Volume 794 10 September 2013 ISSN 0003-2670

ANALYTICA CHIMICA ACTA

AN INTERNATIONAL JOURNAL DEVOTED TO ALL BRANCHES OF ANALYTICAL CHEMISTRY

Review Article

Applications of *in vivo* and *in vitro* solid-phase microextraction techniques in plant analysis: A review

Fang Zhu, Jianqiao Xu, Yuanyuan Ke, Siming Huang, Feng Zeng, Tiangang Luan and Gangfeng Ouyang

(Published on pp. 1–14 of this issue)

Analytica Chimica Acta 794 (2013) 47–54

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

A sensitive flow-batch system for on board determination of ultra-trace ammonium in seawater: Method development and shipboard application

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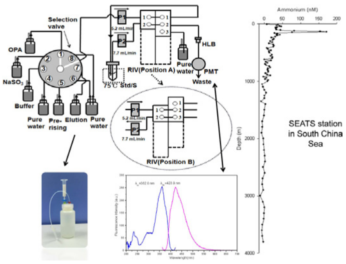
HIGHLIGHTS

- Fluorescence detection was coupled with flow analysis and solid phase extraction for ammonium determination.
- The solid phase extraction technique has been seldom used to enrich compounds for fluorescence detection.
- Different storage methods for ultra-trace ammonium samples were compared during the South China Sea survey.
- This method was applied on board to analyze seawater samples collected from 65 stations in the South China Sea.
- A detailed ammonium profile was obtained in the SEATS station.
- The proposed method offered the benefits of improved sensitivity, reduced reagent consumption, negligible salinity interference and lower cost.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 May 2013
Received in revised form 2 August 2013
Accepted 5 August 2013
Available online 9 August 2013

Keywords:
Ammonium
Seawater
Solid phase extraction
Fluorescence detection
South China Sea

ABSTRACT

Combining fluorescence detection with flow analysis and solid phase extraction (SPE), a highly sensitive and automatic flow system for measurement of ultra-trace ammonium in open ocean water was established. Determination was based on fluorescence detection of a typical product of *o*-phthaldialdehyde and ammonium. In this study, the fluorescence reaction product could be efficiently extracted onto an SPE cartridge (HLB, hydrophilic–lipophilic balance). The extracted fluorescence compounds were rapidly eluted with ethanol and directed into a flow cell for fluorescence detection. Compared with the common used fluorescence method, the proposed one offered the benefits of improved sensitivity, reduced reagent consumption, negligible salinity effect and lower cost. Experimental parameters were optimized using a univariate experimental design. Calibration curves, ranging from 1.67 to 300 nM, were obtained with different reaction times. The recoveries were between 89.5 and 96.5%, and the detection limits in land-based and shipboard laboratories were 0.7 and 1.2 nM, respectively. The relative standard deviation was 3.5% ($n = 5$) for an aged seawater sample spiked with 20 nM ammonium. Compared with the

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0003-2670/$ – see front matter
http://dx.doi.org/10.1016/j.aca.2013.08.009

analytical results obtained using the indophenol blue method coupled to a long-path liquid waveguide capillary cell, the proposed method showed good agreement. The method had been applied on board during a South China Sea cruise in August 2012. A vertical profile of ammonium in the South East Asia Time-Series (SEATS, 18° N, 116° E) station was produced. The distribution of ammonium in the surface seawater of the Qiongdong upwelling in South China Sea is also presented.

1. Introduction

In recent years, more and more oceanographers have been interested in the distribution and concentration changes of ammonium in the oligotrophic ocean during their studies concerning the mechanism and rate of decomposition and denitrification. As one of the most important nutrients in seawater, ammonium in the ocean is a crucial component of the upper ocean nitrogen cycle. The ammonium concentration reflects the balance between production by ammonification and consumption by ammonium assimilation or nitrification, although the inventory of ammonium in the ocean is approximately three orders of magnitude smaller than that of nitrate [1,2]. New production in the open ocean is usually associated with the uptake of nitrate, while regenerated production is associated with the uptake of ammonium. In addition, ammonium that is created by ammonification in the euphotic zone is usually taken up again by the phytoplankton rather than nitrified to nitrate [1,3]. However, compared to nitrate and nitrite, the concentration of ammonium has seldom been measured in open ocean water, resulting in a much more patchy understanding of its distribution. This is because the ammonium consumed by the phytoplankton in the surface waters of the ocean is at nanomolar concentrations and difficult to accurately quantify using conventional analytical techniques [4,5].

To study such nanomolar levels of ammonium distribution in oligotrophic oceans, various methods have been used. The most common one is the indophenol blue (IPB) spectrophotometric method, based on the Berthelot reaction [6,7], which is also used by the U.S. Environmental Protection Agency, which coupled the IPB method with the gas-segmented, continuous flow technique [8]. However, the method has low sensitivity and involves a high reagent blank. In addition, some of the reagents used are toxic [9–11]. To improve the sensitivity, a long-path liquid waveguide capillary cell (LWCC) and solid phase extraction (SPE) coupled with a spectrophotometer are used [4,12]. However, the high reagent blank and toxic reagents still hinder its application in open ocean water. The fluorescence method has higher sensitivity than the colorimetric method [13–15]. The reaction of *o*-phthaldialdehyde (OPA) and borohydride or mercaptoethanol with ammonium or primary amino acids was first introduced in 1971 [16]. This reaction was modified by replacing mercaptoethanol with sulfite and provides a method with better sensitivity for ammonium and greater selectivity for amino acids [17]. However, to adjust the pH of various seawater samples, buffer is needed and the reaction requires 3–4 h to reach a balance at ambient temperature, and the method also suffers from matrix interference, salt effect and low sample throughput [11]. This method was further modified and a shipboard fluorometric flow analyzer developed for high-resolution underway measurement of ammonium in seawater [11,18], and the system has been applied in Florida coastal waters around an oceanic wastewater outfall, where the method showed no refractive index or salinity effect. However, the lowest concentration that can be measured in this application is about 200 nM and the system has not been deployed in the open ocean to measure ammonium at ultra-trace levels. Another approach using gas diffusion to separate ammonium from the sample solution is also used, where the sample is mixed with base to raise the pH above 10.5 and convert ammonium ions to ammonia gas, the gas diffusing across a hydrophobic gas permeable membrane into a receiving solution for spectrophotometric, conductivity [19], fluorescence [20] or chemiluminescence detection [10,21]. Although this method is simple, easy to automate and shows no matrix interference, the transport efficiency of ammonia through the membranes is poor, and most of the detectors are not sensitive enough for the determination of ammonium at nanomolar concentration except fluorescence detection.

