Shipboard Fluorometric Flow Analyzer for High-Resolution Underway Measurement of Ammonium in Seawater

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A shipboard fluorometric flow analyzer has been developed for near-real-time, high-resolution underway measurement of ammonium in seawater. The fluorometric method is based on the reaction of ammonium with *o*-phthaldialdehyde (OPA) and sulfite. The reagents used in this method have been modified to suit seawater analysis. This method shows no refractive index and salinity effect from seawater samples. The potential interferences in seawater have been studied, and their effects have been reduced. The instrument response is linear over a wide range of ammonium concentration. The limit of detection of 1.1 nM was estimated in laboratory using ammonium standards prepared in distilled water. It should be noted that application of this method to low-level ammonium measurement requires a correction of interference species, such as amino acids. The sample throughput is 3600 h^{-1}. The system can be used for both freshwater and seawater samples and has been used to monitor the distribution of ammonium in Florida coastal waters around an oceanic wastewater outfall.

Ammonium is an essential nutrient in the marine environment. Phytoplankton generally prefer ammonium over nitrate, which requires additional energy to reduce to ammonium during assimilation.[1−3] However, high concentrations of ammonia are toxic to marine organisms such as fish,[4] shrimp,[5] abalone,[6] and sea urchin,[7] especially larvae or juveniles of these species. Water quality monitoring requires accurate measurements of the concentration of ammonium in the coastal environment;[8] however, both physical and biological processes, such as biological uptake and release,[9,10] atmospheric deposition, air−sea gas exchange,[11,12]

and wastewater discharge,[13] rapidly change the ammonia concentrations in both space and time. Development of a shipboard analyzer that can perform near-real-time, high-resolution underway measurements of ammonium in coastal water is urgently needed.

The most common technique used to measure ammonia in seawater is the indophenol blue method using the Berthelot reaction;[14,15] however, this technique has low sensitivity (LOD 0.6 μM) and a high reagent blank.[16−18] An ammonia electrode was found easy to operate,[19] but it requires long equilibration times and provides low sensitivity (LOD 0.2 μM). To increase sensitivity, a solvent extraction method[20] was developed, but the procedure is time-consuming and labor-intensive. Recently, a long-path liquid waveguide capillary cell[21] was used to improve the sensitivity (LOD 5 nM), but the problems associated with the indophenol blue still remained. In 1971, Roth[22] developed a fluorescent method for determination of amino acid and ammonia in which *o*-phthaldialdehyde (OPA) reacts with mercaptoethanol to produce a strongly fluorescent compound. This method has been adapted for a variety of applications.[23−25,26] In 1989, Dasgupta[27] modified this method by replacing mercaptoethanol with sulfite and provided a method with higher sensitivity for ammonia and greater selectivity over amino acids. The OPA−sulfite−NH$_3$ reaction has been modified

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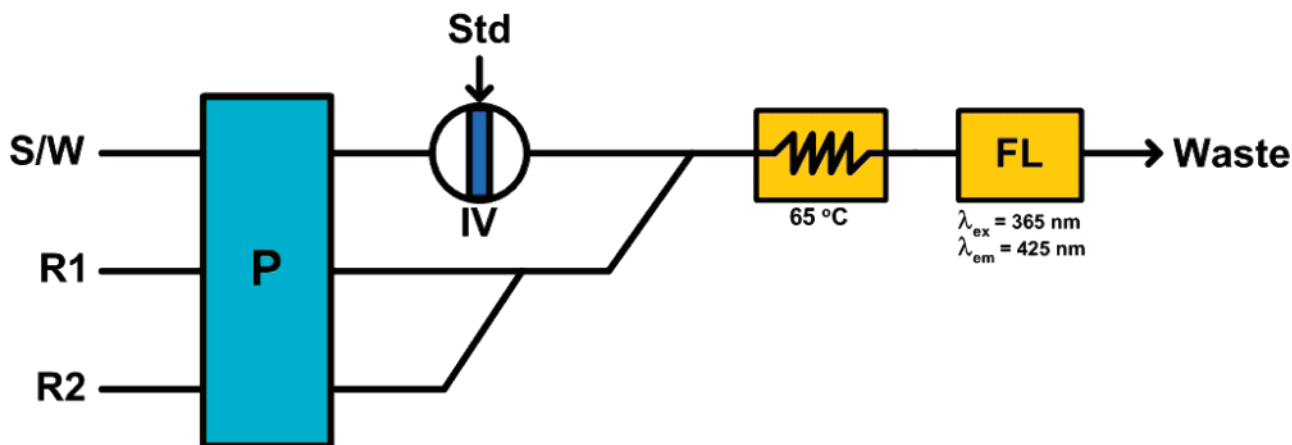(27) Genfa, Z.; Dasgupta, P. K. *Anal. Chem.* **1989,** *61,* 408−412.

