Sequential injection spectrophotometric determination of nanomolar nitrite in seawater by on-line preconcentration with HLB cartridge

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Abstract
The unstable state of nitrite results in its very low concentration in seawater, which is below the limit of detection (LOD) of conventional techniques of analysis. Some sensitivity-enhanced methods have been proposed for the determination of nitrite at nanomolar level to illustrate the role of nitrite in the marine nitrogen cycle. However, most of previous reports are not widely accepted, because of their complexity and cost equipment or intensive labor requirement. In this study, a simple automatic system for the determination of nanomolar level nitrite using on-line preconcentration with spectrophotometric detection was described. An Oasis HLB cartridge was adopted to quantitatively enrich the pink-colored azo compound, formed from nitrite via Griess reaction. The cartridge was rinsed with water and ethanol (volume fraction is 55%, the same below), in turn, then eluted by an eluent containing 50% ethanol and 0.25 M(H2SO4), and determined at 543 nm with a 2 cm path-length flow cell. Under the optimized experimental conditions, the calibration curve showed a good linearity in the range of 1.4–85.7 nM, and the LOD (3σ) was estimated to be 0.5 nM. The relative standard deviations of 7 measurements were 4.0% and 1.0% for the samples spiked at 7.1 and 28.6 nM, respectively. The recoveries for the different natural water samples were between 92.2%–108.4%. Each HLB cartridge could be reused for at least 50 times. As compared with other SPE methods, the advantages of this method included the free of interference from salinity variation and less sample consuming. The results of the application of the proposed method to natural water showed good agreement with liquid waveguide capillary cell detection method.

Key words: nitrite, seawater, on-line preconcentration, sequential injection, solid phase extraction

1 Introduction
It has long been recognized that nitrite is an intermediate product in the oxidation or reduction reactions between ammonium and nitrate, and it is a useful indicator for calculating the nitrification or denitrification processes in the marine nitrogen cycle (Dore and Karl, 1996a; Brandes et al., 2007). As a major form of nitrogen, nitrite is assimilated by phytoplankton for their growth in euphotic zone (Ryther and Dunstan, 1971). Below the surface, nitrite may be excreted by phytoplankton or bacteria as a result of incomplete assimilatory reduction of nitrate (French et al., 1983; Dore and Karl, 1996b). A narrow layer, called primary nitrite maximum, with nitrite concentration exceeding 50 nM, exists in the base of the euphotic zone over many of the world’s oceans (Zafiriou et al., 1992; Dore and Karl, 1996b). However, nitrite concentrations are below 50 nM in more than 90% of oxic deep water column, because of its unstable state (Zafiriou et al., 1992; Dore and Karl, 1996b; Zhang, 2000; Adornato et al., 2007; Chen et al., 2008).

The standard colorimetric assay with a LOD of 30 nM (Grashoff et al., 1983) is unable to provide enough sensitivity to detect the nitrite in most open ocean samples. Consequently, for the aim of biogeochemical research, much effort has been devoted to improve the detection sensitivity and analytical precision for low level nitrite samples. Some sensitive methods applied to analyze the ultra trace nitrite in natural waters include chemiluminescence (Cox, 1980; Garside, 1982; Braman and Hendrix, 1989; Zafiriou et al., 1992), fluorometry (Axelrod and Engel, 1975; Motomizu et al., 1986; Masserini and Fanning, 2000), and HPLC
methods (Kieber and Seaton, 1995). However, the instruments used in these study are expensive, highly specialized and mainly laboratory based, as compared to the spectrophotometric method.

