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On the fluorometric measurement of ammonium in oligotrophic seawater: Assessment of reagent blanks and interferences

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Abstract

Dynamically distributed at trace levels in the open ocean, ammonium is one of the most important and reactive nitrogen compounds in the marine environment. Obtaining reliable measurements of ammonium concentrations is thus a prerequisite to fully understand its role in marine biogeochemical processes, but remains challenging. Among others, quantification and identification of different sources of blanks is an outstanding issue, due partly to the fact that ammonium-free water is very difficult to prepare and/or preserve. Building on a recently developed method using solid phase extraction combined with fluorescence detection (SPE-Flu), we examined the kinetics of the ortho-phthaldialdehyde-sulfite-ammonium reaction and introduce a new approach to quantifying reagent blanks via a "reagents addition" method. The reagent blank of the method, equivalent to 6.7 ± 1.5 nmol L^{-1} of ammonium under our experimental settings, accounted for up to 27% of the ammonium background in seawater samples collected from the oligotrophic ocean. We also showed that the SPE-Flu method is highly specific, with negligible interference from three types of amines and 15 types of amino acids at nanomolar concentrations, which are typical of open ocean regimes. The determination of the reagent blank allowed for optimized data reduction, which was applied to a study in the oligotrophic South China Sea. Water column profiles showed a very well-defined structure and smooth distribution of ammonium concentrations, consistent with the distribution of other parameters. We thus contend that our proposed approach provides a way to further optimize the quantification of ammonium concentrations in natural seawater via the SPE-Flu method.

As the most reactive nitrogen species in marine environments, ammonium (NH_4^+) is an essential component of the marine nitrogen cycle and the key to understanding many marine nitrogen transformations, such as NH_4^+ assimilation, nitrification, and anammox (McCarthy et al. 1977; Kuypers et al. 2005; Ward 2008). The assimilation of NH_4^+ by oceanic biota exerts profound controls on marine primary productivity (regenerated productivity in particular) in oligotrophic waters (Eppley and Peterson 1979). Thus, the accurate determination of NH_4^+ is essential to understand ecosystem metabolism and nitrogen biogeochemistry in marine ecosystems (Holmes et al. 1999). As a specific example, any over- or underestimation of NH_4^+ concentrations would affect the accuracy of estimations of nitrification rates and oceanic new/regenerated production using the commonly adopted ^{15}N isotopic incorporation incubation approach (Yool et al. 2007) as the amount of $^{15}NH_4^+$ added should be optimized to accurately match the ambient trace level NH_4^+ concentration in seawater in order to minimize the perturbation of the incubated microbial community (Lipschultz 2008).

Despite its long-recognized importance, quantifying NH_4^+ remains difficult, especially in oligotrophic waters where nanomolar levels occur. Consequently, the fine structure of NH_4^+ distributions in the ocean has only rarely been determined. Commonly applied techniques, such as the indophenol blue (IPB) spectrophotometric method (Aminot et al. 1997; Hansen and Koroleff 2007) and the fluorometric flow injection method (Zhang and Dasgupta 1989), can only measure micromolar concentrations of NH_4^+. Recently, a more sensitive batch-flow analyzer, combining solid phase extraction with fluorescence detection (SPE-Flu), was developed by Zhu et al. (2013). Briefly, ortho-phthaldialdehyde (OPA) forms a fluorescent complex with NH_4^+ when sulfite is present. This complex is subsequently extracted onto a hydrophilic-lipophilic balance

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cartridge, eluted with ethanol, and quantified with a photomultiplier detector. This method offers excellent precision and is highly sensitive, reaching detection limits of ~ 0.7 nmol L^{-1}.

A difficulty associated with reliably measuring NH_4^+ in seawater, however, is its susceptibility to contamination from the ambient environment. Because eliminating NH_4^+ contamination is almost impossible, it is particularly difficult for standards to be prepared using completely NH_4^+-free solutions. Distinguishing and quantifying different sources of blanks thus becomes critical in any method development, and essential in the data reduction for actual samples. For the SPE-Flu technique, calibrations are made using serial standard solutions prepared using Milli-Q water or low NH_4^+ seawater as the matrix. Determination of NH_4^+ from the (typically linear) calibration curve is represented as $F = a \times [NH_4^+] + F_b$, where F is the fluorescence signal of the standard solutions, a is the slope of the linear regression, and F_b is the intercept, or method blank, which has three components. The first is the instrument blank, which is normally the baseline of the instrument itself. This instrument blank is rather small, $\sim 1\%$ of the fluorescence signal of the samples and standards, and thus is negligible during analysis. The second component is the fluorescence blank of the reagents, including solutions of OPA, Na_2SO_3, $Na_2B_4O_7$ buffer, and methanol. The third component is the matrix blank contributed from the NH_4^+ in either the Milli-Q water or the seawater used to prepare the standards. This matrix blank only affects the standard curves and should not be considered when reducing sample data. In most cases in the present study, the calibration curve slopes were relatively stable, varying by $< 5\%$. However, F_b was highly variable, with values differing by up to threefold (somewhat higher than the twofold variations observed previously by Zhu et al. (2013) and reaching a maximum value equivalent to 50 nmol L^{-1} of NH_4^+ during our 30-d study in the South China Sea (SCS) (Fig. 1). This variance was largely attributed to the matrix-NH_4^+ blanks and their variability. In this regard, the NH_4^+ contents of the deep seawater matrix were lower than that of aged surface seawater and especially laboratory Milli-Q water. Therefore, applying the total calibration standards blank in the data reduction of the samples can easily result in errors, including an over-subtraction leading to an underestimation of sample concentrations which may even reach negative values. It is therefore critical to distinguish between the reagent and matrix-NH_4^+ blanks, especially when sample runs are associated with different calibration curves. This issue is discussed further in the evaluation of our SCS sample concentrations.