Figure 1. Underway analyzer for ammonia determination: S/W, sample or water; Std, standard; P, peristaltic pump; R1, phthaldialdehyde (OPA) solution; R2, sulfite in formaldehyde solution; FL, fluorescent detector; and IV, injection valve.
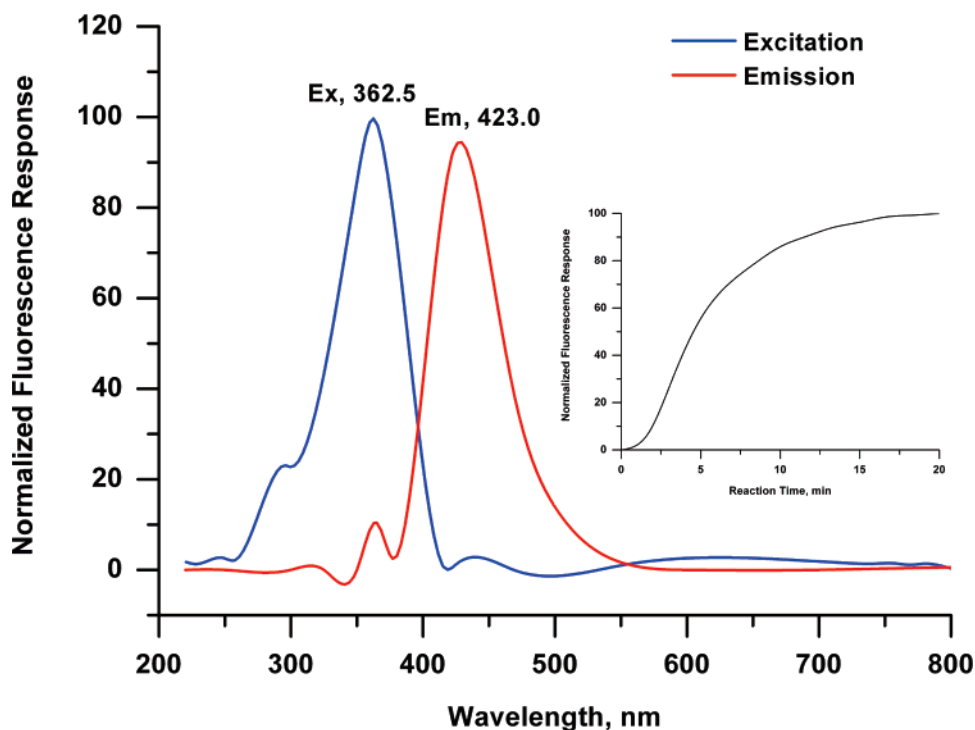

Figure 2. Excitation and emission spectra of reaction product of OPA–sulfite–NH_3. The inset shows the kinetics of the reaction.

for ammonia determination in seawater,[18,28–29] but it suffered from interferences, salt effect, and low sample throughput. Recently, Amornthammarong and Dasgupta[30] developed a hybrid fluorometric flow analyzer for determination of ammonia in air. In their method, the reagent stability was improved from a couple days to two weeks, which is critical for long-term monitoring of atmospheric ammonia.

In this work, we describe an improved OPA–sulfite reaction for determination of ammonia in seawater. By mixing formaldehyde with sulfite solution, we achieved both higher sensitivity and a more stable reagent solution. Moreover, addition of formaldehyde reduced the potential interference species, such as amines and amino acids. The method is highly sensitive for determination of ammonia in seawater with negligible salt effect and no refractive index effect. The method has been used to examine the distribution of ammonium in Florida coastal waters around a wastewater outfall in the Florida Area Coastal Environment (FACE) Program.

EXPERIMENTAL SECTION

Reagents. Reagent 1 (R1) was made by dissolving 3.35 g of OPA (P1378, Sigma) in 200 mL of methanol and diluting to 1 L with deionized (DI) water. The solution is 25 mM in OPA.

Reagent 2 (R2) was made by dissolving 1.26 g of sodium sulfite (1-3922, J.T. Baker Chemical), in 1 L of 5 mM formaldehyde (HCHO) solution. The solution is 10 mM sulfite in 5 mM HCHO.

Standards. A 0.1 M NH_4Cl stock solution was made daily by dissolving 0.535 g of ammonium chloride (12125-02-9, Aldrich), in 100 mL of DI water.

