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Spectrophotometric determination of ammonia nitrogen in water by flow injection analysis based on NH_3- *o*-phthalaldehyde -Na_2SO_3 reaction

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ABSTRACT

The product of the NH_3- *o*-phthalaldehyde (OPA) -Na_2SO_3 reaction is rose red at pH more than 10.4, and its maximum absorption wavelength is 550 nm. Based on this, a novel spectrophotometric method with flow injection analysis has been established to determine ammonia nitrogen in water. Experimental parameters related to the flow injection method and the reaction were optimized throughout the experiments based on univariate experimental design. The length of coil is optimized as 1.6 m for storing up standard or sample solution. The optimal value is 8.20 mL/min for loading flow rate of coloring solution. The OPA concentration, sulfite concentration and reaction pH are chosen as 1.06 g/L, 0.050 g/L and 10.80, respectively. The reaction temperature and stop flow time affects the performances of the method. The linearity range and detection limit of the proposed method are 0.100–0.700 mmol/L and 0.007 mmol/L at the reaction temperature of 55 °C and stop flow time of 340 s, respectively. The sample throughput is more than 8 h^{-1}. Under the optimal experimental conditions, the recovery is 100.4%, 95.2%, 101.7% and 92.4% for the lake water, river water, groundwater and sewage, respectively. Two lake water samples were analyzed using both the proposed method and indophenol blue method, the results show no significant difference between these two methods. In comparison with other spectrophotometric determination method of ammonia nitrogen, the main merits of the proposed method are simplicity, reliability, reproducibility and high sample throughput.

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1. Introduction

Ammonia nitrogen is one of the major nitrogen forms in the nitrogen cycle, especially in natural waters [1]. Ammonia nitrogen consists of ammonia (NH_3) and ammonium (NH_4^+) in natural waters. Ammonium is predominant when the pH is below 8.75, and ammonia is predominant when the pH is above 9.75 [2]. The biogeochemical cycle of nitrogen is one of the main research topics in environment science [3], and accurate analysis of the ammonia nitrogen in various water samples is thus very important.

The common methods for quantitative analysis of ammonia nitrogen in natural water are the indophenol blue (IPB) spectrophotometric methods and *o*-phthalaldehyde (OPA) fluorometric methods [4]. The IPB spectrophotometric methods are based on the Berthelot reaction [5–11]. In the catalysis of nitroprusside, ammonia nitrogen reacts with hypochlorite and phenol forming indophenol blue, which has a maximum absorbance of 640 nm. The hypochlorite solution must be prepared just before use because it is unstable. The equilibration time of the Berthelot reaction is more than 120 min at room temperature [9]. Several on-line IPB methods [10,11] were reported, but not widely adopted in field due to the defects of the reaction. The OPA fluorometric methods are based on the reaction of NH_3-OPA-Na_2SO_3 [12–20]. Since it was firstly reported that OPA could react with amino acid and ammonia nitrogen in the existence of mercaptoethanol producing a strongly fluorescent compound in 1971 [15], the OPA fluorometric method had never stopped being reported [16]. The OPA fluorometric method has many merits, such as good selectivity, low to nanomolar's detection limit, not difficult to operate and so on. It was widely applied to determine trace and ultra trace level of ammonia nitrogen in seawater, especially in open ocean seawater [12,17–20]. However, if the OPA fluorometric method is used to analyze

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continental water where ammonia nitrogen is usually more than micromolar level, even up to millimolar level [21], the water sample must be diluted many times to fit the linearity range of the method. Too much dilution leads to serious errors, so the OPA fluorometric method is not usually fit for micromolar and millimolar level analysis of ammonia nitrogen. It is necessary to develop a novel method for high concentration ammonia nitrogen samples.

It was found that the product of the NH_3-OPA-Na_2SO_3 reaction was not only fluorescent but also colored. It is rose red at pH more than 10.4. Sensibility of spectrophotometric method is approximately three orders of magnitude less than that of the fluorescence method, so the OPA spectrophotometric method is theoretically fit to analyze the continental water with high concentration ammonia nitrogen. Flow injection analysis (FIA) has many merits, such as high sample throughput, minimum sample and reagent use, high precision measurement, and so on [22]. It was widely used in environmental monitoring [23]. Based on the above mentioned, a novel spectrophotometric method with FIA has been established.

2. Experimental

2.1. Reagents and solutions

All the chemicals used in this study were of analytical grade, supplied by Aladdin Chemical Reagent Co., China, unless stated otherwise. All solutions were prepared in ultrapure water (resistivity ≥18.2 MΩ cm at 25 °C).