The sensitivity-enhanced spectrophotometric approaches include increasing the path length of the detector cell using liquid waveguide capillary cell (LWCC) devices (Yao et al., 1998; Zhang, 2000, 2006; Adornato et al., 2007; Gimbert and Worsfold, 2007; Patey et al., 2008) and preconcentration of the azo compound formed from nitrite with normal spectrophotometric detection (Pai et al., 1996; Miró et al., 2000; Chen et al., 2008). Although the LWCC detection technique become more popular in recent years (Gimbert and Worsfold, 2007; Patey et al., 2008), the interferences, including variation of salinity between samples (Zhang, 2000), blockage of particle and adsorption from surface-reactive species (Gimbert and Worsfold, 2007), to the performance of LWCC were still concerned. The separation techniques, including liquid-liquid extraction (LLE) (Ren et al., 2008) and solid-phase extraction (SPE) (Liang et al., 2007; Chen et al., 2008), are widely used for the isolation and concentration of the target analytes from the seawater. In the published preconcentration methods, the C18 cartridge was the primary choice for enriching the azo compound (Pai et al., 1996; Miró et al., 2000; Chen et al., 2008). Nevertheless, the matrix interference from seawater leading to low recoveries (70%-80%) has been observed (Chen et al., 2008), and moreover, a low batch-to-batch reproducibility is obtained among the different C18 cartridges.

The aim of this study was to develop an on-line preconcentration method with normal spectrophotometric detection to analyze ultra trace nitrite in natural water samples. A sequential injection (SI) system was employed to carry out on-line preconcentration and desorption. An Oasis HLB cartridge was used as the enrichment column to minimize the matrix interferences. Experimental parameters in reaction, preconcentration, along with desorption, were investigated in order to achieve satisfactory sensitivity and precision.

2 Experiment and methods

2.1 Reagents

All the chemicals used in the study were of analytical grade, supplied by Sinopharm Chemical Reagent Co., Ltd., China, unless stated otherwise. All solutions were prepared with fresh ultra-pure water (18.2 MΩ/cm), obtained from a Millipore Purification Water System (Millipore Co., MA, USA.). All bottles and vessels used were soaked in 3 M HCl for at least 1 h, and rinsed with the pure water thoroughly.

The sulfanilamide (SAM) solution containing 0.20 M of SAM and 2.4 M HCl, and the 4 mM N-1-naphthylethylenediamine dihydrochloride (NED) solution were prepared as color developing solutions. A 55% ethanol (volume fraction, the same below) was prepared as the pre-rinsing solution. A solution containing 50% (volume fraction, the same below) ethanol and 0.25 M H2SO4 was prepared as the eluent. The pre-rinsing and eluent solutions were degassed in an ultrasonic bath for 10 min prior to use.

Nitrite stock standard solution (100 mg N/dm3, GBW(E)-080223-0610) was purchased from National Research Center for Certified Reference Materials (Beijing, China) and stored at 4°C. Nitrite working solutions (50 µg N/dm3) were obtained by appropriate dilution of the stock solution daily.

2.2 Apparatus

The SI manifold proposed for nitrite determination is schematically shown in Fig. 1.

The equipments employed to automatically carry out on-line preconcentration operations included two peristaltic pumps (Beijing Titan Instruments Co., Beijing, China), a rotary valve (Beijing Titan Instruments Co., Beijing, China), and a VICI C25-3188EMH 8-position selection valve (Valco Instruments Co. Inc., Houston, TX., USA). All instruments were controlled by a homemade single-chip controller. All the pump tubings were of silicon-latex, and the other tubings (0.8 mm i.d.) were made of PTFE material.

The sample held in a 200 ml polyvinylchloride reaction vessel was incubated in a homemade thermostat water bath at (40 ±0.2)°C. All the solution bottles along with the reaction vessels were put in a closed cabinet with an air filtration unit to eliminate the nitrogen oxides (NOx) contamination from the surrounding air. The air filtration unit was assembled by an air pump and two bottles connected in series both containing the mixture solution of SAM and NED. Air was continuing pumped through the bottles and the NOx was absorbed by the mixture solution. With the filtration unit, no significant effect from the air was observed on the reagent blank.

An Oasis HLB cartridge (200 mg, Waters Corporation, Milford, MA., USA) was connected to the