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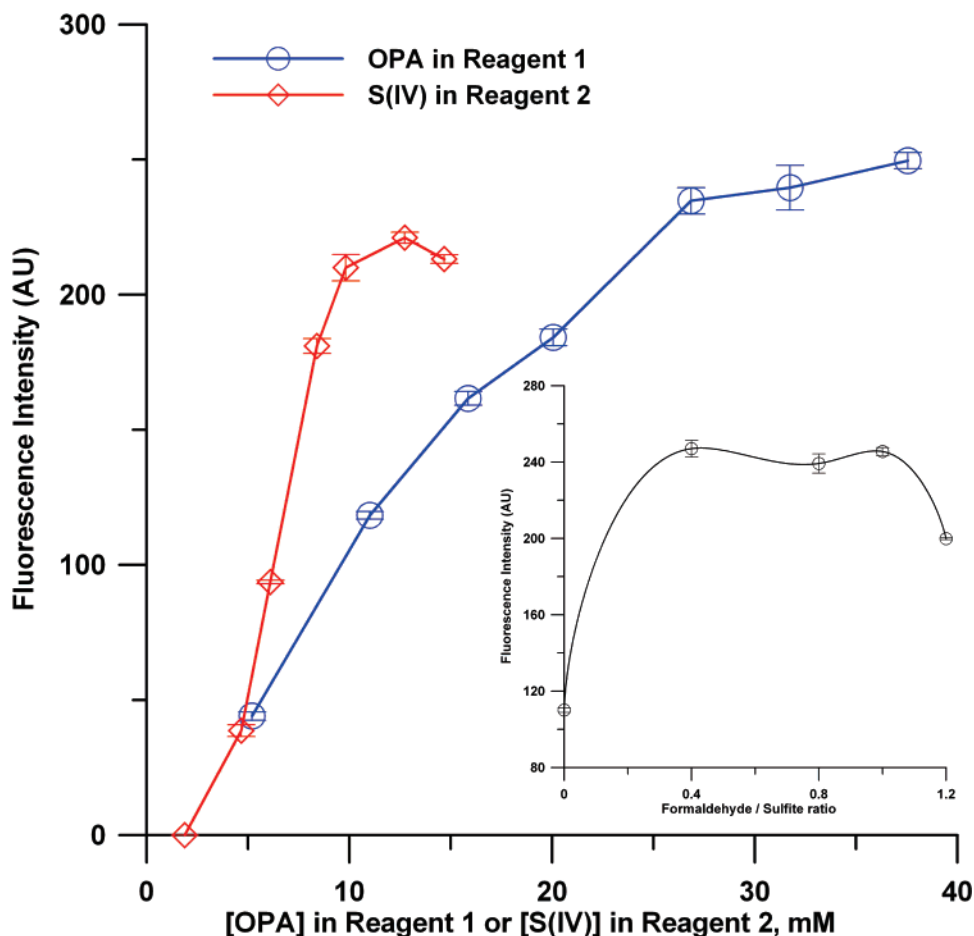
Figure 3. Effect of reagent concentrations on fluorescence intensity. The inset shows the effect of formaldehyde/sulfite (f/s) ratio. Test NH_4^+ concentration was 1 μM.

All chemicals were ACS reagent grade. Exposure of reagents and standards to ambient air was minimized to avoid NH_3 contamination from ambient air. Acidic traps (made of acid-washed silica) were used to protect reagent and standard solutions from the atmospheric ammonia.

Analytical System. The flow injection analysis (FIA) technique was used for this work, as shown in Figure 1. A peristaltic pump, P (Ismatec), was used to pump (160 μL/min) reagents and samples (S) during high-resolution sample analysis. To calibrate the system, water was used as a carrier solution, and a set of standard solutions was aspirated through an electromechanically actuated six-port injection valve (IV) (V-1541-DC, Upchurch) to a high-volume injection loop. The injection loop constituted a 1.0 × 1220 mm PTFE tube (Zeus Industrial Products, NJ), and measured injection volume was 958 μL, which was large enough to achieve a flattop peak from any concentration of standard solutions (Std) within the dynamic range of the instrument. For low-resolution sample analysis, the samples were injected via the injection valve and stored in the sample loop before analysis. The sample throughput is 8 h^{-1} by using the injection valve (IV). For high-resolution analysis, samples are continuously pumped as the carrier solution, and the sample throughput of this system is 3600 h^{-1}.

To produce a fluorescent product, the sulfite solution R2 merged with the OPA reagent stream R1 and then mixed with the sample or standard stream in a heated reaction coil (65 °C,

1.0 × 2000 mm). The final product was detected by a fluorescence detector (FL) (F1080, Hitachi). In-house software was developed using LabVIEW program (National Instruments, TX) to automate the injection valve IV and acquire data from the fluorescence detector.

RESULTS AND DISCUSSION

Spectral Characteristic, Reagent Composition, and Stability. A scanning fluorescence spectrophotometer (F-2500, Hitachi) was used to study the excitation and emission spectra of the reaction product formed from reaction of ammonium with the reagents. The emission−excitation spectra shown in Figure 2 indicate that the maximum of excitation and emission are located at 362.5 and 423.0 nm, respectively. The time necessary for completion of the OPA−sulfite−NH_3 reaction under this condition is typically 15 min at room temperature, as shown in the inset of Figure 2.

The effect of OPA and sulfite concentration in the reagents was studied by analysis of ammonium in freshwater and seawater. The effects of OPA and sulfite concentration are similar for both freshwater and seawater samples. As stated in the Experimental Section, 25 mM OPA and 10 mM sulfite were chosen on the basis of the results of the optimization experiments shown in Figure 3. It is well-known that the sulfite solution is not stable and is subject to oxidation by oxygen[31] and that addition of formaldehyde (HCHO) can stabilize the sulfite solution. It has been shown that
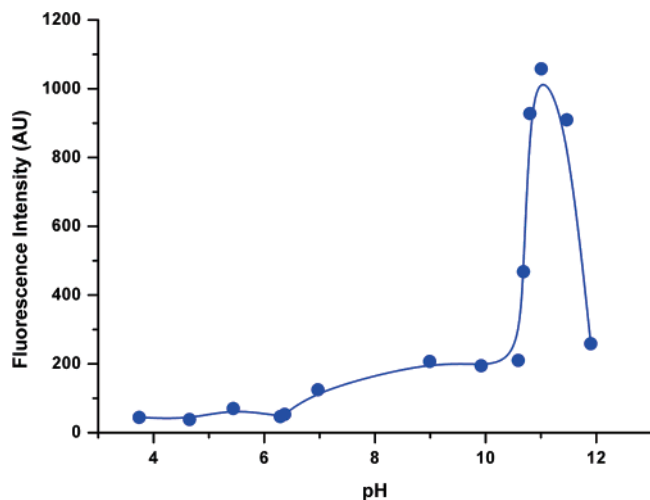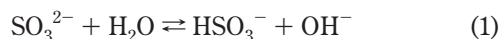
Figure 4. Effect of reaction pH on fluorescence intensity.