Ammonia Nitrogen Standard Solution. Ammonia nitrogen standard stock solution (20 mmol/L) was made by dissolving 0.6608 g of ammonium sulfate dried for at least 2 h at 110 °C in 500 mL ultrapure water, stored at 4 °C in a refrigerator while not in use. Ammonia nitrogen standard solution was made by diluting the stock solution.

OPA-Na_2SO_3 Mixed Reagent Solution.10.6 g/L OPA solution (Reagent A) was made by dissolving 2.65 g of OPA in 50 mL of methanol (HPLC grade) and diluting to 250 mL with ultrapure water. 1.0 g/L sodium sulfite solution (Reagent B) was made by dissolving 0.5 g of Na_2SO_3 in 500 mL ultrapure water and adding 0.20 mL HCHO to prevent the solution from being oxidized. Reagent A and Reagent B were stored at 4 °C in a refrigerator while not in use. OPA- Na_2SO_3 mixed reagent solution was daily made by diluting the mixture of a certain number of Reagent A and a certain number of Reagent B to 250 mL with ultrapure water.

EDTA-NaOH Buffer Solution. EDTA-NaOH buffer solution was made by dissolving 25.5 g disodium ethylenediaminetetraacetate (EDTA, ACS grade) and a certain number of NaOH (ACS grade) in 500 mL ultrapure water.

2.2. Apparatus

A FIA system was fabricated with the following parts: a FIA-3110 FIA processor (Beijing Instrument Co., Ltd., Beijing, China) including two 7-channel peristaltic pumps and a 8-way rotary valve; a thermostatic water bath (Shanghai He De Experimental Equipment Co., Ltd., Shanghai, China); and a UV-2550 spectrophotometer (Shimadzu Co., Japan) equipped with a 1 cm × 1 mm (i.d.) optical flow cell.

2.3. Flow injection manifold and procedures

The arrangement of the FIA system is shown in Fig. 1. All the pump tubing is silicon-latex, the other tubing is made of PTFE. Before analysis, injection tube was put into an ammonia nitrogen standard solution (or water sample), and coil 2 was heated in water bath. The detection wavelength of the spectrophotometer was set as 550 nm. Once starting the FIA program, the instrument run according to the steps listed in Table 1. In step 1, the valve was switched to “Fill” position (see Fig. 1 (a)). Ammonia nitrogen standard solution (or water sample) and ultrapure water were pumped to each tube at the flow rate of 5.0 mL/min by the pump 1.

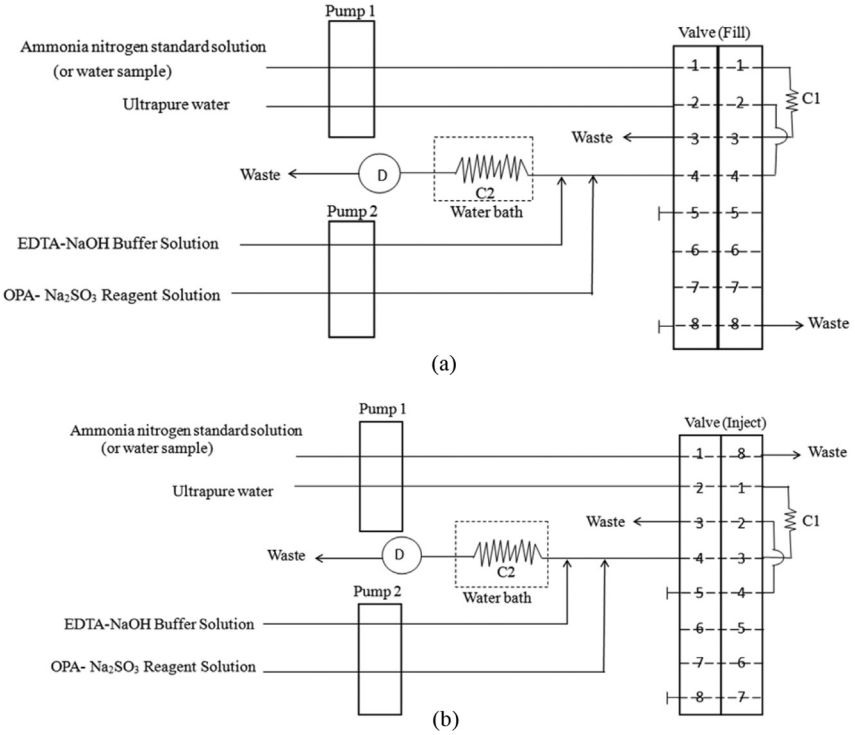

Fig. 1. Schematic diagram of the FIA system (a) Fill, (b) Inject, D: UV–Vis spectrophotometer detector, C1: Coil 1, C2: Coil 2.