Solid phase extraction (SPE) is popular for application in trace analysis for the isolation and concentration of target analytes, clean-up of samples or removal of matrix interference, and can be easily applied for on-line operation [12,22–24]. Among various sorbent materials, the Waters Oasis hydrophilic–lipophilic balance (HLB) is a macroporous copolymer made from a balanced ratio of two monomers, lipophilic divinylbenzene and hydrophilic N-vinylpyrrolidone. The HLB provides reversed-phase capability with a special “polar hook” for enhanced capture of polar analytes, and excellent wettability [25].

To establish a simple, low-cost and highly sensitive method for the analysis of ultra-trace ammonium in open ocean seawater, the typical fluorescence reaction was adopted together with the SPE technique in our work. Fluorescence compounds could be efficiently extracted onto the HLB cartridge and separated from the seawater matrix. The extracted fluorescent compounds were then rapidly eluted with ethanol and delivered to a fluorescence detector. After careful optimization of various parameters, the proposed method was applied on board during a Southern China Sea cruise from 29 July to 21 August 2012.

2. Experimental

2.1. Reagents and solutions

All the chemicals used in this study were of analytical grade, unless stated otherwise. All solutions were prepared in purified water (>18.0 MΩ cm) from a pure water purification system (Millipore). Plastic ware for storing reagent and standard solutions as well as seawater samples was high-density polyethylene (HDPE, Nalgene) bottles and these were carefully washed before use [12]. Reagent bottles were sealed with air inlet clean-up devices, which consisted of a syringe filled with acidic silica gel. The acidic silica gel, an effective trap for basic ammonia gas, was made by adding 50 mL 6 M H_2SO_4 into 200 g of 20-mesh silica gel then dried overnight at 70 °C [18].

A 100 mM NH_4Cl (ammonium chloride) stock solution was made by dissolving 0.5349 g of NH_4Cl (Fluka), previously dried at 105 °C for 2 h, in 100 mL pure water. The solution was stored at 4 °C while not in use. The working solution was diluted daily from the stock solution. 25 mM OPA (*o*-phthaldialdehyde) solution was made by dissolving 0.8383 g of OPA (Alfa Aesar) in 50 mL of methanol and diluted to 250 mL with pure water. 10 mM Na_2SO_3 (sodium sulfite) solution was made by dissolving 0.3151 g Na_2SO_3 (Alfa Aesar) in 250 mL pure water, and 0.1 mL HCHO (formaldehyde, GR grade, Honeywell Burdick & Jackson) was added to prevent the Na_2SO_3 from oxidation, and, moreover, addition of this formaldehyde could reduce the potential interference species, such as amines and amino acids [11]. 15 g L^{-1} $Na_2B_4O_7{\cdot}10H_2O$

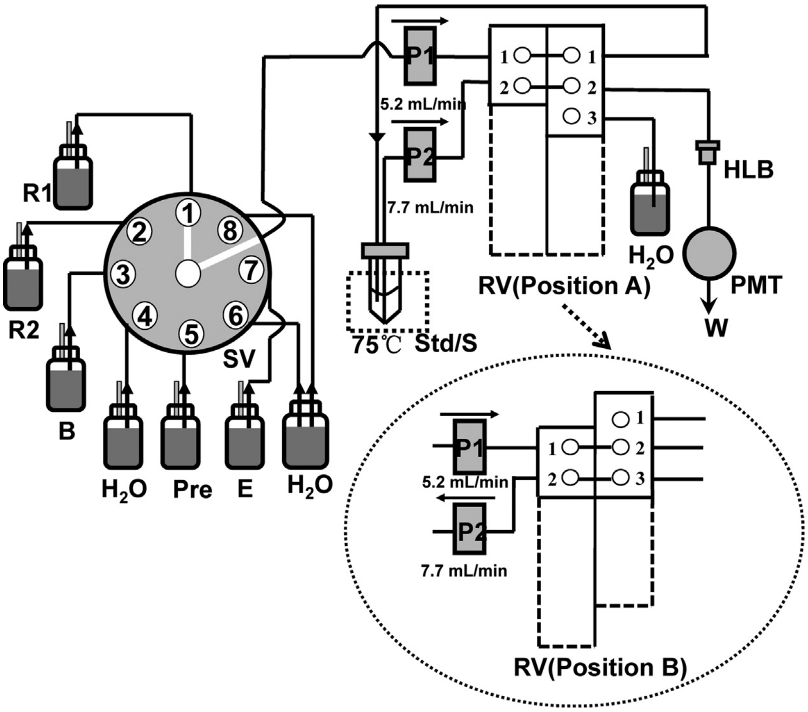

Fig. 1. Manifold of the automatic system for ammonium determination (SV: eight-position selection valve; RIV: two-position rotary injection valve with position A and B); R1: OPA solution; R2: sulfite solution; B: buffer, sodium tetraborate solution; E: elution, ethanol; Pre: pre-rinsing solution, 40% (v/v) ethanol; P1: pump 1; P2: pump 2; Std/S: standard solution or sample; HLB: HLB cartridge; PMT: photomultiplier tube detector; W: waste).