Some previous work has either advised to make no blank correction at all or did not mention the problem (Zhang and Dasgupta 1989; Aminot et al. 2001; Zhu et al. 2013), which could cause a significant bias in the reported NH_4^+ concentrations in samples. Other studies have attempted to measure reagent blanks at "time-zero" (time < 10 s), when reagents have not yet reacted with any solvents (Taylor et al. 2007; Abi Kaed Bey et al. 2011). However, because the complete reaction of OPA-ammonium-sulfite usually takes a long time (several hours) and Milli-Q water in reagents always contains at least some NH_4^+, these "time-zero" reagent blank estimates are not appropriate and may underestimate the real blank of the reagents. Therefore, a better way to evaluate the "real" reagent blank is needed.

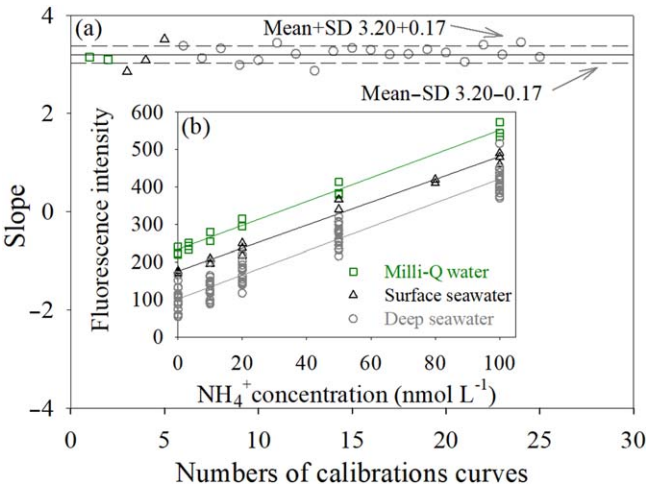

Fig. 1. (**a**) Variations of the slopes of calibration curves prepared using three matrixes: Milli-Q water, aged surface seawater, and deep seawater. The inset panel (**b**) shows the series calibration curves. Note that the deep seawater was obtained from the SCS cruise ($n = 20$). The ammonium concentrations of standard solutions were 0 nmol L^{-1}, 10 nmol L^{-1}, 20 nmol L^{-1}, 50 nmol L^{-1}, 100 nmol L^{-1}, and 100 nmol L^{-1}. The mean slope of the total 26 calibration curves was 3.20 with a standard deviation of 0.17. Note that this dataset obtained from the cruise to the SCS in May–July 2014 used the same fluorescence detector as Zhu et al. (2013) (detection limit ~ 0.7 nmol L^{-1}), while the dataset of reagent blanks experiment was obtained from a new fluorescence detector with relatively lower sensitivity (detection limit ~ 2.2 nmol L^{-1}).

Another concern associated with many NH_4^+ measurement techniques, including the SPE-Flu method, is interference by amino acids and amines, which could react with sulfite-OPA to produce non-NH_4^+ fluorescence signals. Previous work, which tested this problem using select amino acids and NH_4^+ concentrations in the range of $\sim$ 1–10 μmol L^{-1} obtained inconsistent results (Zhang and Dasgupta 1989; Aoki et al. 1997; Aminot et al. 2001; Watson et al. 2005; Amornthammarong and Zhang 2008; Abi Kaed Bey et al. 2011). Moreover, it remains unexamined as to whether amino acids interfere with the measurement of NH_4^+ in the oligotrophic ocean where both occur at nanomolar levels.

This study sought to address these challenges associated with NH_4^+ analyses. First, we describe a new approach to quantify reagent blanks. Second, we examine potential interference from amino acids and amines on NH_4^+ measurements. By accounting for these two critical factors, this study presents approaches that improve both the accuracy

and precision of measuring trace levels of NH_4^+ in the ocean using the SPE-Flu method.

Materials and procedures

Reagents, solutions, and equipment

The analytical equipment, reagents, and procedures used in this study were similar to those in Zhu et al. (2013). Milli-Q water was freshly drawn from a water purification system with a resistivity $\geq$ 18.2 MΩ cm. In order to minimize contamination, instead of using volumetric flasks, reagents were prepared by weighing them directly into precleaned dark brown HDPE bottles, and adding Milli-Q water gravimetrically as well. The concentrations of the OPA, Na_2SO_3, and $Na_2B_4O_7{\cdot}10H_2O$ solutions were 25 mmol L^{-1}, 10 mmol L^{-1}, and 39.3 mmol L^{-1}, respectively. Formaldehyde was added to the Na_2SO_3 solution at a ratio of $\sim$ 1 : 1. To prevent contamination and maintain stability, reagents were stored in sealed bottles (250 mL) using traps made of acid-washed silica gel (Amornthammarong and Zhang 2008; Zhu et al. 2013). The concentration of the NH_4Cl stock solution was 100 mmol L^{-1}, which was diluted with Milli-Q water or seawater to prepare the standard solutions. The pH values of the solutions were measured at 25°C using a pH electrode meter (Thermo) calibrated with NBS buffers. The precision of pH measurements was 0.005.