Table 1
Flow injection program.

Step	Time(s)	Valve	Flow rate (Pump1) (mL/min)	Flow rate (Pump 2) (mL/min)	Illustration
1	40	Fill	5.0 (ultrapure water, ammonia nitrogen standard solution (or water sample))	0	Coil 1 was filled with ammonia nitrogen standard solution (or water sample), the spectrophotometer was set zero.
2	37	Inject	2.6 (ultrapure water)	2.6 (OPA- Na_2SO_3 mixed reagent solution, EDTA-NaOH buffer solution)	OPA- Na_2SO_3 mixed reagent solution and EDTA-NaOH buffer solution mixed with ammonia nitrogen standard solution (or water sample) in the coil 2.
3	0–340	Inject	0	0	Stopped to react
4	50	Fill	8.2 (ultrapure water)	0	Detecting

Coil 1 was filled with ammonia nitrogen standard solution (or water sample). Ultrapure water was pumped through the flow cell of the spectrophotometer, the absorbance value (A) of the spectrophotometer was set to zero at the moment. In step 2, the injection valve switched to the "Inject" position (see Fig. 1 (b)). Pump 1 and Pump 2 run at the same time. Ammonia nitrogen standard solution (or water sample) stored in coil 1 was pushed forward by ultrapure water at the flow rate of 2.6 mL/min (called sample flow). At the same time, OPA-Na_2SO_3 mixed reagent solution and EDTA-NaOH buffer solution were separately pumped to join the sample flow in front of Coil 2, and mixed with ammonia nitrogen standard solution (or water sample) in the Coil 2. The mixed solution in coil 2 stopped to be heated for a certain time at a certain temperature, and ammonia nitrogen reacted with OPA and Na_2SO_3 forming rose red matter in step 3. After the stopped flow time, the injection valve switched to "Fill" position again, and pump 1 started. The coloring solution was propelled through the flow cell by ultrapure water, the absorbance (A) was detected by the spectrophotometer at 550 nm, and the detector output was continuously recorded with a computer as a flow curve. The peak height (H_A) of the curve was used to quantify the concentration of ammonia nitrogen in water sample. The ammonia nitrogen standard solutions were determined from low to high concentration. One or two blank samples should be run after a higher concentration sample. A standard solution was used as a quality control sample to check the signal every 20 injections. If the signal of the quality control sample changed more than 10% in comparison with the previous average value, the system should be checked and adjusted. The samples between this two control samples should be analyzed again.

3. Results and discussion

3.1. Parameters optimization

Some parameters, including detection wavelength, reagent concentrations, reaction time, pH and those related to the FI method, were investigated and discussed based on univariate experimental design. In this section, the stop flow time and temperature was set as 160 s and 55 °C, respectively.

3.1.1. Absorption spectra of the reaction solution

According to section 2.3, 0.500 mmol/L ammonia nitrogen standard solution was allowed to react with OPA and sodium sulfite forming coloring matter. The rose red solution flowing from the detector was collected using a 1 cm cuvette in step 4. The absorbance of the rose red solution was detected at wavelength of 450–600 nm by the spectrophotometer, and the absorption spectra was obtained as Fig. 2. An approximate absorption platform is observed in the range of 520–560 nm, and the maximum absorbance appears at 550 nm. So 550 nm is chosen as detection wavelength of this proposed method.

3.1.2. Effect of OPA and Na_2SO_3 concentrations

OPA and Na_2SO_3 are the main reagents of the NH_3-OPA-Na_2SO_3 reaction. The effect of OPA and Na_2SO_3 concentrations on the reaction were separately investigated below.

To investigate the effect of OPA concentration on the reaction, seven aliquots of OPA-Na_2SO_3 mixed reagent solutions with OPA concentration in the range of 0.106–1.272 g/L and Na_2SO_3 concentration of 0.050 g/L were prepared, and separately allowed to react with 0.500 mmol/L ammonia nitrogen standard solution according to the procedures described in section 2.3. Thus, the OPA concentrations in the reaction solution ranged from 0.035 to 0.424 g/L. Seven flow curves were recorded. The relationship of H_A of each curve and OPA concentration in the OPA-Na_2SO_3 mixed reagent (or in the reaction solution) is shown in Fig. 3. H_A firstly increases as OPA concentration increasing from 0.106 to 0.848 g/L in the OPA-Na_2SO_3 mixed reagent, and then kept stable in the range of 0.848–1.272 g/L. It illuminates that more than 0.848 g/L OPA in the reagent is enough to the NH_3-OPA-Na_2SO_3 reaction. Consequently, an OPA concentration of 1.06 g/L in the OPA- Na_2SO_3 mixed reagent solution is chosen for the following experiments, which means that OPA concentration is 0.353 g/L in the reaction solution.