sulfite forms a stable complex with formaldehyde as α-hydroxymethanesulfonate[32] that has an intrinsic stability constant of 6.6×10^9 ($K_1 = [\text{HCH(OH)SO}_3^-]/[\text{HCHO}][\text{HSO}_3^-]$). The mechanism for the formation of the hydroxymethanesulfonate complex was given in the following equations:

$$\text{SO}_3{}^{2-} + \text{H}_2\text{O} \rightleftharpoons \text{HSO}_3{}^- + \text{OH}^- \qquad (1)$$

$$\text{HCHO} + \text{HSO}_3{}^- \rightleftharpoons \text{HCH(OH)SO}_3{}^- \qquad (2)$$

The optimal concentration of formaldehyde in the sulfite solution was studied. The effect of the formaldehyde/sulfite ratio (f/s) is shown in the inset picture of Figure 3. The appropriate ratio of formaldehyde to sulfite is 0.4 − 1.0, and an f/s ratio of 0.5 was chosen in this study. We also found that adding formaldehyde to the sulfite solution can increase the signal by 2 times. The optimum concentration of solution R2 is 10 mM sulfite in 5 mM formaldehyde. The measured pH of R2 is 11.8 and agrees with a calculated pH of 11.7. With these recipes, the reagent solutions (R1 and R2) can be used continuously without replacement for at least 1 month.

Effect of pH, Salinity, and Temperature. Under the operating conditions, the pH effect is shown in Figure 4. The best pH was 11, and the sensitivity of the system would be substantially decreased when the pH was lower than 10.7 or higher than 11.5. This pattern is similar to the previous study.[27] However, using a high pH can cause precipitation from seawater. This problem has been circumvented by eliminating the phosphate buffer used in the previous method.[30] Even though no phosphate buffer was in the reagents (R1 and R2), the pH of the final mixture of seawater sample with reagents was still around 11.0 because of the high concentration of sulfite and the high pH in reagent R2. No precipitation was observed from seawater analysis, possibly due to a combination of the effects of complex ions of Mg^{2+} by a high concentration of sulfite,[33,34] dilution of seawater by reagents (1/3), and kinetic hindrance of the precipitation.

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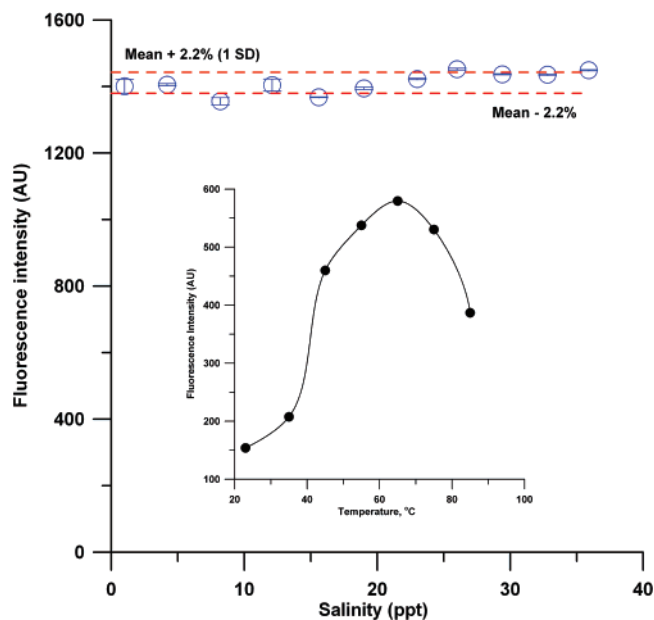(33) Roy, R. N.; Zhang, J. -Z.; Millero, F. J. *J. Solution Chem.* **1991**, *20*, 361−373.

Figure 5. Effect of sample salinity from 0 to 36. The inset shows the effect of the reaction temperature. The salinities were adjusted by mixing with low nutrient seawater.

Table 1. Relative Response of This Method Compared with Previous Method[27] to High Potential Interferences after Mixing Solutions 50 Min, as in Batch Analysis, and Relative Response of This Method to the Interferences after Mixing Solutions 5 Min, as in the FIA Method

sample	previous method (50 min)	this method (50 min)	this method (5 min)
ammonia	100.0 ± 0.0	100.0 ± 0.1	100.0 ± 0.2
methylamine	122.5 ± 0.2	25.2 ± 0.1	32.0 ± 0.0
ethanolamine	64.8 ± 0.1	42.1 ± 0.3	71.8 ± 0.2
glycine	63.6 ± 0.0	30.8 ± 0.1	29.6 ± 0.1
β-alanine	66.2 ± 0.3	34.0 ± 0.2	13.1 ± 0.1
L-phenylalanine	84.1 ± 0.5	56.6 ± 0.3	14.1 ± 0.2