For testing potential interferences caused by amino acids and amines, the following compounds were used: histidine, isoleucine, arginine, glycine, serine, alanine, valine, threonine, tryptophan, aspartic acid, phenylalanine, proline, lysine, leucine, tyrosine, methylamine hydrochloride, ethylamine hydrochloride, and ethanolamine hydrochloride. All chemicals were of ACS reagent grade (Sigma Aldrich), and all solutions were prepared using Milli-Q water and then stored at 4°C.

Due to many possible sources of contamination, special care is essential when measuring trace levels of NH_4^+ throughout sampling, processing, storage, and analysis (Meeder et al. 2012). During this study, both the sampling bottles (125-mL HDPE) and bottle caps were precleaned by soaking in 2 mol L^{-1} HCl for $>$ 12 h, and then extensively rinsed with Milli-Q water. Powder-free gloves and masks were worn at all times. Both sampling bottles and caps were rinsed three times with $\sim$ 50 mL seawater before sample collection, and the seawater was sampled from a Niskin spigot as quickly as possible to minimize contamination from the atmosphere. Even in land-based laboratories, reproducibility can be very poor at low concentrations. The variation of NH_4^+ concentrations determined in Milli-Q water samples can be $>$ 10% (Watson et al. 2005), and occasionally $>$ 20% based on our experience.

Contamination is of less concern if experiments are conducted in a shipboard "clean lab," containing adequate ventilation and a filtration system to remove particulates such as smoke from the ship or marine aerosols (Watson et al. 2005). However, optimal clean lab environments are not always available, and most experiments are done under normal laboratory conditions. In this study, to minimize contamination from the ambient environment, shipboard sample preparation was carried out under a helium atmosphere using glove bags, in which 30 mL seawater sample was transferred from sampling bottles to testing bottles using a pipette (Eppendorf, 10 mL). Helium gas was chosen based on the results of replicate measurements ($n = 10$) of NH_4^+ concentration in Milli-Q water under nitrogen, helium, and ambient air. These showed that the lowest and most stable NH_4^+ concentration values were obtained under helium, with concentrations averaging 25.1 $\pm$ 1.9 nmol L^{-1} as compared to 46.8 $\pm$ 6.6 nmol L^{-1} under nitrogen gas and 37.9 $\pm$ 3.5 nmol L^{-1} under ambient air.

Procedures

Reaction kinetics

We first examined how the pH of the Milli-Q water varied under differing amounts of reagents, ranging from 0.5× to 2.0× their original published levels (Zhu et al. 2013). We also examined how pH changed as each reagent was sequentially injected into the standard solutions, under both 1.0× and 2.0× their original levels.

Second, we examined the kinetics of the OPA-sulfite-ammonium reaction at the optimized temperature of 75°C (Zhu et al., 2013) to determine the time required to complete the reaction at two levels of reagent concentrations: the original concentrations of Zhu et al. (2013) (or 1.0× the original level); and half these concentrations (or 0.5× the original level). The concentrations of reagents at the 1.0× the original level in prepared samples or standards were 1.5 mmol L^{-1} for OPA, 0.3 mmol L^{-1} for Na_2SO_3, and 0.3 mmol L^{-1} for $Na_2B_4O_7$. Note that both of the reagent levels were in excess for the reaction. The experiments were conducted in triplicate using a standard NH_4^+ solution of 100 nmol L^{-1}.

Stability of blanks and reagents

The stability of the reagents was examined in control experiments using (1) week-old reagents and freshly drawn Milli-Q water; and (2) daily freshly prepared reagents and freshly drawn Milli-Q water. Fluorescence signals were measured for six consecutive days, five successive times each day for each group. Next, we examined the reproducibility of the fluorescence background of Milli-Q water at the reaction completion phase under normal laboratory conditions with good ventilation.

Determination of the reagent blank

The reagent blanks for fluorescence measurements were constrained by comparing the responses under different levels of reagents (the so called "reagents additions method") at the moment of completion of the OPA-sulfite-ammonium reaction, which was determined by examining the kinetics

of the reaction. To do so, three sets of experiments were conducted as explained below.

The first set of tests examined the fluorescence response throughout the reaction in Milli-Q water under both levels of reagent concentrations. By doing so, we were able to discern the difference in fluorescence between the two levels of reagent concentrations in the stationary phase, which distinguishes the "real" fluorescence difference caused solely by variations in the amounts of reagents similar to the "standard addition method," so as to constrain the fluorescence background of the reagents. We assumed that the relationship between the fluorescence signal and reagent concentration was linear during the stationary phase, in the range of 0.5× to 1.0× the original reagent level, as described by Eq. 1.

$$F_{\text{reagent: 1.0}\times\text{ original level}} = \left(F_{\text{total: 1.0}\times\text{ original level}} - F_{\text{total:0.5}\times\text{ original level}}\right)\times 2 \quad (1)$$

Next, we ran a calibration curve (0–100 nmol L^{-1}) with reagents at the 1.0× original level at the completion stage of the reaction, to convert the reagent blank from fluorescence units into an equivalent NH_4^+ concentration.

