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A modified method for on-line determination of trace ammonium in seawater with a long-path liquid waveguide capillary cell and spectrophotometric detection

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ABSTRACT

An automated colorimetric method for the on-line determination of trace ammonium in seawater was established using a flow injection technique coupled with a 2.5-m liquid waveguide capillary cell. Using low ammonium seawater as a carrier, a sample was injected into the carrier and mixed with reagents to form indophenol blue dye, which was monitored at a wavelength of 690 nm. Different strategies of reagent injection were investigated to obtain a lower reagent blank and a higher detection sensitivity. Experimental parameters were optimized using a univariate experimental design, and the matrix effect of seawater was preliminarily investigated. The proposed method had high sensitivity with a detection limit of 3.6 $\text{nmol}\cdot\text{L}^{-1}$. The linearity was 10 to 500 $\text{nmol}\cdot\text{L}^{-1}$ and the upper limit could be extended to 30 $\mu\text{mol}\cdot\text{L}^{-1}$ by choosing a less sensitive detection wavelength or lower reaction temperature. The recoveries were between 95.0 and 104.3% and the relative standard deviation was 4.4% ($n = 7$) for an aged seawater sample spiked with 50 $\text{nmol}\cdot\text{L}^{-1}$ ammonium. The sample throughput was 22 h^{-1}. The analytical results obtained with the proposed method showed good agreement with those using reference fluorescence methods. Compared with the normal indophenol blue (off-line) method, the proposed method was superior due to its lower reagent consumption, greater convenience, higher sample throughput, wider linear range (10 $\text{nmol}\cdot\text{L}^{-1}$ to 30 $\mu\text{mol}\cdot\text{L}^{-1}$), as well as higher sensitivity. The method was applied in-field in Wuyuan Bay for 24 h on-line monitoring of ammonium concentrations in the surface seawater. In addition, it was also used to analyze surface seawater samples collected from the South China Sea for the study of ammonium distribution.

1. Introduction

Ammonium, the most reduced form of inorganic nitrogen in seawater, is known as an important nutrient in the marine environment nitrogen cycle. In the ocean, the ammonium ion is the dominant form of ammonium, while ammonia is a minor component. The ammonium concentration reflects the balance between production via ammonification and consumption via ammonium assimilation or nitrification. Although the ammonium inventory in the ocean is approximately three orders of magnitude lower than that of nitrate (Brandes et al., 2007; Capone et al., 2008), the phytoplankton generally prefer ammonium rather than nitrate because additional energy is required to reduce nitrate to ammonium during assimilation (Harrison et al., 1996; Wheeler and Kokkinakis, 1990). Accurate measurement of ammonium in seawater is needed in many research areas, such as the study of new production and the rate of decomposition and denitrification. Knowledge concerning the ammonium concentration in seawater is also necessary for characterization of the water masses in modeling nutrient fluxes and the estimation of algal growth potential (Horstkotte et al., 2011). However, from coastal water to the open ocean, surface water ammonium concentrations are greatly changed from micromolar dozens to nanomolar, and most of the analytical methods can accurately determine micromolar ammonium but fail to detect nanomolar ammonium. Additionally, the residence time of ammonium in the surface ocean is typically hours or even less (Clark et al., 2008) and, from day to day, the ammonium concentration often varies little, suggesting a close balance between removal and production processes (Dodds, 1993). Therefore, in order to better understand the role of ammonium in the marine nitrogen cycle, a rapid ammonium measurement method with the advantages of high sensitivity and wide linear range for on-line determination is necessary.