In colorimetric flow analysis, the standard solutions for ammonia measurement in seawater are usually prepared in low-nutrient seawater (LNSW), which was also used as a blank. However, most LNSW has a trace amount of ammonium. By the standard addition method, it was found that different batches of LNSW could vary from 0.01 to 0.3 μM. Moreover, when the LNSW is kept in a plastic bottle, the ammonia concentration can increase up to 0.5 μM after a couple of weeks. Therefore, LNSW cannot be used as a blank. In this system, we used deionized water as a blank instead of LNSW. We therefore tested the effect of salinity up to 36 upon the response due to 10 μM NH_4^+. The sample salinities were measured by a salinity meter (Oakton Salt 6, Eutech instruments, IL), which was calibrated against IAPSO standard seawater (salinity = 34.997, batch P140, Ocean Scientific International, UK). We found that our method has no apparent salinity effect within the $\pm 2.2\%$ of the measurement error, as shown in Figure 5. This observation is in agreement with previous findings.[27]

The inset of Figure 5 shows the effect of reaction temperature. Increasing the temperature was effective in accelerating the

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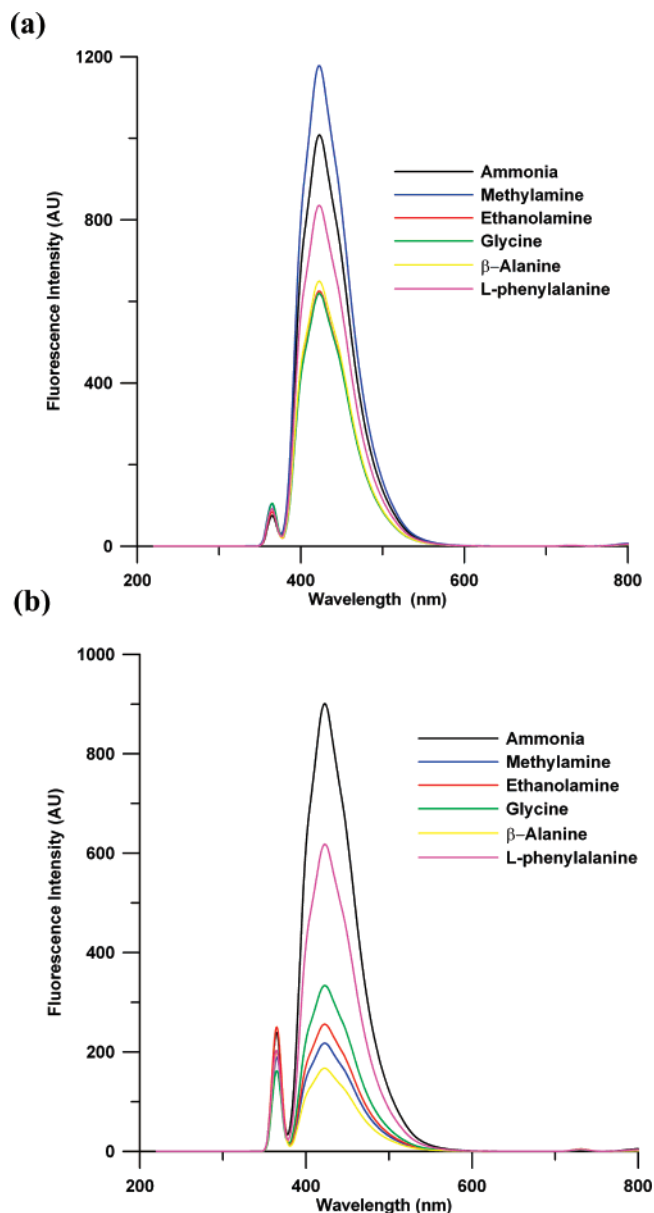
Figure 6. Response signals of high-potential interference species, which have a concentration 1 μM, the same as ammonium. Methylamine, ethanolamine, glycine, β-alanine, and L-phenylalanine were recorded in the Hitachi F-2500 scanning fluorescence spectrophotometer at 50 min after mixing with reagents used in (a) the previous method[27] and (b) this method.

desired reaction. The optimum temperature was 65 °C, at which the system performs with higher sensitivity. If the temperature is higher than 65 °C, bubbles will form inside the system and cause erroneous signals.