(sodium tetraborate decahydrate) solution was prepared by dissolving 3.75 g $Na_2B_4O_7{\cdot}10H_2O$ (Sinopharm Chemical Reagent Co.) in 250 mL pure water.

2.2. *Apparatus*

The arrangement of the system is illustrated in Fig. 1. The flow-batch system was constructed with the following parts: a flow injection analysis processor (model: FIA 3110, Beijing Jitian Instruments Co.) including two six-channel peristaltic pumps, a two position and eight-way rotary injection valve. An eight-position selection valve (VICI, Valco Instruments Co.), a thermostatic water bath (Jintan Shunhua Instruments Co.), an HLB cartridge (Waters Associates) and a PMT-FL detector (FIA Lab Instruments Inc.) with a 360 nm UV LED as the excitation light source.

2.3. *Manifold and procedures*

All manifold tubing was 1.58 mm o.d. × 1.00 mm i.d. transparent PTFE tubing (VICI, Valco Instruments Co.) except for the peristaltic pump tubing, where Tygon tubing (Baoding Longer Precision Pump Co.) was used.

The flow-batch program is shown in Table 1. During Steps 1–3, appropriate amounts of R1, R2 and buffer were added to a 30 mL sample and the concentrations of R1, R2 in the final solution were 1.5 μM and 0.3 μM. In Step 4, the buffer solution remaining in the tubing was sent into the sample solution using pure water. After all the reagents were added, pure water and 40% (v/v) ethanol were used in sequence to rinse the HLB cartridge. In Step 7, pumps were stopped to allow the fluorescent compounds to form at 75 °C. After 300 s or longer, depending on the concentration of ammonium in the sample, the sample solution was pumped through the HLB

Table 1
Flow analysis program for fluorescence detection of ammonium in seawater samples.

Step	Duration time (s)	8-position stream selector position	Rotary injection valve position	Flow rate (mL min^{-1})		Comments
				Pump 1	Pump 2	
1	15	1	A	5.8	0	Adding OPA solution into sample solution
2	10	2	A	5.8	0	Adding Na_2SO_3solution into sample solution
3	10	3	A	5.8	0	Adding $Na_2B_4O_7$solution into sample solution
4	15	4	A	5.8	0	Sending the $Na_2B_4O_7$solution remaining in the tubing into the sample solution and filling the tubing with pure water
5	40	5	B	11.5	0	Rinsing HLB cartridge with pure water
6	25	6	B	11.5	0	Rinsing HLB cartridge with 40% (v/v) ethanol
7	300	6	B	0	0	Forming fluorescent compounds
8	120	6	A	0	11.5	Loading fluorescent compounds on HLB cartridge
9	35	6	B	11.5	8.5	Rinsing HLB cartridge with pure water
10	45	7	B	7.2	0	Eluting with ethanol
11	60	8	B	8.5	0	Washing all tubing with pure water

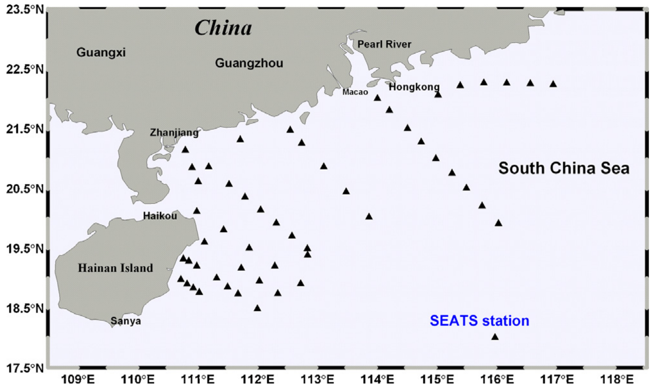

Fig. 2. Location of sampling stations in the South China Sea.

cartridge to allow the fluorescent compounds formed to be extracted onto the cartridge. The cartridge was then washed with pure water to remove impurity, and then in Step 10, the fluorescent compounds enriched on the cartridge were rapidly eluted with ethanol. The eluted compounds were detected with a PMT, and the signal was recorded with a computer using software written in Labview 2010 for the quantification. The sample throughput was 5 h^{-1}.

2.4. Sampling

Seawater samples were collected from various depths at the SEATS station using a 12-bottle CTD rosette sampler system (SBE-911/917 Plus CTD, Seabird Co.) with which the temperature, salinity, depth and fluorescence measurements were also obtained, during a cruise on board *R*/*V* Dongfang Hong 2 in the South China Sea from 29 July to 21 August 2012. The location of the sampling stations is shown in Fig. 2.

The sample collectors wore gloves, which were changed between samples. Before collecting the sample, the sampling bottle and the bottle cap were washed three times or more with the sample, and then the sample was collected as quickly as possible to avoid contamination from the atmosphere. If the samples are collected from coastal area where depth is less than 200 m, it is suggested that the sample should be filtered through a 0.45 μm pore size membrane before analysis. Otherwise samples could be directly analyzed in the shipboard laboratory. Some samples, which could not be analyzed immediately, were kept frozen and thawed just before analysis.

3. Results and discussion

3.1. Parameter optimization

The excitation and emission spectra of the reaction product shown in Fig. 3 indicate that the maximum of excitation and emission are located at 362.0 nm and 423.9 nm. The fluorescence intensities as a function of reaction time at different temperature are shown in the inset of Fig. 3.