Many techniques have been developed to analyze ammonium in seawater. The ion selective electrode method (Beckett and Wilson, 1974; Garside et al., 1978) is easy to operate and suitable for continuous determination, but long equilibration time, low sensitivity and matrix interference hinder its application. The fluorescence method is widely used because of its higher sensitivity than the colorimetric method (Amornthammarong et al., 2013; Roth, 1971). A shipboard fluorometric flow analyzer is developed for high-resolution underway measurement

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of ammonium in seawater (Amornthammarong et al., 2006; Zhang and Dasgupta, 1989), and combining fluorescence detection with flow analysis and solid phase extraction (SPE), the detection limit can be as low as 0.7 $\text{nmol}\cdot\text{L}^{-1}$ (Zhu et al., 2013). However, the method is time consuming and unsuitable for application in high coastal water concentrations of ammonium. Another approach uses a gas-permeable membrane to separate ammonium from the sample solution, where the sample is mixed with a base to raise the pH to 10.5 and to convert ammonium ions to ammonia gas, which can diffuse across a hydrophobic gas permeable membrane into a receiving solution for detection (Oliveira et al., 2007, 2008; Plant et al., 2009; Terry et al., 2008). Although this method is easy to automate and shows no matrix interference, the mass transfer efficiency is not high enough and the analysis is time consuming. The indophenol blue (IPB) spectrophotometric method based on the Berthelot reaction (Grasshoff et al., 1999; Pai et al., 2001) is still the most common and widely adopted method for ammonium determination in seawater and is also adopted by the U.S. Environmental Protection Agency (EPA, 1997). However, low sensitivity and high levels of toxic reagent blank are its main problems. SPE coupled with a spectrophotometer is developed to improve the sensitivity (Chen et al., 2011); however, the method still suffers from the high levels of reagent blank and its toxicity.

In recent years, the liquid waveguide capillary cell (LWCC) has played an important role in the measurement of chemical species at nanomolar concentrations (Huang et al., 2012; Páscoa et al., 2012; Zhang and Chi, 2002). According to the Lambert–Beer law, the absorbance of a sample will increase with the extension of the optical path length, thus enhancing the sensitivity. Extending the optical path length can be easily achieved by applying an LWCC, which involves a capillary made of a flexible fluoropolymer material (Teflon AF, DuPont) with a refractive index (1.29) lower than that of water (1.33). The light introduced into the capillary core is totally internally reflected and there is constrained propagation within the capillary towards the detector (Gimbert and Worsfold, 2007). With an LWCC, a gas-segmented continuous flow colorimetric method has been developed for the analysis of ammonium in seawater (Li et al., 2005). However, the system is complex and involves a nitrogen gas supplier.

A modified method using a 2.5-m long LWCC together with flow injection analysis and the IPB method was developed in our work. The effects of the reagent blank were greatly reduced owing to the lower concentration of reagents used, and the sensitivity was enhanced with LWCC. Additionally, a wider linear range of 10 $\text{nmol}\cdot\text{L}^{-1}$ to 30 $\mu\text{mol}\cdot\text{L}^{-1}$ was obtained by adjusting the detection wavelength and reaction temperature. The method was capable of measuring ammonium in the open ocean as well as in coastal and shelf waters.

2. Materials and methods

2.1. Reagents and solutions

All the chemicals used in this study were of analytical grade, and were supplied by Sinopharm Chemical Reagent Co., China (www.reagent.com.cn), unless stated otherwise. All solutions were prepared in purified water (resistivity > 18.20 $\text{M}\Omega\cdot\text{cm}$) from a water purification system (Millipore Co., MA, USA, www.millipore.com). Plasticware for storing reagents and standard solutions as well as seawater samples were high-density polyethylene (Nalgene®, Nalge, NY, USA, www.nalgene.com) bottles and carefully washed before use (Chen et al., 2011). Reagent bottles were sealed with an air inlet clean-up device, which consisted of a syringe filled with acidic silica gel (Amornthammarong et al., 2006). The acidic silica gel inside the clean-up device, as an effective trap for basic ammonia gas, was prepared by adding 50 mL 6 $\text{mol}\cdot\text{L}^{-1}$ H_2SO_4 into 200 g of 20-mesh silica gel then dried overnight at 70 °C.