Interference Study, Intercomparison Experiment with Standard Method, and Performance. The potential interference species, such as methylamine, ethanolamine, glycine, β-alanine, and L-phenylalanine, were studied to estimate the responses of this method and the previous method to these species. Response signals of methylamine, ethanolamine, glycine, β-alanine, and L-phenylalanine were recorded in the Hitachi F-2500 scanning fluorescence spectrophotometer at 50 min after mixing with reagents used in this method and previous method. Their relative responses to ammonium at a concentration of 1 μM are listed in
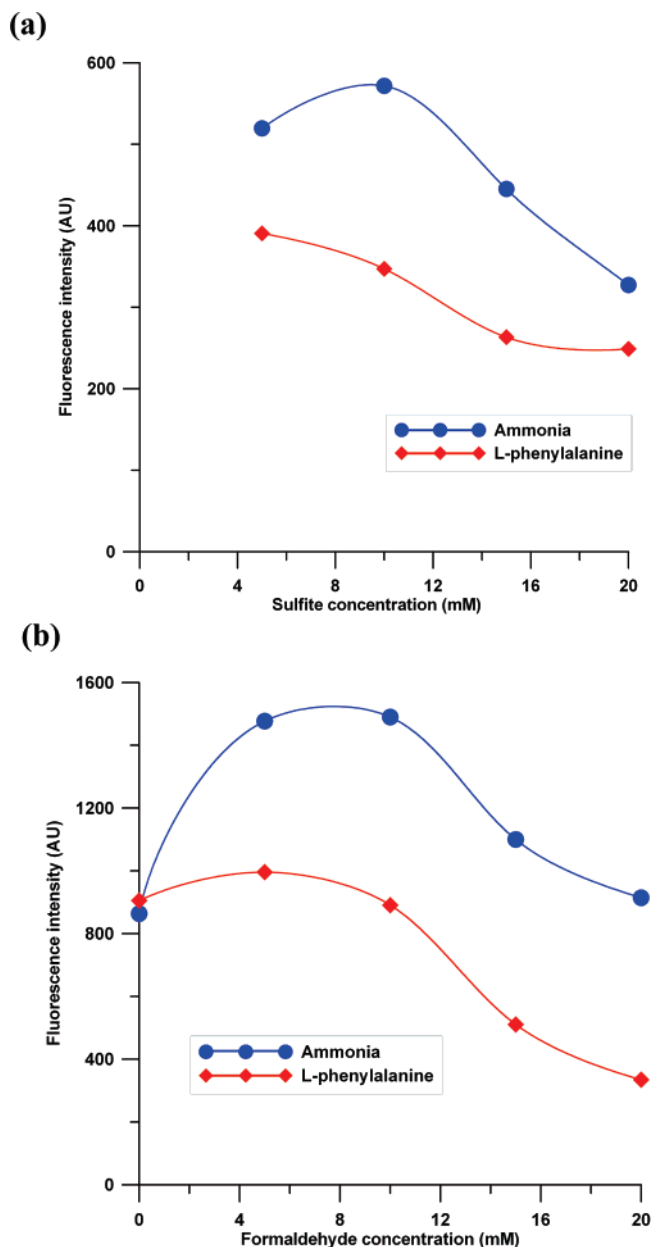

Figure 7. Effect of (a) sulfite and (b) formaldehyde concentration on discrimination against L-phenylalanine.

Table 1, and the response signals of 50-min reaction time are shown in part a of Figure 6 for the previous method[27] and in part b for this method. The interference of these compounds in our FIA analysis of a typical 5-min reaction time is also listed in Table 1. The method is obviously more selective for NH_3 than the previous method[27] in every selected interference species. In addition, the effects of sulfite and formaldehyde concentration on the sensitivity of L-phenylalanine and ammonium measurements were investigated. The results shown in Figure 7a and b, respectively, indicate that optimal sulfite and formaldehyde concentrations chosen for this method result in the discrimination of L-phenylalanine relative to ammonium. From these results, it clearly shows that low-level ammonium measurement must be corrected for these potential interference species, which normally exist in seawaters at concentrations of less than 20 nM.[35−37]

The EPA (Environmental Protection Agency, USA) standard method[14] (Method 349.0, Determination of Ammonia in Estuarine
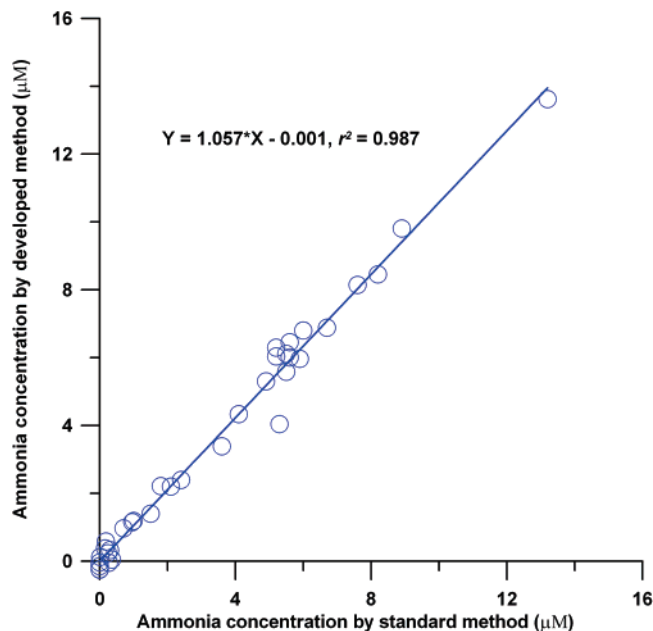
Figure 8. Intercomparison data with the EPA's standard method.[14] Seawater samples were from Florida Bay collected in May 2007.
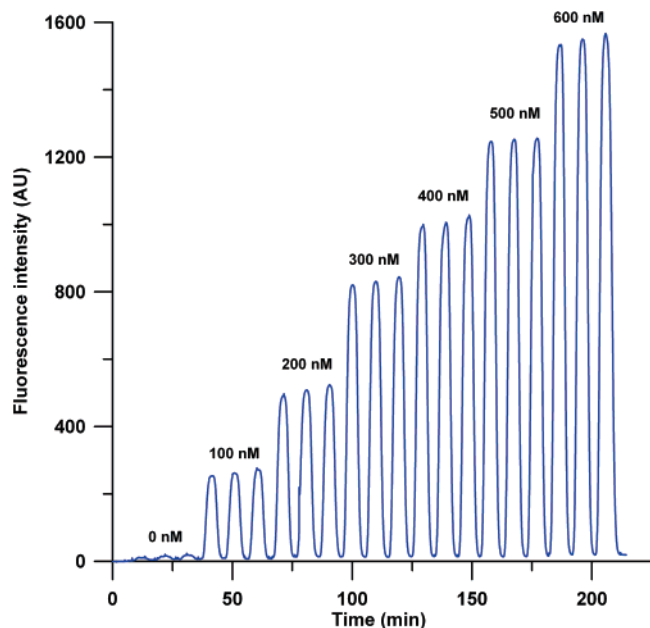