To investigate the effect of Na_2SO_3 concentration on the reaction, Na_2SO_3 concentration in the OPA-Na_2SO_3 mixed reagent solution was changed from 0.005 to 0.070 g/L, and OPA concentration was fixed at 1.06 g/L. The other parameters were controlled as description in section 2.3. The relationship of H_A and Na_2SO_3 concentration was studied. The results are displayed in Fig. 4. H_A increases as the Na_2SO_3 concentration increasing from 0.005 to 0.040 g/L. While the Na_2SO_3 concentration is in the range of 0.040–0.070 g/L, H_A does not change as the Na_2SO_3 concentration increasing. 0.050 g/L Na_2SO_3 concentration is chosen as the optimal value in the OPA-Na_2SO_3 mixed reagent solution, illuminating that the concentration of Na_2SO_3 is 0.0167 g/L in the reaction solution.

3.1.3. Effect of pH

The NH_3-OPA-Na_2SO_3 reaction takes place under alkaline conditions [15]. Precipitation usually occurs under pH more than 10.4

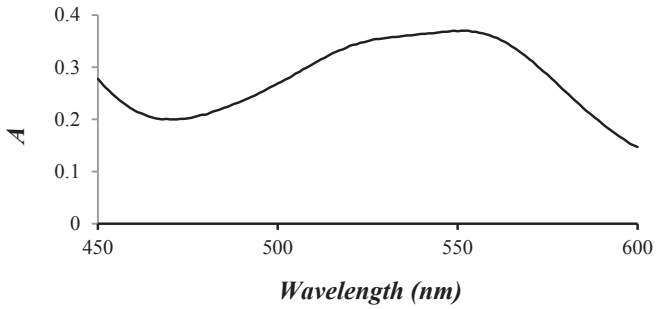

Fig. 2. Absorption spectra of the reaction solution.

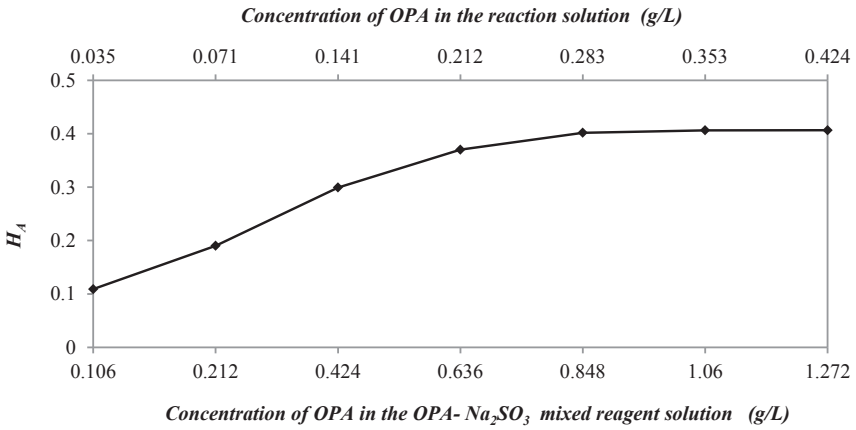

Fig. 3. Effect of OPA concentration on H_A.

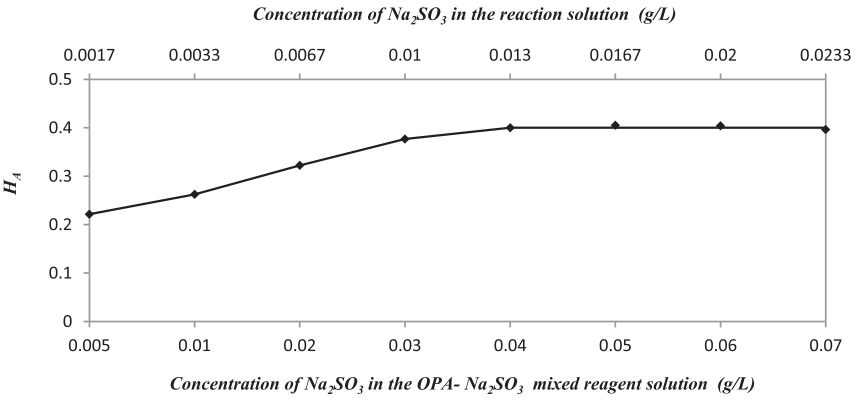

Fig. 4. Effect of Na_2SO_3 concentration on H_A.