Finally, we measured the ratio, denoted as p (Eq. 2), which allows for conversion of the reagent blank determined at the completion of the reaction to that at 365 s for the actual sample runs:

$$p = \frac{\bar{F}_{\text{incompletion}}}{\bar{F}_{\text{completion}}} \times 100\% \quad (2)$$

where $\bar{F}_{\text{incompletion}}$ represents the average fluorescence signal at the reaction time of 365 s (incomplete reaction) under 1.0× original reagent levels, and $\bar{F}_{\text{completion}}$ represents the average fluorescence signal at the final stationary phase (complete reaction) under the same reagent levels.

Amino acid and amine interference tests

Three sets of tests were conducted to examine possible interferences with NH_4^+ signals by amines (methylamine, ethylamine, and ethanolamine) and a variety of naturally occurring amino acids. The first test, following the approach of many previous studies, was to examine the instrument response to amines and amino acids at micromolar levels. Thus, two calibration curves at the micromolar level were created, with a working range of 0–2.76 μmol L^{-1} for amino acids and 0–2.9 μmol L^{-1} for amines.

In the second test, taking advantage of the high sensitivity of our method to address concentrations appropriate to the oligotrophic ocean, we mixed NH_4^+ with amino acids and amines at nanomolar levels in different ratios. We compared a range of NH_4^+ concentrations (0–333 nmol L^{-1}) mixed separately with a 276 nmol L^{-1} amino acid standard and a 483 nmol L^{-1} amine standard. By doing so, we were able to determine the ratios of amines and amino acids to NH_4^+ at which interference will be negligible.

In the last test, we compared the instrument response to solutions containing a range of concentrations of amino acids at micromolar levels (0.5–2.2 μmol L^{-1}) mixed with nanomolar levels of a 267 nmol L^{-1} NH_4^+ standard.

Field application

Discrete seawater samples for nutrient analyses were collected from the northern basin of the SCS at 111°E, 18°N, using a rosette sampler equipped with Conductivity-Temperature-Depth (CTD) sensors (SBE-911, Seabird) onboard the R/V *Dong Fang Hong II* during a July 2014 cruise. Temperature and salinity were obtained from the CTD recorder. Chlorophyll was estimated from the fluorescence detector attached to the CTD. NH_4^+ concentrations were measured onboard using the Flu-SPE method (Zhu et al. 2013). Samples with NO_2^- and/or NO_3^- at nanomolar levels were measured using a home-made flow injection system with a liquid waveguide capillary cell (LWCC, World Precision Instruments) modified from Zhang (2000), based on the standard pink azo dye method (Pai et al. 1990). The detection limit was 2.0 nmol L^{-1}. Samples with NO_3^- at micromolar levels were analyzed onboard using a Technicon AA3 Auto-Analyzer (Bran-Lube, GmbH) (Hansen and Koroleff 2007). The detection limit was 100 nmol L^{-1}.

Assessment

Reaction kinetics

The kinetics of the OPA-sulfite-ammonium reaction is largely influenced by pH and temperature. The optimal pH value for the reaction is 11 (Zhang and Dasgupta 1989; Amornthammarong and Zhang 2008). However, in our protocol, the pH was adjusted to 10 with a $Na_2B_4O_7$ buffer to avoid the precipitates that form in seawater samples (Zhu et al. 2013). Figure 2a shows that the pH was stable at 10.0 ± 0.15 for reagent levels from 0.5× to 2.0× their original concentrations in the final ~ 30 mL solutions. Figure 2b further shows how pH changed as reagents were sequentially injected into Milli-Q water. OPA did not change the pH. When Na_2SO_3 was added, the pH increased to as high as 10.7. The pH then quickly adjusted to ~ 10 when $Na_2B_4O_7$ was added. In this study, the temperature was set to 75°C to speed up the reaction (Zhu et al. 2013), since at room temperature the reaction is very slow, typically taking several hours to complete (Holmes et al. 1999).

We then examined the reaction kinetics under two reagent concentration levels: 1.0× and 0.5× original levels. The fluorescence intensity increased rapidly as the reaction proceeded, reaching a short-stationary phase within 20 min followed by a gradual decrease with time (Fig. 3). The reaction reached maximum fluorescence after 765 s at the 1.0× reagent level and 965 s at the 0.5× reagent level.

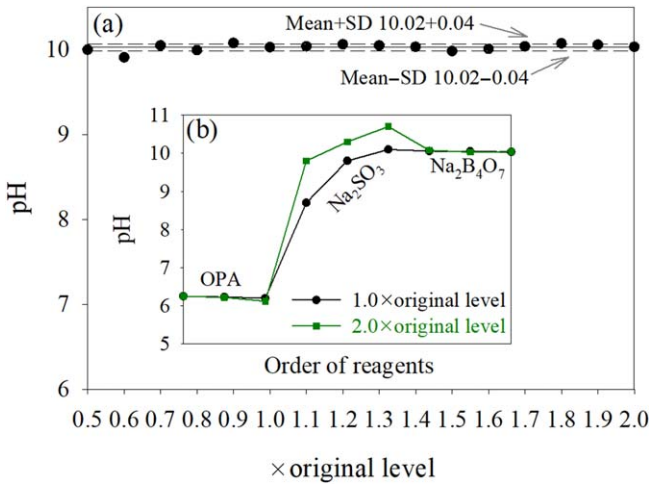

Fig. 2. (**a**) pH variations using different reagent concentrations, ranging from 0.5× to 2.0× their original level in final solutions. Each interval denotes 0.1× original level. The inset panel (**b**) shows the changes of pH value when OPA, Na_2SO_3, and $Na_2B_4O_7$ buffer were sequentially injected into 30 mL Milli-Q water.