Figure 9. Typical system output of 0, 100, 200, 300, 400, 500, and 600 nM ammonium samples.

and Coastal Waters by Gas Segmented Continuous Flow Colorimetric Analysis) was used to compare with this method for measurement of ammonia in Florida Bay water collected in May 2007. The result in Figure 8 shows a good agreement between these two methods with a wide concentration range from 0.1 to 12 μM.

A typical calibration series performed by the system is shown in Figure 9. With the heated reactor maintained at 65 °C, the system showed a linear response in a range of 0−600 nM NH_4^+,

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Table 2. Performance Comparison with Previous Method[27]

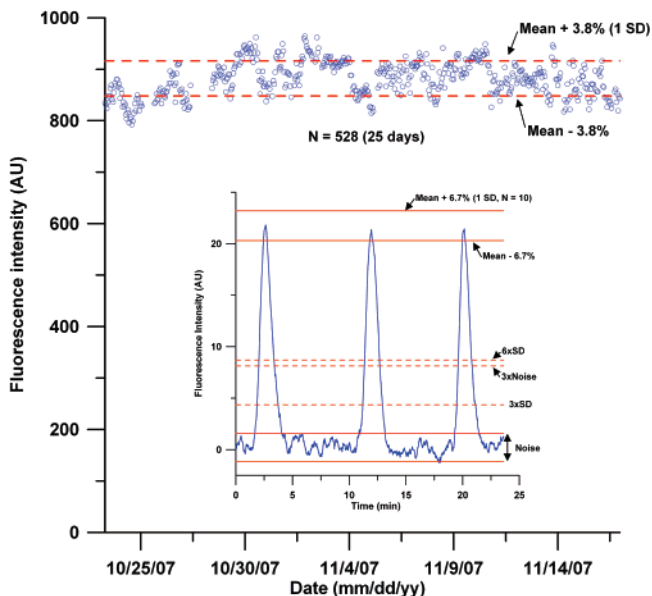| characteristic	previous method	this method
calibration equation	$y = (1498.6 \pm 26.0)x + (19.7 \pm 15.7)$	$y = (1802.3 \pm 37.8)x + (45.7 \pm 18.5)$
linear regression, r^2	0.9988	0.9987
detection limit (3SD)	2.02 nM	1.15 nM

Figure 10. Stability of response for about 25 days by measuring a 0.5 μM ammonium solution every hour. The inset shows typical signals (shown, 3 out of 10 signals) of a 1 nM ammonium solution and baseline with some statistical data, such as noise level, standard deviation (SD) of 10 injections, 3 and 6 times the SD, and 3 times the noise level.

with a linear correlation coefficient of 0.998, as shown in Table 2. The long-term stability of the system is shown in Figure 10 (top), which is the continuous data trace for a ~25-day period with one measurement per hour at an ammonium level of 0.5 μM; 95% of the results fall within the ±3.8% (one relative standard deviation) of the average. Reproducibility is 2.2 and 6.7% at an ammonium level of 200 and 1.0 nM, respectively. The limit of detection was estimated in the laboratory using ammonium standards prepared in distilled water. To ensure the system can detect at a 1 nM ammonium concentration level, the system was injected 10 times with a 1 nM ammonium solution. The response signals shown in the inset picture of Figure 10 are higher than the level of 3 times the noise level (S/N = 3), 3 times the standard deviation (3SD), and 6 times the standard deviation (6xSD). These statistical data show the system is able to measure at a nanomolar ammonium level; however, this detection limit estimated in the laboratory using ammonium solutions prepared in pure water is an idealized detection limit and is likely lower than the practical detection limit when using this instrument for natural water measurements because of the interference species in natural waters.

Field Application. The system has been used in the Florida Area Coastal Environment program, which is primarily concerned with anthropogenic discharges in Florida's coastal ocean. There are several probable sources of nutrients to the Florida coastal waters, including coastal inlets, groundwater seepage, oceanic
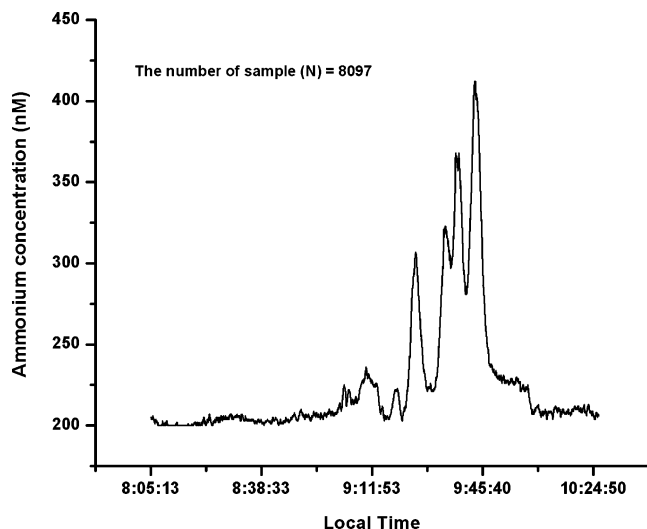
Figure 11. Temporal data (1-Hz sampling frequency) of underway measurement of ammonium at the plume of Delray Outfall in Florida coastal waters during Feb 2007.

upwelling, atmospheric deposition, and outfalls. Currently, the FACE program is focused on the coastal waters of Florida from Miami-Dade to Brevard Counties because this region includes a variety of economically significant recreational activities and several coral reef tracks.