condition in natural water sample in absence of chelating agent. According to our previous work [16], EDTA-NaOH buffer can be used to avoid the precipitation appearing and control the reaction pH. When the concentration of EDTA in the reaction solution is more than 15 g/L, precipitation do not appear in natural water samples, such as river water, mountain spring water and groundwater. EDTA concentration of 17 g/L in the reaction solution is controlled in this proposed method.

To investigate the effect of the pH on H_A, the pH was changed in the range of 8.9–10.95 by altering the concentration of NaOH in the EDTA-NaOH buffer solution. The H_A was detected according to the procedures in section 2.3 under different pH conditions. The relationship of the H_A and pH is illuminated in Fig. 5. As shown in Fig. 5, the H_A fast increases as pH changing from 8.9 to 10.77, and then closes to maximal constant in the pH range of 10.77–10.95. To gain better method sensitivity, the pH of the reaction solution should be controlled in the range of 10.77–10.95. When the concentrations of EDTA and NaOH are prepared as 51 g/L and 20 g/L in the EDTA-NaOH buffer solution, the pH and EDTA concentration of the reaction solution can be controlled as the optimal value above described.

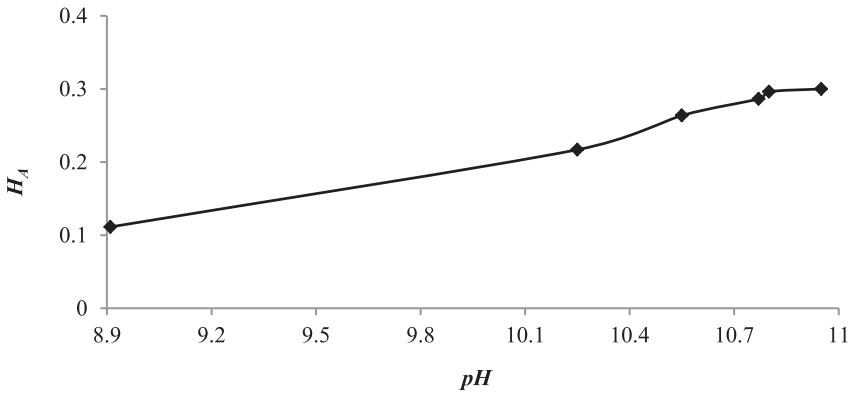

Fig. 5. Effect of pH on H_A.

3.1.4. Effect of coil 1 length

In the proposed analysis procedures, ammonia nitrogen standard solution (or water sample) was stored in coil 1, then was propelled by ultrapure water to mix and react with reagent solution, and was detected by UV–Vis spectrophotometer. During the process, diffusion occurs on the interface between ultrapure water and ammonia nitrogen standard solution (or water sample), and affects the signals of flow curve. Theoretically, diffusion would not affect the H_A of flow curves when coil 1 is long enough. To make sure how long coil 1 should be used, the effect of coil 1 length on the H_A of flow curves was studied in the range of 0.6–2.0 m. The result is shown as Fig. 6. When the length of coil 1 is more than 1.4 m, H_A tended to maximum constant, and the diffusion on the interface does not affect the concentration of ammonia nitrogen standard solution (or water sample) in the middle part of coil 1. The longer of coil 1, the larger volume of ammonia nitrogen standard solution (or water sample) required, and the more analysis time required. By balancing H_A, analysis time and reproducibility, coil 1 length of 1.6 m is selected in this method.