To optimize the parameters to be used in the experiment, the effects of pH, reaction temperature, reaction time, eluting flow rate, reagent concentrations in the final solution, and salinity were investigated, based on a univariate experimental design. A standard solution of 20 nM ammonium was used in the tests.

3.1.1. Effects of pH, reaction temperature, reaction time and eluting flow rate

The optimal pH value for the reaction was 11 [11,17]. In order to adjust the pH of the various coastal seawaters and the open ocean waters, buffer was needed in this study. Phosphate and $Na_2B_4O_7{\cdot}10H_2O$ are the most common buffers [26–28], but precipitation easily occurred when phosphate buffer was used, and so $Na_2B_4O_7{\cdot}10H_2O$ was selected as the buffer in our study.

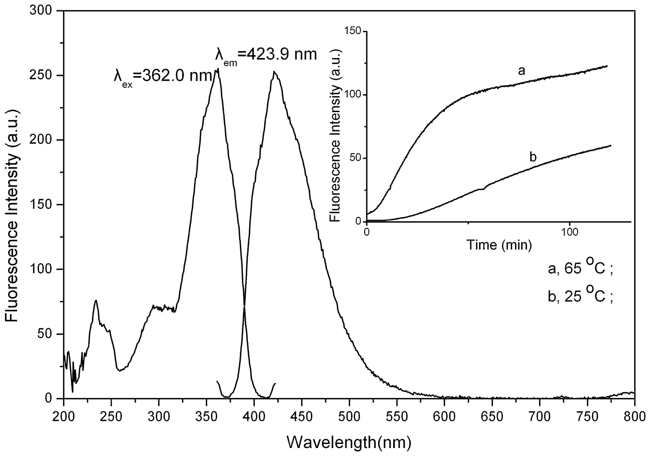

Fig. 3. Excitation and emission spectra of the reaction product of OPA–sulfite–$Na_2B_4O_7$–NH_3. (Inset shows fluorescence intensity vs. reaction time.)

Fig. 4(a) shows the effect of reaction temperature. The optimal temperature was selected based on a consideration of the net signal, reproducibility and the problem of air bubbles in the detector cell. Increasing temperature accelerated the reaction rate, but the blank signal also increased. The net fluorescence signal was highest at 75–85 °C. Nevertheless, the reproducibility was worse at 85 °C, therefore, the optimal temperature was selected as 75 °C.

Based on a previous study [23], longer reaction time can effectively improve the sensitivity. The influence of different reaction times was studied and is shown in Fig. 4(b).

The effect of the eluting flow rate was tested by varying the flow rate from 3.1 to 9.2 mL·min^{-1}. The result is shown in Fig. 4(c). The eluting flow rate was optimized from the viewpoint of complete elution, peak shape, column pressure and net signal intensity. The signal peak became wider as the flow rate decreased (Fig. 4(d)). In order to maintain a proper sensitivity and a narrower peak, an eluting flow rate of 7.7 mL min^{-1} was selected.

3.1.2. Concentration of OPA and sulfite in the final solution

The effect of OPA concentration on a signal intensity ranging from 0.4 to 1.8 μM is illustrated in Fig. 4(e). The net fluorescence intensity increased with the increase of OPA concentration in the final solution, and 1.5 μM OPA was selected as the optimum. Fig. 4(f) shows that the effect of sulfite concentration in the final solution ranged from 0.15–0.71 μM. The net fluorescence intensity decreased when the sulfite was more than 0.3 μM, and so 0.3 μM sulfite was chosen.

3.1.3. Effect of salinity

Higher salinity corresponds to larger ionic strength in the sample solution. According to the Debye–Hückel equation, the activity coefficients for ions in a sample solution decrease as ionic strength increases [24]. Since the salinity of open ocean seawater is almost constant as 35, in order to investigate the salinity effect, several different salinity seawaters (salinity ranged from 0 to 35) were prepared by diluting aged seawater collected from the surface of the South China Sea at the SEATS station with pure water as the matrix. Calibration curves were obtained by spiking standard ammonium solution into different salinity seawaters. Fig. 5 shows the calibration curves prepared with different salinities and the relationship between the slope of the calibration curves and salinity. The