During the survey cruise (from February 21 to March 2, 2007), the 24-m-long research ship R/V Coral Reef II was used to sample

seawater in the coastal area of Boynton Beach, Florida. To measure the ammonia concentration, a submersible water pump was towed to pump seawater from approximately 1-m depth below the surface up to the ship. The seawater was sent through 40-meter nylon tubing (1.25-cm o.d.) to a second pump on the deck of the ship, then the second pump pushed water into the laboratory, where the system was set up. Ship speed was slowed to about 3 knots to ensure match-up with the speed of instrument analysis. A part of the results is shown in Figures 11 and 12, in which the ship was sailing from Boynton inlet down to Delray outfall, made a circle survey around the outfall, and then passed through the plume of the outfall from west to east. The seawater sample was measured every second during sailing from 8:05 am to 10:30 am (~2 h). Seawater samples at the center of the outfall were sampled by towing an open bucket and were measured on the desk. Continuously recorded data (1-Hz sampling frequency) of underway measurement of ammonium at the plume of Delray Outfall in Florida coastal waters during Feb 2007 is shown in Figure 11. The rapid changes in ammonium concentrations were observed when the ship transited in and out of the plume. It clearly demonstrates the need for high-frequency measurement to capture the plume dynamics.

The highest ammonia concentration was about 10 μM at the center of the outfall, which was analyzed from a discrete sample taken at a visible boil at the surface of the outfall. As shown in Figure 12, the highest ammonia concentration was diluted by 50-fold in the ambient current and became less than 0.2 μM with a
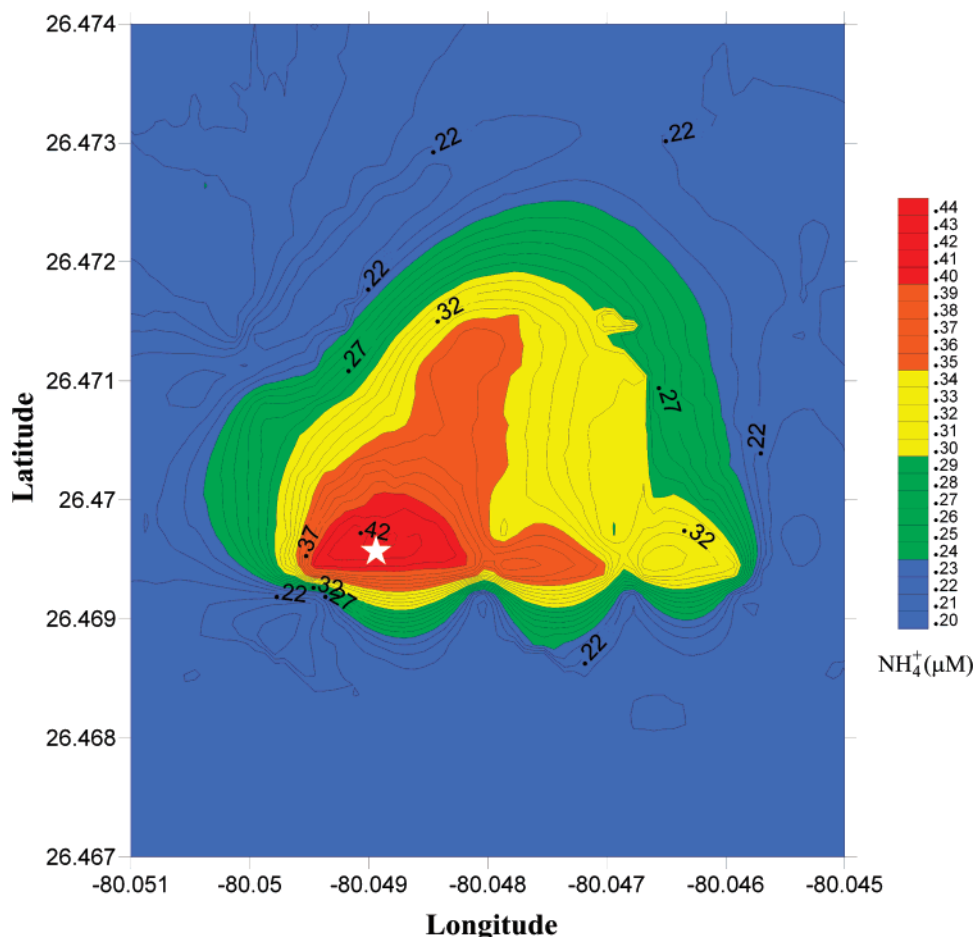

Figure 12. Contour plotting of underway measurements of ammonium at the plume of Delray Outfall in Florida coastal waters during Feb 2007.

traveling distance of ~300 meters from the center of the outfall. In addition, the plume of the outfall seemed to move northward, in agreement with the prevailing current direction of the Gulf Stream.