3.1.5. Reaction temperature and time

Reaction temperature depends on the heating temperature of coil 2, affects the rate and equilibration time of the NH_3-OPA-Na_2SO_3 reaction. Stop flow time decides how long the reaction can last and thus affects the extent of the reaction. The flow curves of 0.500 mmol/L ammonia nitrogen standard solution were determined under different reaction temperature and different stop flow time. The relationships between H_A and stop flow time in different reaction temperature are displayed in Fig. 7, where curve a, b, c, d and e are the results under 27, 35, 45, 55 and 65 °C conditions, respectively. H_A increases as the stop flow time ranging from 0 to 580s in curve a, b and c, and firstly increases and then closes to constant in curve d and e. This means that the reaction equilibration times are 220 s, 340 s and more than 580 s at the heating temperatures of 65 °C, 55 °C and less than 45 °C, respectively. It is obvious that heating can accelerate the reaction. H_A increases as heating temperature increasing in the range of 27–55 °C at the same stop flow time. However, H_A at 65 °C was slightly less than that at 55 °C when the stop flow time was more than 300 s, due to the coloring solution fading at 65 °C. Besides, a number of bubbles appears in the tube, and disturb the determination at 65 °C. Therefore, the heating temperature of coil 2 had better set as less than 55 °C. To get better method sensitivity, 55 °C is chosen as the heating temperature in this work.

The stop flow time can be accurately controlled by the FIA instrument, ensuring that the reaction product can be measured in a non-equilibrium condition. As above described, the reaction equilibration time is 340 s at 55 °C. The stop flow time in the range of 0–340 s can be chosen depending on the ammonia nitrogen concentration in water samples. In detail, higher concentration ammonia nitrogen samples should use shorter stop flow time and vice versa.

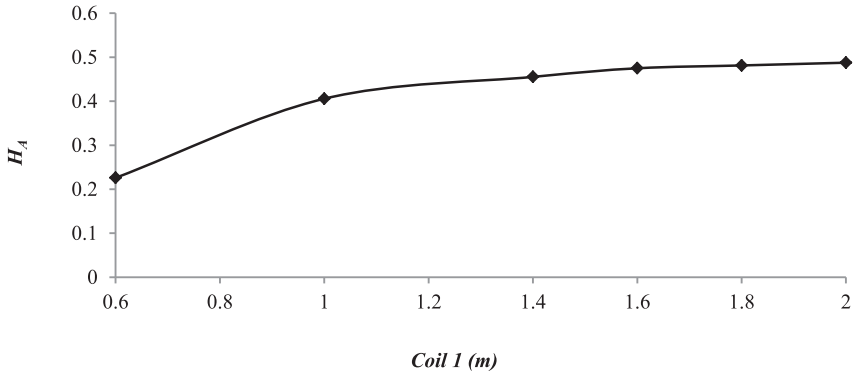

Fig. 6. Effect of coil 1 on H_A.

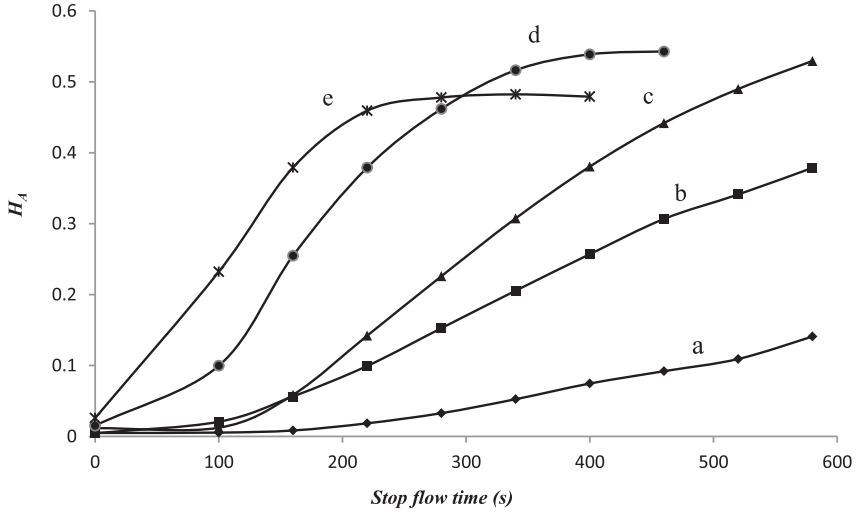

Fig. 7. Effect of different stop flow time in different reaction temperature on H_A. (a) 27 °C, (b) 35 °C, (c) 45 °C, (d) 55 °C, (e) 65 °C.

3.1.6. Coloring solution loading flow rate

Coloring solution loading flow rate is the flow rate of coloring solution propelled by ultrapure water at step 4 in Table 1. The flow curves of 0.900 mmol/L ammonia nitrogen standard solution were measured at different coloring solution loading flow rate, and are shown in Fig. 8. As shown in Fig. 8, the flow curve becomes more and more narrow as the flow rate ranging from 2.4 to 8.2 mL/min, and H_A of the flow curve slightly increased as the flow rate increasing. To get more sample throughput and better sensitivity, 8.2 mL/min is selected as the coloring solution loading flow rate in this work.