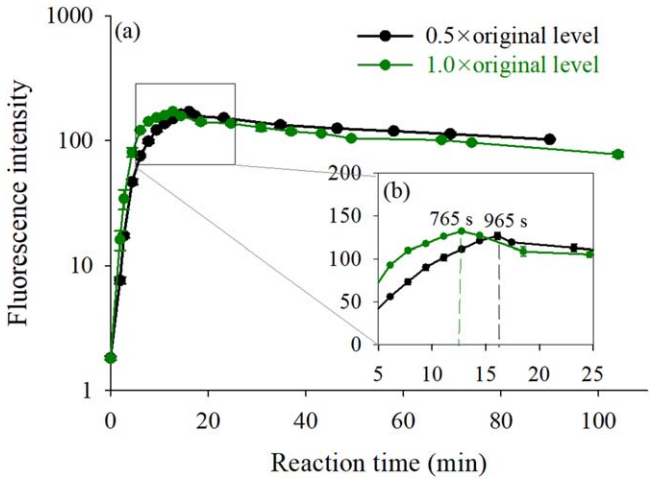

Fig. 3. (**a**) Averaged time-series of fluorescence intensity during the reaction of OPA-sulfite-ammonium with original (1.0×) reagent levels and 0.5× reagent levels at 75°C. Each time-series analysis was conducted three times. The inset (**b**) expands the 5–25 min interval.

Stability of blanks and reagents

Time-series measurements in Milli-Q water during the stationary phase (765 s) were examined at the 1.0× reagent level (Fig. 4). The relative standard deviation (RSD) of 17 measurements was ± 4.2%, suggesting that the background variation introduced by Milli-Q water was minor during the measurement. Figure 5 shows identical fluorescence signals (RSD = 1.3%) between the daily freshly prepared reagents and the week-old reagents throughout the 6 d of tests, suggesting that the reagents were stable throughout our experiments and can last for at least 2 weeks at room temperature.

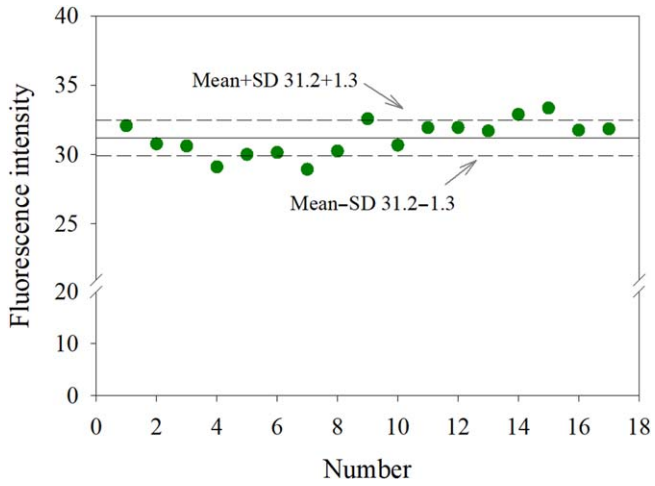

Fig. 4. Reproducibility of the fluorescence background of Milli-Q water under original (1.0×) reagent levels at the reaction time of 765 s ($n = 17$).

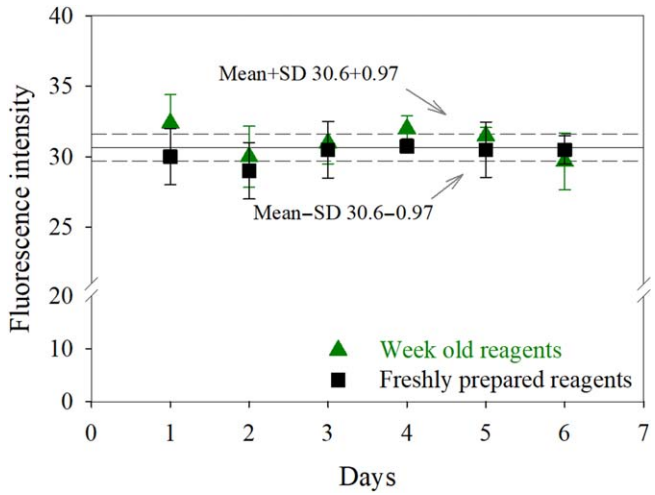

Fig. 5. Time-series measurements of reagent stability. Each group was measured five successive times each day for 6 d.

Reagent blank evaluation

First, reagents at 1.0× and 0.5× their original levels were added to Milli-Q water, and allowed to react with NH_4^+ for 765 s and 965 s, respectively (Fig. 6a,b). Each batch was continuously measured a total of seven times. The average fluorescence intensities at the completion stage were 27.5 ± 1.0 at the 0.5× reagent level and 31.9 ± 0.1 at the 1.0× level. Therefore, using 1.0× reagent levels would produce 8.8 ± 2.0 units of fluorescence (or reagent blank) at the stationary phase, as calculated with Eq. 1.