A 100 $\text{mmol}\cdot\text{L}^{-1}$ NH_4Cl (ammonium chloride) stock solution was made by dissolving 0.5349 g of NH_4Cl (Fluka, USA, www.sigmaaldrich.com), 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. The complexing reagent solution was prepared by dissolving 20 g of $Na_3C_6H_5O_7\cdot 2H_2O$ (sodium citrate, Merck, Germany, www.merck-china.com), 2.0 g of NaOH (sodium hydroxide, GR, Merck) and 2.5 g of $C_{10}H_{14}N_2Na_2O_8\cdot 2H_2O$ (EDTA-Na_2, ethylenediaminetetraacetic acid disodium salt) in 250 mL pure water. To prepare a 0.53 $\text{mol}\cdot\text{L}^{-1}$ C_6H_5OH (phenol) stock solution 5.0 g of C_6H_5OH (Tedia, USA, www.tedia.com) was dissolved and diluted to 100 mL with pure water, and then, 21.3 $\text{mmol}\cdot\text{L}^{-1}$ C_6H_5OH was prepared by dilution of this stock solution as the working solution. A 0.16 $\text{mmol}\cdot\text{L}^{-1}$ $NaC_3Cl_2N_3O_3$ (SDIC, sodium dichloroisocyanurate) stock solution was made with 0.3517 g of $NaC_3Cl_2N_3O_3$ (Alfa Aesar, UK, www.alfachina.cn) dissolved in pure water and diluted to 100 mL. The working solution of 0.64 $\mu\text{mol}\cdot\text{L}^{-1}$ SDIC was obtained by diluting the stock solution. A 18.46 $\text{mmol}\cdot\text{L}^{-1}$ $Na_2Fe(CN)_5NO\cdot 2H_2O$ (sodium nitroferricyanide) solution, acting as a catalyst in the reaction, was made by dissolving 0.55 g $Na_2Fe(CN)_5NO\cdot 2H_2O$ (Alfa Aesar) in 100 mL of pure water and the working solution of 0.22 $\text{mmol}\cdot\text{L}^{-1}$ $Na_2Fe(CN)_5NO\cdot 2H_2O$ was produced by diluting the stock solution.

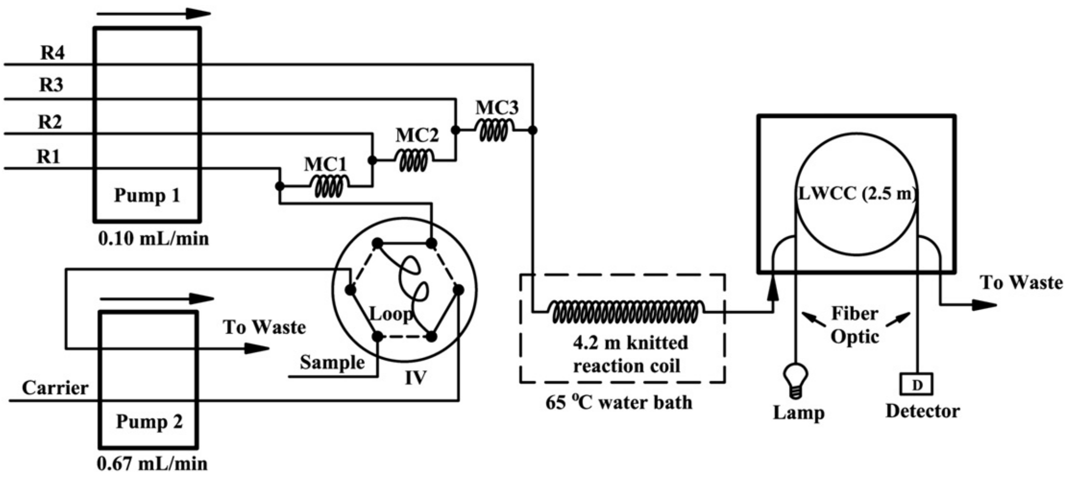

Fig. 1. Flow injection manifold configuration (R1, complexing reagent solution composed of citrate, NaOH and EDTA-Na_2; R2, phenol solution; R3, sodium dichloroisocyanurate solution; R4, sodium nitroferricyanide solution; carrier, low ammonium seawater; pump, peristaltic pump; MC1–MC3, mixing coils of 40 cm length each; IV, 6-port, two-position injection valve, in "fill" position, s/std solution was loaded into the loop: 60 s; in "inject" position, the carrier pushed the injected s/std solution into the analytical system: 80 s).