3.2. Calibration curves and method detection limit

Under the optimal conditions chosen above, the typical calibration curves were determined at 55 °C. The flow curves of different concentration of ammonia nitrogen standard solution are shown in Fig. 9 at stop flow time of 160 s. H_A is linear with the concentration of ammonia nitrogen (C_N) in the range of 0.100−0.900 mmol/L, the equation of correlation is $H_A = 0.6831C_N$-0.0725 (n = 6, R^2 = 0.9953). The calibration curves were also determined at stop flow time of 250 s and 340 s. The results are listed in Table 2. The linearity ranges were 0.100−0.900, 0.100−0.900 and 0.100−0.700 mmol/L at the stop flow time of 160 s, 250 s and 340 s, respectively. The method detection limits, estimated as the ratio of 0.01 to the slope of calibration curves [24] are 0.015, 0.012 and 0.007 mmol/L, respectively. The corresponding sample throughput were 12, 10 and 8 h^{-1}, respectively. Ammonia nitrogen concentration regulated in the first level criterion for surface water quality in China (GB 3838-2002) is 0.011 mmol/L. So the proposed method is available for the determination of ammonia nitrogen concentration in most of surface water.

3.3. Validation of the method

3.3.1. Recovery

A series of fresh water samples, such as lake water, river water and groundwater collected at Yaoshan Scenic Area in Guilin, and sewage removed ammonia nitrogen were spiked with ammonia nitrogen at 0.100, 0.200, 0.300, 0.500 and 0.700 mmol/L, respectively. The spiked samples were analyzed using the proposed method at stop flow time of 160 s, together with the calibration curve. The linear equations of the matrix spike curves and corresponding calibration curves are showed in Table 3. The average

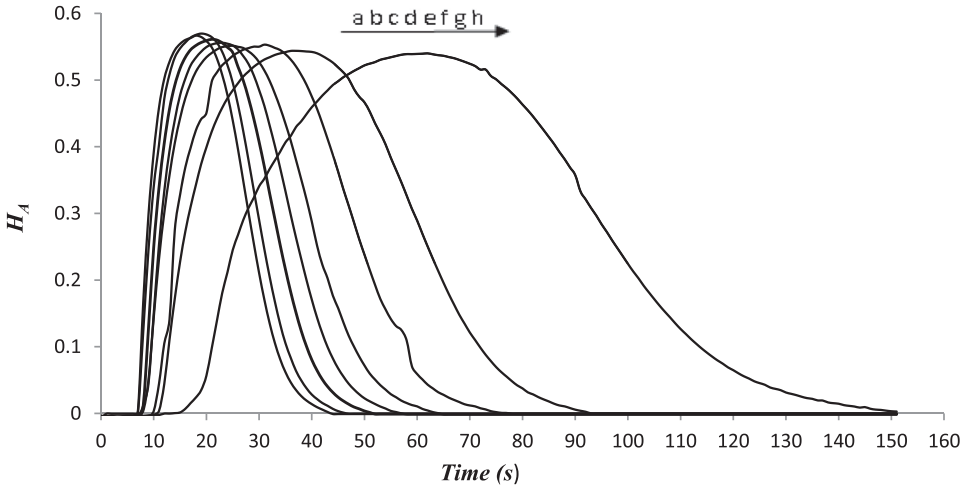

Fig. 8. Effect of coloring solution loading flow rate on the flow curves. (a) 8.2 mL/min, (b) 8.0 mL/min, (c) 7.4 mL/min, (d) 6.6 mL/min, (e) 5.8 mL/min, (f) 4.9 mL/min, (g) 3.7 mL/min, (h) 2.4 mL/min.