A calibration curve was then made at the 1.0× reagent level with fluorescence intensity at 765 s (the time of maximum fluorescence) plotted as a function of NH_4^+ concentrations in

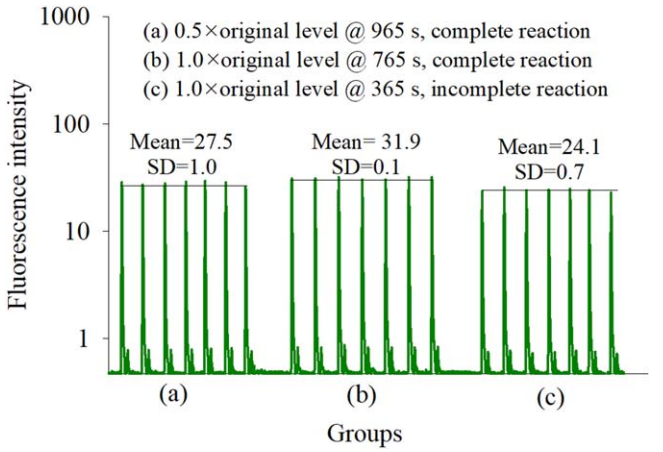

Fig. 6. Successive measurements of the background fluorescence of Milli-Q water under (**a**) 0.5× reagent levels at a reaction time of 965 s, (**b**) original (1.0×) reagent levels at a reaction time of 765 s, and (**c**) 1.0× reagent levels at a reaction time of 365 s. The small peaks following each of the main peaks were prerinsed peaks using ethanol.

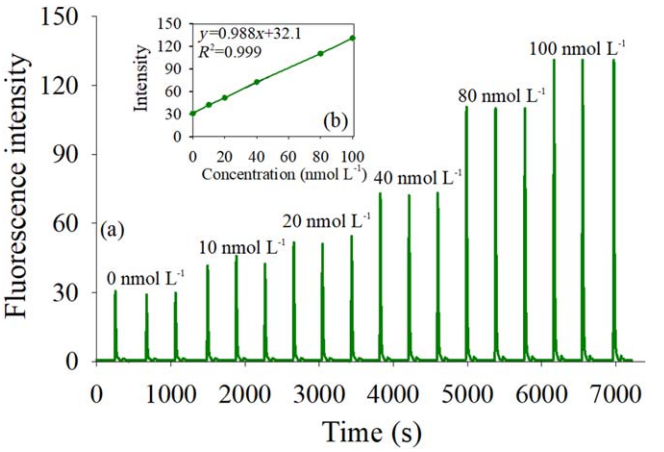

Fig. 7. (**a**) Triplicate measurements of fluorescence intensity of 0 nmol L^{-1}, 10 nmol L^{-1}, 20 nmol L^{-1}, 40 nmol L^{-1}, 80 nmol L^{-1}, and 100 nmol L^{-1} ammonium standard solutions prepared in Milli-Q water under original (1.0×) reagent levels at the reaction time of 765 s. The inset panel (**b**) shows the linear regression of the averaged ammonium output signals.

standard solutions. The slope of the resulting curve (0.988 ± 0.020 fluorescence per unit nmol L^{-1}, Fig. 7b) was then used to calculate the NH_4^+ concentration of the reagent blank, which was 8.9 ± 2.0 nmol L^{-1}.

Finally, in order to obtain p values from Eq. 2, fluorescence measurements were taken from Milli-Q water that was allowed to react with original (1.0×) reagent levels for 365 s (Fig. 6c). This shorter time period, occurring before the reaction was complete, was chosen because our protocol followed sample reaction times in Zhu et al. (2013). The average fluorescence intensity was 24.1 ± 0.7, which accounted for 75.6% ± 2.21% of the signal at the completion of the reaction as calculated with Eq. 2. Thus, for a reaction time of 365 s, the concentration of the reagent blank was 6.7 ± 1.5 nmol L^{-1}, which accounted for ~ 27% of the total blank (~ 24.3 nmol L^{-1}), based on an average of the values in Fig. 6c.

Amino acid and amine interferences

Figure 8 shows the fluorescence responses of the ammonium analyzer to NH_4^+, amino acids, amines, amino acids + 267 nmol L^{-1} NH_4^+, NH_4^+ + 483 nmol L^{-1} amines, and NH_4^+ + 276 nmol L^{-1} amino acids. No significant response was observed for amines between 0 μmol L^{-1} and 2.9 μmol L^{-1} since the slope of the amine regression was two orders of magnitude lower than that of NH_4^+. The slope of the amino acid regression (0.5–2.76 μmol L^{-1}) was around one magnitude lower than that of NH_4^+, which were 0.75 and 0.087, respectively, showing that the analyzer would only respond to amino acid concentrations higher than 500 nmol L^{-1} but was much more sensitive to NH_4^+.

The identical slopes of the regressions for NH_4^+, NH_4^+ + 276 nmol L^{-1} amino acids and NH_4^+ + 483 nmol L^{-1} amines, indicated insignificant interference of NH_4^+ analysis when nanomolar levels of amino acids and amines co-occurred with NH_4^+. However, the slope of the calibration curve for amino acids (0.5–2.2 μmol L^{-1}) + 267 nmol L^{-1} NH_4^+ was the same as for amino acids alone, indicating that the NH_4^+ signal was not discernable when higher micromolar concentrations of amino acids were present. The reported concentrations of dissolved free amino acids and of dissolved primary amines are generally lower than 300 nmol L^{-1} in the open ocean and coastal areas (Lee and Bada 1977; Sipler and Bronk 2015). Thus, we concluded that our present method was suitable for measurements of nanomolar levels of NH_4^+ in the open ocean and most coastal areas. However, the potential exists for interference in eutrophic waters where environmental amino acid concentrations can be very high. An alternative method for these areas could be the classic IPB spectrophotometric method combined with a long-path LWCC (Zhu et al. 2014).