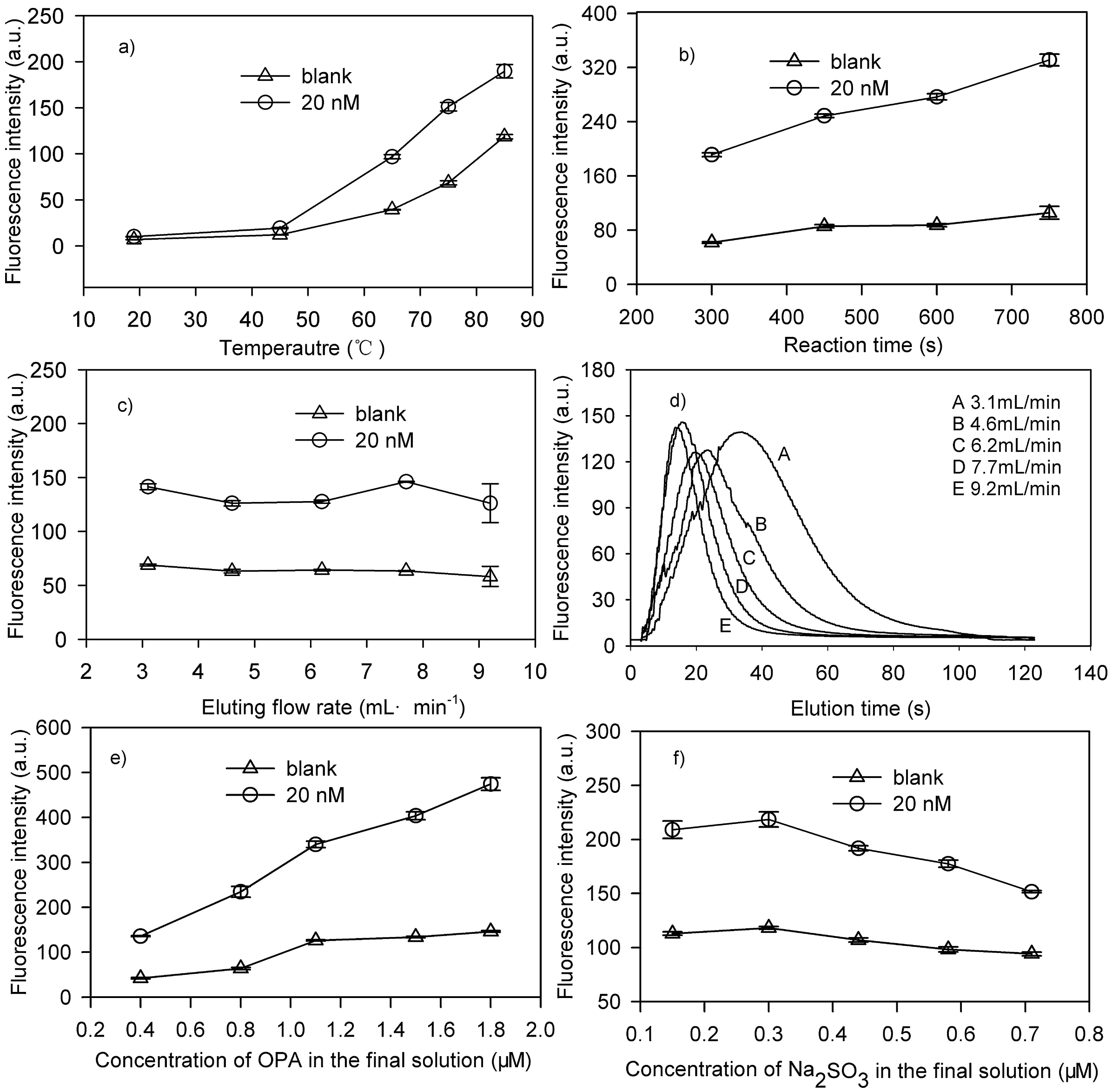

Fig. 4. Variation of fluorescence intensity with different parameters ($n = 3$). (a) temperature; (b) reaction time; (c) elution flow rate; (d) the elution curves with different eluting flow rates; (e) concentration of OPA in final solution; and (f) concentration of Na_2SO_3 in final solution.

deviation for seven different salinities was within ±3.8% indicating that the salinity effect was negligible.

3.2. *Storage method for seawater samples with ultra-trace ammonium*

A suitable storage method was very important for the ultra-trace ammonium seawater samples, especially when a large number of samples were collected and could not be measured on time. The samples must be preserved in a manner that successfully maintains the original concentrations of the species interested until analysis [29]. In previous studies, researchers suggest keeping ammonium samples frozen and thawing them before analysis [30,31]. In our study, different storage methods for ultra-trace ammonium samples were compared during the South China Sea survey in August 2012. The surface seawater samples collected at the SEATS station were kept under three different conditions: room temperature, cold storage (4 °C) and freezing (−20 °C). The proposed method was used to measure the concentration of ammonium every 12 h and to compare the results of each storage method. The change of ammonium concentration with different storage methods is shown in Fig. 6. It was found that the ammonium seawater samples were adequately kept for at least 5 days if they were immediately frozen and stored at −20 °C in clean HDPE bottles.

3.3. *Linearity, reproducibility and method detection limit*

The standard solutions for ammonium measurement in seawater should be prepared in ammonium-free seawater. However, no ammonium-free seawater exists at present, and deionized water has been used as a blank matrix instead of low nutrient seawater (LNSW) [11]. Another method to prepare low-ammonium seawater involves the removal of background ammonium in LNSW [4]. However, the method is complicated and the preparation process may result in contamination. In our study, artificial seawater and 26 aged seawaters collected from the Atlantic Ocean, Indian Ocean, and the South China Sea were used to evaluate the blank with the proposed method. The aged seawater with the lowest blank

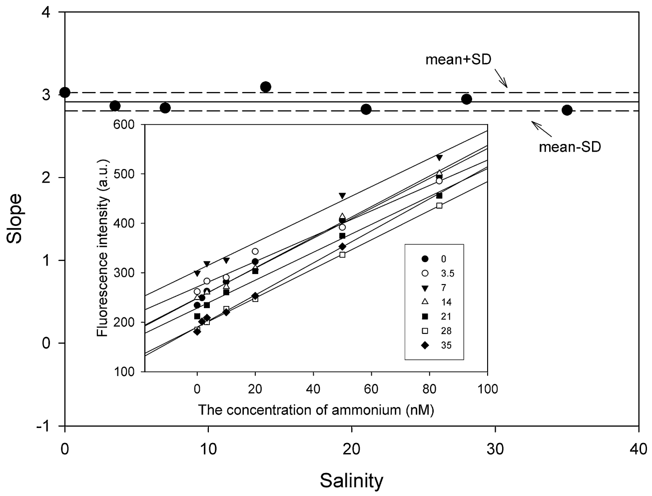

Fig. 5. The relationship between slope of calibration curve and salinity. (The inset shows the calibration curves for ammonium in samples with different salinities (0, 3.5, 7, 14, 21, 28 and 35).).

was considered to be ammonium-free seawater, for use in our work.