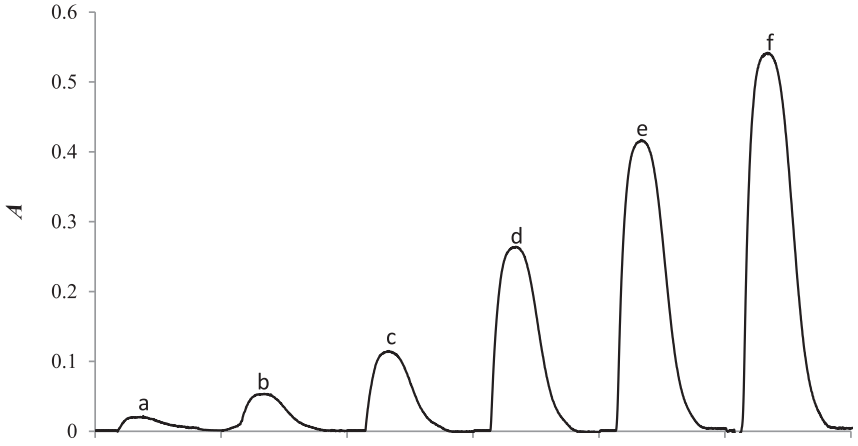

Fig. 9. The flow curves of different concentrations of ammonia nitrogen (a) 0.000 mmol/L, (b) 0.100 mmol/L, (c) 0.300 mmol/L, (d) 0.500 mmol/L, (e) 0.700 mmol/L, (f) 0.900 mmol/L.

Table 2
Calibration curves and the corresponding performances.

Stop flow time	Calibration Curves	n	R^2	Detection limit (mmol/L)	Linearity range (mmol/L)	Throughput (h^{-1})
160s	$H_A = 0.6831C_N$ - 0.0725	6	0.9953	0.015	0.100–0.900	12
250s	$H_A = 0.8456C_N$ - 0.0806	6	0.9929	0.012	0.100–0.900	10
340s	$H_A = 1.4358C_N$ - 0.1553	5	0.9924	0.007	0.100–0.700	8

Table 3
The matrix spiked recovery.

Matrix	Matrix spiked curve	Corresponding calibration curve	The average matrix spiked recovery
Lake water	$H_A = 0.7343C_N - 0.0614$ ($n = 5$, $R^2 = 0.9969$)	$H_A = 0.7314C_N - 0.0654$ ($n = 5$, $R^2 = 0.9952$)	100.40%
River water	$H_A = 0.5735C_N - 0.046$ ($n = 5$, $R^2 = 0.997$)	$H_A = 0.6025C_N - 0.0455$ ($n = 5$, $R^2 = 0.9957$)	95.19%
Groundwater	$H_A = 0.5719C_N - 0.0428$ ($n = 5$, $R^2 = 0.9957$)	$H_A = 0.5623C_N - 0.0347$ ($n = 5$, $R^2 = 0.9913$)	101.71%
Sewage	$H_A = 0.6048CN + 0.0021$ ($n = 5$, $R^2 = 0.9978$)	$H_A = 0.6546CN - 0.0566$ ($n = 5$, $R^2 = 0.9954$)	92.4%

Table 4
Analytical results of the proposed method and classical indophenol blue method.

Lake water	The proposed method (mmol/L)	The classical indophenol blue method (mmol/L)	Calculated *t*-value	Critical *t*-value ($P = 0.05$)
1	0.470 ± 0.000374 (n = 3)	0.484 ± 0.0367 (n = 3)	1.86	2.78
2	0.663 ± 0.00165 (n = 3)	0.671 ± 0.0276 (n = 3)	1.42	

recovery of the ammonia nitrogen in spiked samples is represented as the ratio of the slope of matrix spike curve to that of corresponding calibration curve [25]. As shown in Table 3, the average recovery is 100.4%, 95.2%, 101.7% and 92.4% for lake water, river water, groundwater and sewage, respectively. The average recovery between 90% and 110% illuminates that the matrix does not disturb the determination of ammonia nitrogen. The proposed method is available for both fresh water and sewage.

3.3.2. *Comparison with classical indophenol blue method*

Two lake water samples, collected from Huajian River in Guilin, were analyzed using the proposed method. At the same time, ammonia nitrogen concentrations were determined using indophenol blue method [4]. The results are compared in Table 4. Using the paired Student's *t*-test at 95% confidence level to test the difference between these two methods, the calculated *t*-values are lower than the critical *t*-value. This indicates that there was no statistically significant difference between the proposed method and classical indophenol blue method.

4. Conclusion

Ammonia nitrogen can rapidly react with OPA and Na_2SO_3 producing rose red matter under strong alkaline condition. Based on this, a novel FI method is established to determine ammonia nitrogen concentration in fresh water and sewage samples. The method detection limit is low to 0.007 mmol/L. The average recovery are 100.4%, 95.2%, 101.7% and 92.4% for lake water, river water, groundwater and sewage, respectively. The sample throughput is more than 8 h^{-1}. There are no significant difference between the results obtained from the proposed method and classical indophenol blue method. The method is available for most of surface water. In comparison with other spectrophotometric determination method of ammonia nitrogen, the main merits of the proposed method are simplicity, reliability, reproducibility and high sample throughput.

Acknowledgments

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