In this study, the sensitivity of the fluorescence response to amino acid concentrations at micromolar levels was ~ 10 times lower compared to NH_4^+, as seen in the differences in their slopes in Fig. 8. This suggests that the method will experience negligible interferences (< 10% bias) as long as amino acids are less abundant than 0.5 μmol L^{-1}. The high sensitivity of the SPE-Flu method for NH_4^+ might be due to the fact that formaldehyde was added to the Na_2SO_3 solution at a ratio of ~ 1 : 1, which can form a stable complex, α-hydroxymethanesulfonate, to protect sulfite from oxidation by air (Amornthammarong and Zhang 2008). Furthermore, the NH_4^+ signal can be substantially enhanced and, most importantly, the potential interference species, such as amino acids and amines can also be reduced (Amornthammarong and Zhang 2008). Our tests agreed with these previous results and demonstrated that the method was free from major interferences caused by amino acids and amines at very low NH_4^+ levels.

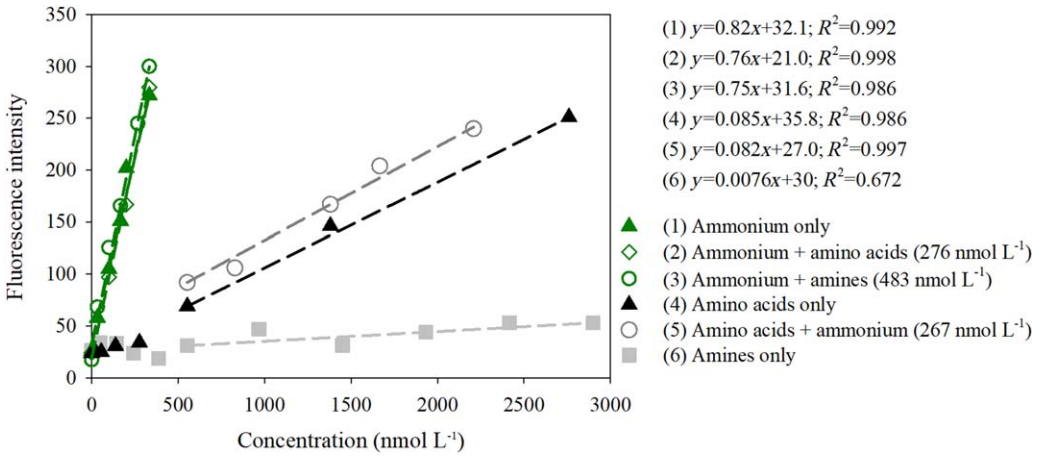

Fig. 8. Response of the ammonium analyzer to amino acid and amine additions. Ammonium only samples were not measured at micromolar levels since the SPE-Flu method is linear only at nanomolar levels. The average slope of the two regression lines of amino acids (alone and with 267 nmol L^{-1} of ammonium) was 0.088 and the RSD was 0.6%. The average slope of three regression lines of ammonium (alone, with 276 nmol L^{-1} of amino acids and with 483 nmol L^{-1} of amines) was 0.77 and the RSD was 6.3%. No significant response to amines was detected.

Field application and discussion

To apply the techniques described above to natural seawater, we prepared calibration curves using SCS deep water. Two standards with designated NH_4^+ concentration of 110 nmol L^{-1} and 200 nmol L^{-1} were measured to examine the accuracy and precision during the cruise. The average precisions for seven repeated measurements of the 110 nmol L^{-1} and 200 nmol L^{-1} standards were 6.4% and 2.7%, respectively, with associated accuracies of 5.8% and 3.8%. Overall precision of the dataset was 4.2%, which was derived from the time series of the calibration curves runs during the cruise in collecting the dataset reported in Fig. 9.

It must be pointed that the variation of the intercepts among 20 calibration curves during a 30-d cruise to the SCS was very large (Fig. 1) and thus it is of critical importance to discuss different options to calculate NH_4^+ concentration using calibration curves, which has been rarely reported in the literature. Clearly, applying the total blank in the data reduction of the samples is not appropriate since the matrixes possess more or less NH_4^+, which would lead to an underestimation of sample concentrations. Three other options could then be considered. The first option is to use an average calibration curve to calculate NH_4^+ concentration for all stations, which may be too arbitrary because of the large variations in intercepts in our cases and many others in the course of a cruise campaign. This approach could produce negative NH_4^+ concentration in deep water at some stations and substantial high NH_4^+ concentration in deep water at other stations. A second option is to use a calibration curve made at each station but use the slope only to calculate the NH_4^+ concentration. This method assumes the SPE-Flu method does not have blanks, or all the blanks come from matrix-NH_4^+ so that they can be discarded. However, the present study proved at least reagent blanks are non-negligible and should be considered in data reduction of samples. Thus, the last choice is to use the reagent blank we evaluated in this paper as the "true" intercept and use different slope at each station. We adopted this approach and subtracted only the reagent blank (6.7 nmol L^{-1}) as the "true" intercept and used slopes at each station in the calculation of NH_4^+ concentrations in the samples under study. This approach assumes that the reagent blank was stable throughout the voyage, which was not directly evaluated but is supported by the stability of the results observed in the laboratory as discussed above.