Under the optimal conditions chosen, the typical calibration curves ranged from 1.67 to 300 nM with two different reaction times (300 s and 120 s) are presented as: $y = 3.4340x + 215.87$ ($R^2 = 0.9912$, $n = 7$) and $y = 1.4317x + 121.14$ ($R^2 = 0.9935$, $n = 6$). The quantification range could be broadened by choosing a different reaction time, depending on the concentration of ammonium in the seawater samples.

The reproducibility of the method was evaluated with five repetitive determinations of an aged seawater sample at a concentration of 20 nM ammonium. The relative standard deviation was 3.5%. During the experiment, a 20 nM ammonium solution was used as the data quality control sample and inserted after the analyses of every 10 samples, in order to check the calibration curve and make sure the cartridge was still good to be used.

The method detection limits, estimated as three times the standard deviations of the blank ($n = 7$), were 0.7 nM for land-based and 1.2 nM for shipboard laboratories, which were low enough for the determination of ammonium in most open seas.

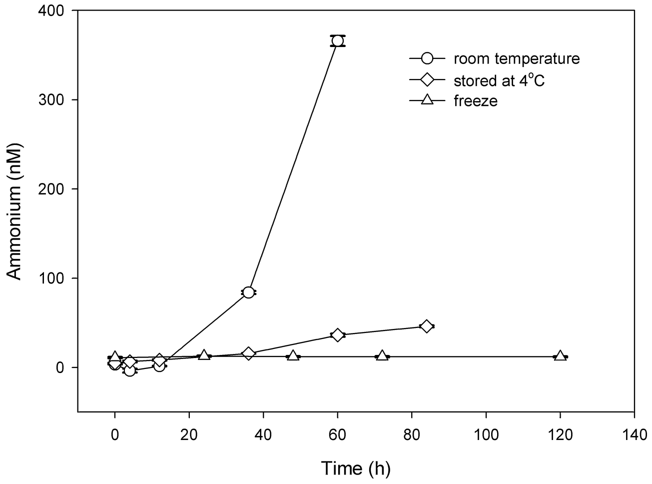

Fig. 6. Influence of storage method on the concentration of ultra-trace ammonium in seawater samples ($n = 3$).

Table 2
Recovery of ammonium from seawater samples using the proposed method.

Sample	Added (nM)	Determined conc. (nM)	Rec. (%)
SW1	–	55.9 ± 2.6	–
	50	102.5 ± 3.5	93.2
SW2	–	260.8 ± 6.9	–
	200	442.9 ± 4.6	91.0
SW3	–	34.8 ± 3.9	–
	20	52.7 ± 5.7	89.5
SW4	–	93.4 ± 4.8	–
	80	170.6 ± 3.2	96.5

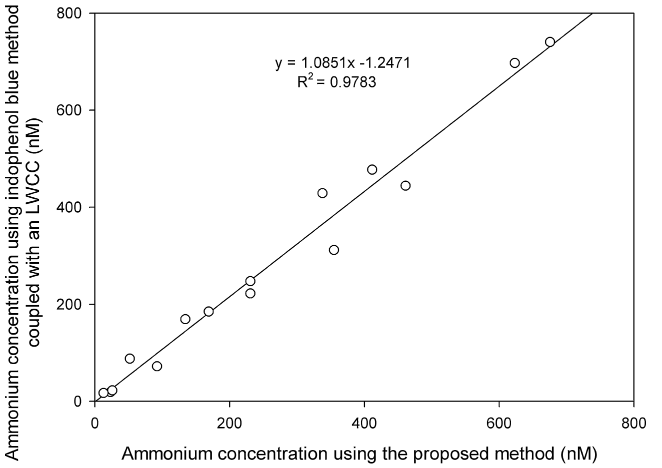

Fig. 7. Comparison of the proposed method and the IPB method coupled with an LWCC. (Seawater samples were collected from the South China Sea near Sanya port in August 2012.).

3.4. Validation of the method

3.4.1. Recovery

In order to examine the method recovery, a series of LNSW samples collected from the South China Sea were spiked with ammonium at different concentrations and analyzed using the proposed method. The results are shown in Table 2. The recoveries of the ammonium in spiked samples were between 89.5 and 96.5%. Therefore, it was considered that the determination of ammonium would not be interfered with by the seawater matrix.

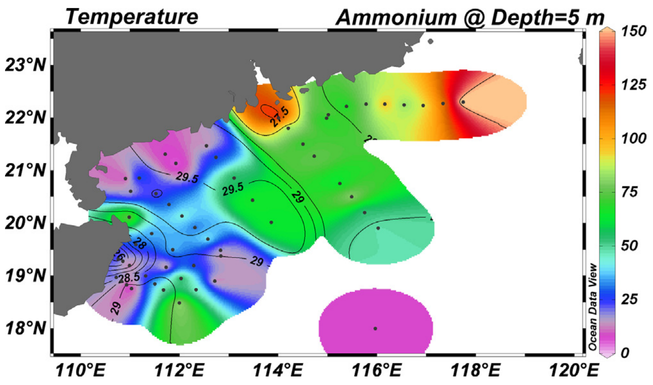

Fig. 8. Distribution of ammonium and temperature in the surface water of the Qiongdong upwelling in August 2012 (unit: nM, the figure was created with the software Ocean Data View [32]).

