	$NiCl_2$	1×10^{-4}
Cu^{2+}	$CuCl_2$	2×10^{-5}
Fe^{2+}	$FeSO_4$	2×10^{-6}
Fe^{3+}	$Fe(NO_3)_3$	2×10^{-6}
NO_3^-	$NaNO_3$	2×10^{-3}
SO_4^{2-}	Na_2SO_4	4×10^{-3}
SO_3^{2-}	Na_2SO_3	5×10^{-5}
CO_3^{2-}	Na_2CO_3	5×10^{-3}

*Defined as the concentration causing less than ±5% relative error.

Fig. 8 Effect of pH of the reagent solution on signal intensity of ammonia. Ammonia concentration: □ : blank; ▲: 1×10^{-5} M; ■ : 2×10^{-5} M; ∗ : 4×10^{-5} M; ● : 8×10^{-5} M.

The effect of phosphate buffer concentration was studied by varying the concentrations from 0.2 to 0.8 M, while the acetylacetone concentration and pH were kept constant at 0.24 M and 6.3, respectively. As shown in Fig. 9, the buffer concentrations did not affect the peak intensity. Considering the buffer capacity and shorter blank peak, 0.4 M was chosen.
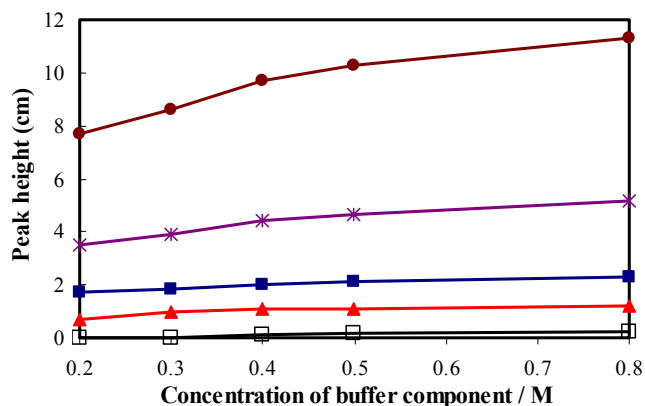
3.3 Analytical characteristics

In this work, the collection of ammonia in air samples into water was examined by a batchwise collection, and the ammonia

collected in the absorbing solution was measured by the proposed FIA. More than 99% ammonia in the air samples was collected in ultrapurified water, and therefore the standard aqueous solutions of ammonium chloride were used for preparing the calibration graph at the concentration range of 1 to 50×10^{-6} M. The peak profile of ammonia is shown in Fig. 10. The equation of the calibration graph was expressed as $Y = 0.2663X$, where Y was peak height (cm) and X was ammonia concentration in 10^{-6} M. The correlation coefficient was 0.9995 and the relative standard deviation (RSD) was less than 0.6% for 1×10^{-5} M ammonia (n=8). The limit of detection (LOD) was 10^{-7} M, and the sample throughput was 21 injections h^{-1}.

Fig. 11 Effect of shaking time on the collection of ammonia from air by a batchwise collection method.
Absorbing solution: ultrapurified water; volume of absorbing solution: 3 ml; volume of air sample: 68.9 ±0.4 ml; air sample: sampled in our lab on November 4, 2004.

3.5 Application of the proposed method to real samples

The proposed method was applied to the determination of ammonia in indoor and outdoor air. The results obtained by the proposed method are shown in Table 2 with enough sensitivity and precision. Since the allowed concentration of air NH$_3$ is 1-5 ppm [17], our results from indoor air are a little higher than this level. It is possible due to the emission from building materials, persons in the house, etc.

Table 2   Analytical results of ammonia in air samples

Sampling location	Ammonia conc.*1 / μM	Ammonia*2 / μg l^{-1}
Laboratory A	7.00 ± 0.10	5.20 ± 0.10
Laboratory B	5.22 ± 0.05	3.86 ± 0.04
Outdoor A	5.50 ± 0.10	4.10 ± 0.10
Outdoor B	6.30 ± 0.10	4.66 ± 0.09

∗1   Air samples were sampled at Faculty of Science, Okayama University, on November 22, 2004. Air sample volume: 68.9 ml; absorbing solution: 3 ml of ultrapurified water; ammonia concentration in absorbing solution.
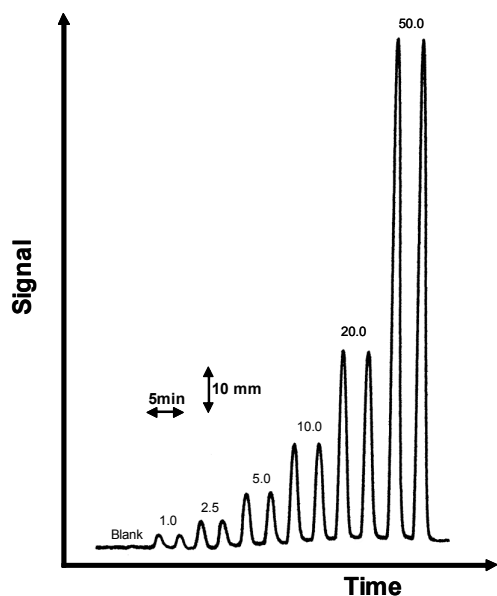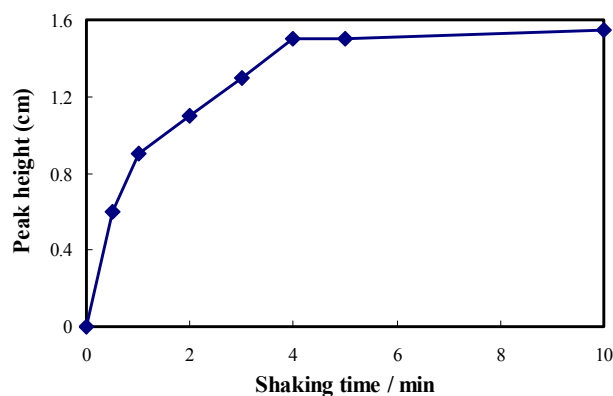∗2   Ammonia concentrations in air.

Fig. 10 Flow signals for ammonia determination.
Carrier solution: ultrapurified water; reagent solution: 0.3 M acetylacetone, 0.13 M formaldehyde and 0.4 M phosphate buffer at pH 6.3; ammonium chloride concentration: $0-50 \times 10^{-6}$ M; sample injection volume: 300 μl; temperature: 70 °C.

3.4 Optimization of experimental variables of batchwise collection/concentration method

The batchwise collection method for air pollutants, such as formaldehyde [14, 15] and ammonia [17] was reported by using a plastic syringe. However, it was necessary for examining the collection efficiency of ammonia in air for determining ammonia in air by FIA. The effect of shaking time on the absorption efficiency of ammonia from air sample into the purified water was examined from 1 to 10 min, using 3.00 ml of the purified water as an absorbing solution in a 50-ml plastic syringe (the accurate volume: 71.92±0.43 ml). The result obtained (Fig. 11) shows that the peak height became almost constant when the shaking time was longer than 4 min, which suggests that ammonia from the air sample was completely transferred into the absorbing solution after shaking for more than 4 min. Therefore, in the present study, 5 min was selected.

4. Conclusion

In the present work, a new fluorometric method based on the Hantzsch reaction for ammonia determination was developed using flow injection (FI) technique. The FI fluorometric method proposed here has advantages in the simplicity, rapidity and sensitivity of the method and in the use of non-toxic reagents. The FI fluorometric method can be successfully applied to the determination of ammonia in environmental air samples, as well as in aquatic environment.

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