Using this optimized data reduction approach, we obtained the high-resolution profile of NH_4^+ concentrations at Sta. X5 located in the western basin of the SCS shown in Fig. 9, which had a very well-defined structure and smooth distribution of values, and was overall consistent with previous oceanographic studies. We emphasize that if instead we had subtracted the total blank (all three components as described earlier), all NH_4^+ concentrations at Sta. X5 would have been negative. The value of using our approach to remove the reagent blank is demonstrated by obtaining low, yet always non-negative, NH_4^+ concentration estimates for the deep-water samples.

The fluorescence profile indicated a typical deep chlorophyll maximum (DCM) of 1.2 mg m^{-3} at 50 m (Fig. 9). Below 50 m, chlorophyll concentrations decreased rapidly. NO_3^- concentrations were depleted to nanomolar levels ($\sim$ 10 nmol L^{-1}) from the surface down to $\sim$ 45 m, where a shallow nitracline started. They then rapidly increased to a value of $\sim$ 36.8 μmol L^{-1} at $\sim$ 1000 m, followed by a slow

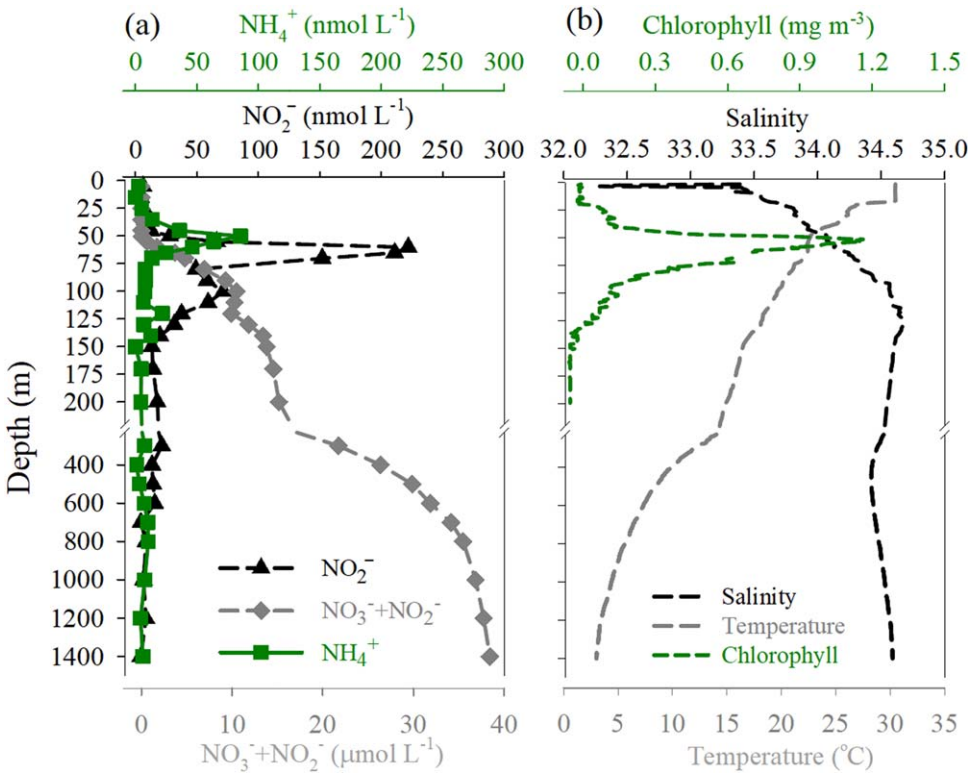

Fig. 9. Vertical profiles of nitrite, nitrate + nitrite, and ammonium concentrations (**a**) and chlorophyll, salinity, and temperature (**b**) at Sta. X5, located at 111°E, 18°N in the oligotrophic SCS. The sampling resolution was 10 m in the upper 200 m. The reagent blank was used as the "true" blank during data reduction to calculate ammonium concentrations.

increase and eventually reaching 38.4 μmol L^{-1} in deeper waters. The concentration of NH_4^+ peaked at 50 m, with a value of 85.4 nmol L^{-1}, and the NO_2^- concentration reached a peak at 60 m, with a value of 222.0 nmol L^{-1}. Both NH_4^+ and NO_2^- concentrations decreased with depth and eventually nearly reached their limits of detection.

Relatively invariable NH_4^+ concentrations in the deep seawater samples further suggested that sample contamination was minor. The fine structures we observed of the different nitrogen species, which showed an NH_4^+ and a primary nitrite maximum that correlated with the depth of the DCM, have important biogeochemical implications, showing the combined influences of NH_4^+ assimilation and NH_4^+ and NO_2^- oxidation. Similar distribution patterns have also been observed in the Red Sea (Meeder et al. 2012).

In this study, we demonstrated further improvements to the SPE-Flu method for the measurement of low NH_4^+ concentrations in oligotrophic seawater. We showed that the SPE-Flu method can work well in the oligotrophic ocean without interferences from amino acids and amines. Notably, we developed a new method to quantify the method's reagent blank. This is a critical step in advancing NH_4^+ measurement techniques, allowing a long-standing and difficult problem to be resolvable in practice. This approach was successfully applied to samples collected during a cruise to the SCS, yielding a high-resolution profile of NH_4^+ concentrations with high precision and accuracy.

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Conflict of Interest

None declared.

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