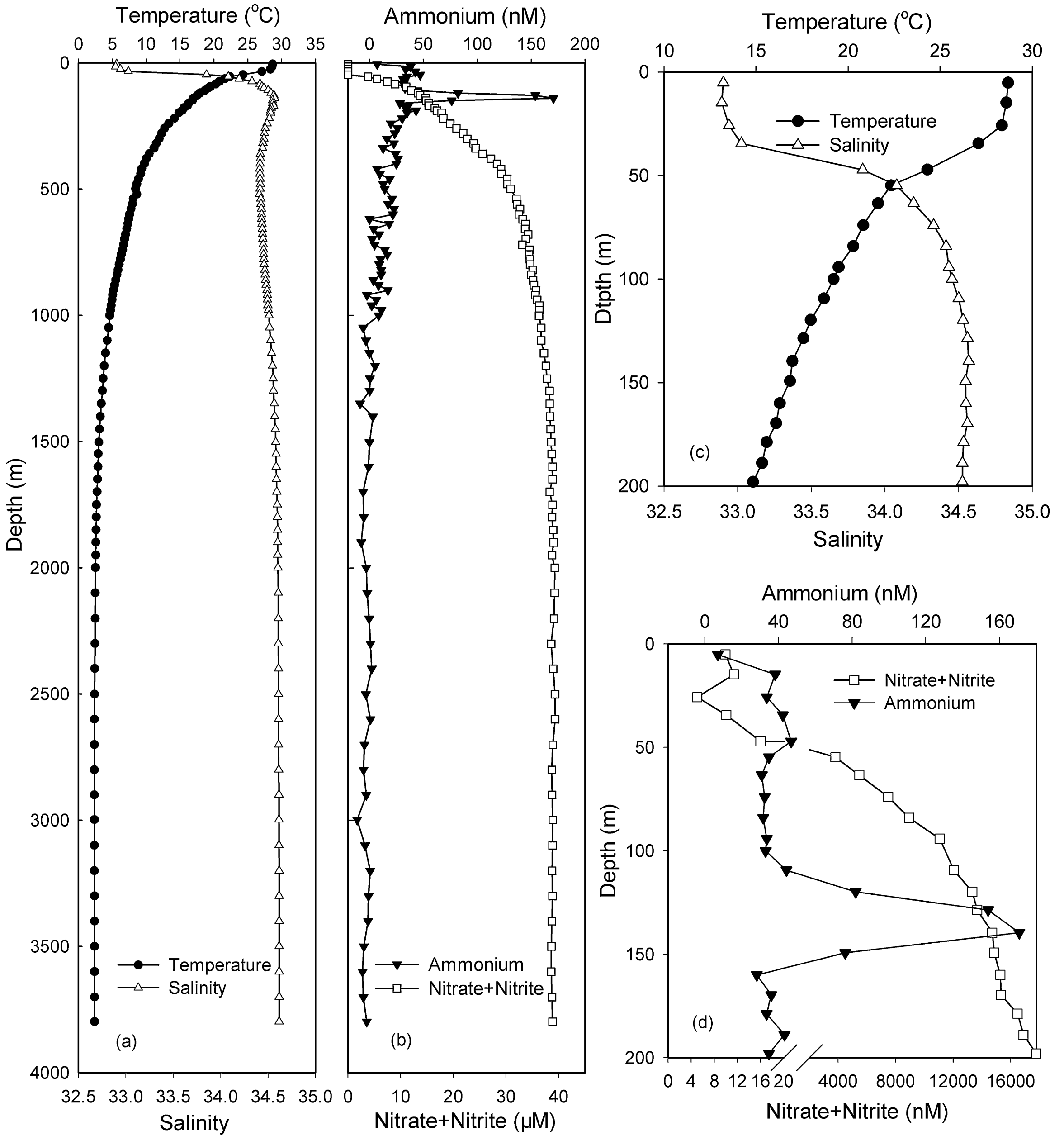

Fig. 9. The vertical profile of temperature, salinity, nitrate + nitrite, and ammonium at the SEATS station (18°N, 116°E) with 92 depth levels (a) temperature and salinity; (b) nitrate + nitrite and ammonium; (c) enlargement of (a) above the depth of 200 m; and (d) enlargement of (b) above the depth of 200 m.

3.4.2. Comparison with the indophenol blue method coupled with an LWCC

The IPB method is widely used to determine ammonium in seawater. Another method developed uses segmented flow analysis coupled with an LWCC, and the detection limit is 5 nM [4]. The ammonium samples collected in the South China Sea were determined with both the proposed method and the IPB method using a 1 m LWCC. As shown in Fig. 7, the linear regression equation (with a regression coefficient of 0.9783) for the two sets of data gave a slope of 1.0851, suggesting good agreement between the two methods.

3.5. Field application

Our method was applied on board to analyze seawater samples collected from 65 stations in the South China Sea, including the SEATS station. The seawater samples were analyzed immediately after collection. It was found that one HLB cartridge could be used for the analyses of more than 50 seawater samples.

Fig. 8 shows the distribution of ammonium in the surface water of the Qiongdong upwelling. The typical vertical profiles of temperature, salinity, nitrate + nitrite and ammonium at the SEATS station are shown in Fig. 9. Fig. 9(a) and (b) indicates the vertical profiles in 3980 m of water at 92 levels. The vertical profiles from 5 m to 200 m are enlarged in Fig. 9(c) and (d). Ammonium had a low concentration of about 7 nM in the surface water, but the concentration increased in the mixed layer, and the maximum was about 47 nM at the bottom of the mixed layer (Fig. 9(d)). The thermocline appeared at about 45–65 m. The maximum ammonium caused by organic matter mineralization was observed at about 140 m with a concentration of about 170 nM. From 160 m to 1000 m, the concentration of ammonium slowly decreased (Fig. 9(b)). In the deep zone from 1500 m to 3980 m, there were no obvious changes in temperature, salinity, nitrate + nitrite and ammonium. This is the first time that a detailed vertical profile of ammonium concentration had been obtained at the SEATS station.

4. Conclusions

A highly sensitive and automated analytical method, which coupled fluorescence detection with flow analysis and the SPE technique, was established for on board determination of ultra-trace ammonium in open ocean water. There was a good agreement in the results between the proposed method and the IPB method coupled with an LWCC. Compared with the conventional fluorescence method, this method offers the benefits of improved sensitivity, reduced reagent consumption, negligible salinity effect, and it is suitable for on board determination. Furthermore, the proposed method was successfully applied on board in the South China Sea. The results demonstrated the feasibility of the proposed method for the determination of nanomolar ammonium in seawater.

Acknowledgments

We would like to thank Mr. Chuanjun Du and Mr. Tao Huang for their help in sampling. We also thank the OCG research group in the State Key Laboratory of Marine Environmental Science for providing nitrate and nitrite data. This work was financed by the Science Fund for Creative Research Groups of the National Natural Science Foundation of China (Grant No. 41121091) and the China National Basic Research Program ("973" Program) "Carbon cycling in China Seas-budget, controls and ocean acidification (CHOICE-C Project, NO. 2009CB421200)". Professor John Hodgkiss is thanked for his assistance with English.

References

[1] D.G. Capone, D.A. Bronk, M.R. Mulholland, E.J. Carpenter, Nitrogen in the Marine Environment, second ed., Academic Press, 2008.
[2] J.A. Brandes, A.H. Devol, C. Deutsch, Chem. Rev. 107 (2007) 577.
[3] A. Yool, A.P. Martin, C. Fernández, D.R. Clark, Nature 447 (2007) 999.
[4] Q.P. Li, J.Z. Zhang, F.J. Millero, D.A. Hansell, Mar. Chem. 96 (2005) 73.
[5] D.R. Clark, T.W. Fileman, I. Joint, Mar. Chem. 98 (2006) 121.
[6] K. Grasshoff, M. Ehrhardt, K. Kremling, T. Almgren, Methods of Seawater Analysis, third completely rev. and extended ed., Wiley-VCH, Weinheim, 1999.
[7] S.C. Pai, Y.J. Tasu, T.I. Yang, Anal. Chim. Acta 434 (2001) 209.
[8] J.Z. Zhong, P.B. Ortner, C.J. Fischer, L.D. Moore, EPA Method 349.0: Determination of Ammonia in Estuarine and Coastal Waters by Gas Segmented Continuous Flow Colorimetric Analysis, US Environmental Protection Agency, Washington, DC, 1997, EPA/600/R/97/072.
[9] A. Aminot, D. Kirkwood, R. Kerouel, Mar. Chem. 56 (1997) 59.
[10] S. Willason, K. Johnson, Mar. Biol. 91 (1986) 285.
[11] N. Amornthammarong, J.Z. Zhang, Anal. Chem. 80 (2008) 1019.
[12] G. Chen, M. Zhang, Z. Zhang, Y. Huang, D. Yuan, Anal. Lett. 44 (2011) 310.
[13] N. Amornthammarong, J.Z. Zhang, P.B. Ortner, Anal. Meth. 3 (2011) 1501.
[14] N. Amornthammarong, J.Z. Zhang, P.B. Ortner, J. Stamates, M. Shoemaker, M.W. Kindel, Environ. Sci. Processes Impacts (2013).
[15] S. Xue, K. Uchiyama, H.-f. Li, J. Environ. Sci. 24 (2012) 564.
[16] M. Roth, Anal. Chem. 43 (1971) 880.
[17] G.F. Zhang, P.K. Dasgupta, Anal. Chem. 61 (1989) 408.
[18] N. Amornthammarong, J. Jakmunee, J. Li, P.K. Dasgupta, Anal. Chem. 78 (2006) 1890.
[19] J. Plant, K. Johnson, J. Needoba, L. Coletti, Limnol. Oceanogr.: Methods 7 (2009) 144.
[20] R.J. Watson, E.C.V. Butler, L.A. Clementson, K.M. Berry, J. Environ. Monit. 7 (2005) 37.
[21] J.M. Terry, J.L. Adcock, D.C. Olson, D.K. Wolcott, C. Schwanger, L.A. Hill, N.W. Barnett, P.S. Francis, Anal. Chem. 80 (2008) 9817.
[22] R.D. Jones, Limnol. Oceanogr. 36 (1991) 814.
[23] J. Ma, D. Yuan, Y. Liang, Mar. Chem. 111 (2008) 151.
[24] Y. Liang, D. Yuan, Q. Li, Q. Lin, Anal. Chim. Acta 571 (2006) 184.
[25] SPE Chapter from the Chromatography Columns and Supplies Catalog, Waters Corp., 2013, pp. 13–16.
[26] G. Huang, J. Hou, X. Zhou, Environ. Sci. Technol. 43 (2009) 5851.
[27] S.K. Abi Kaed Bey, D.P. Connelly, F.-E. Legiret, A.J.K. Harris, M.C. Mowlem, Ocean Dyn. 61 (2011) 1555.
[28] B. Horstkotte, C.M. Duarte, V. Cerda, Talanta 85 (2011) 380.
[29] J.E. Dore, T. Houlihan, D.V. Hebel, G. Tien, L. Tupas, D.M. Karl, Mar. Chem. 53 (1996) 173.
[30] R.M. Holmes, A. Aminot, R. Kérouel, B.A. Hooker, B.J. Peterson, Can. J. Fish. Aquat. Sci. 56 (1999) 1801.
[31] R. Kérouel, A. Aminot, Mar. Chem. 57 (1997) 265.
[32] R. Schlitzer, http://odv.awi.de (2009).