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Standard addition/absorption detection microfluidic system for salt error-free nitrite determination

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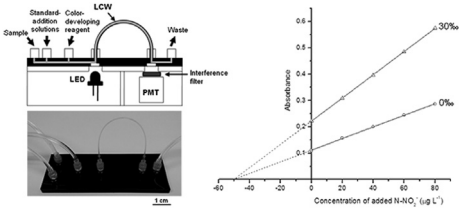
HIGHLIGHTS

- Microfluidic chip-based absorption detection system for salt error-free determination of nitrite in water.
- Standard addition, Griess reaction, and absorption detection play on the microchip in a continuous-flow mode.
- The salt error is caused by the reduction in the yield of Griess reaction.
- The use of black PDMS secures a wide linear range in detection.
- Externally mounted liquid-core waveguide detection flow cell facilitates optical path length change.

GRAPHICAL ABSTRACT



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ABSTRACT

A continuous-flow microfluidic chip-based standard addition/absorption detection system has been developed for accurate determination of nitrite in water of varying salinity. The absorption detection of nitrite is made via color development using the Griess reaction. We have found the yield of the reaction is significantly affected by salinity (e.g., −12% error for 30‰ NaCl, 50.0 μg L^{-1} $N-NO_2^-$ solution). The microchip has been designed to perform standard addition, color development, and absorbance detection in sequence. To effectively block stray light, the microchip made from black poly(dimethylsiloxane) is placed on the top of a compact housing that accommodates a light-emitting diode, a photomultiplier tube, and an interference filter, where the light source and the detector are optically isolated. An 80-mm liquid-core waveguide mounted on the chip externally has been employed as the absorption detection flow cell. These designs for optics secure a wide linear response range (up to 500 μg L^{-1} $N-NO_2^-$) and a low detection limit (0.12 μg L^{-1} $N-NO_2^-$ = 8.6 nM $N-NO_2^-$, S/N = 3). From determination of nitrite in standard samples and real samples collected from an estuary, it has been demonstrated that our microfluidic system is highly accurate (<1% RSD, n = 3) and precise (<1% RSD, n = 3).

1. Introduction

Estuary, a transitional zone where freshwater and seawater meet and mix, is one of the most biologically productive ecosystems and creates various habitats for humans and a wide range of

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animal and plant species [1,2]. In addition, wetlands and plants in estuary function as a filter, removing pollutants from industry and agriculture [3–5]. However, excessive nitrite runoffs from natural and human-made sources in estuary result in eutrophication causing algal blooms, massive fish kills, and red tides [5–7]. Furthermore, nitrite in humans converts hemoglobin to methemoglobin which cannot transport oxygen, and forms carcinogenic nitroso compounds resulting from reaction with amides and amines [8–11]. United States Environmental Protection Agency (U.S. EPA) has set the maximum allowable concentration for nitrite in drinking water at 1 mg L^{-1} $N-NO_2^-$ [12]. Consequently, it is important to monitor nitrite in estuary in order to protect human health and aquatic ecosystems, and to understand estuarine ecology [13–16].

Griess assay, a U.S. EPA-approved analytical method, is the most popular approach for nitrite determination, where nitrite is converted into a highly absorbing azo compound through diazotization/coupling reaction and the absorbance at 540 nm is measured [17–20]. Notable popularity of the method is attributed to its high nitrite-selectivity and wide applicability to a broad range of environments and natural products: for examples, vegetables, soil, various aquatic systems, etc [9,11,15,16,20–22]. For monitoring of nitrite in natural waters, conventional tubing-based flow injection analysis (FIA) systems have mainly been employed because they facilitate automated, high-throughput analysis [20–23]. However, since such tubing-based systems are big and therefore, consume reagents for analysis at high rates, maintenance in short intervals is demanded for continuous monitoring, which has made deploying more systems for high spatial-resolution monitoring difficult [16,22,23]. Recently interests in developing microfluidic chip-based monitoring systems become increasing because to use microchips could reduce the size and price of a system substantially [16,22–28]. Such small systems can be loaded in patrol boats, underwater vehicles, buoys, etc. for mobile or unmanned monitoring [29–32]. Moreover, the microchip-based systems consume analysis reagents and power at very low rates, and therefore, frequent maintenance is not required, which is very favorable to unmanned telemonitoring [16,22–27]. These advantages would also make monitoring with high spatial-resolution possible [16,22–25].

Microfluidic system for the nitrite analysis using the Griess method was first introduced by Greenway et al [33]. They developed a glass microchip-based FIA system using electroosmotic flow pumps. Sieben and co-workers reported a sensitive microfluidic absorbance detection system based on a tinted poly(-methylmethacrylate) (PMMA) absorption cell for making a simple, efficient optical coupling with a light-emitted diode (LED) light source and a photodiode phototransducer [22]. Sensitive nitrite determination was achieved by the use of the colored PMMA that absorbs light reaching the detector via paths undesirable in quantitative absorption spectrometry. Recently, the same group developed an unmanned in situ monitoring system based on the tinted PMMA microchip and reported high spatial-resolution monitoring of nitrite and nitrate in a tidal estuary [16]. These reported FIA and microfluidic systems employed external-standard calibration for nitrite determination [16,20–22]. In this calibration mode, it is practically impossible to make the matrix composition of the standard solutions identical to that of complex samples, which may cause unacceptable error in quantitation [34,35]. This kind of issue would be very serious when nitrite monitoring is demanded in estuarine water because the salinity of water can vary from 0 to 35‰ (Salinity is the total amount of dissolved salts in water. The common unit of salinity is per mil (‰) that is parts per thousand.) depending on locations, daily tides, seasons, current patterns, etc [1,2,36,37]. The U.S. EPA method (Method 353.4) also notes that the salinity difference between samples and standards could generate errors in the Griess-reaction-based determination (so called salt error) [38]. Moreover, estuary is, at high probability, exposed to other interfering species (e.g., Fe^{3+}, Pb^{2+}, Hg^{2+}, Ag^{+}, Cu^{2+}, Cr^{6+}, etc.), owing to human activities [19,20,39,40].

In this work, we report the first development of salt error-free nitrite determination system, where standard addition, color development by the Griess reaction, and sensitive absorption detection are integrated on a continuous-flow poly(-dimethylsiloxane) (PDMS) microchip. We have found the use of standard-addition calibration in determination of nitrite in estuarine water can effectively compensate for matrix effect from salts and other interfering species in the water. It has been also found that the salt error arises from the reduction in the yield of the color development reaction. The absorption detection employs an 80-mm liquid-core waveguide (LCW) flow cell to provide an extended absorption length, black PDMS as microchip substrate to minimize stray light contribution in detection, and a housing to isolate the light source and the detector parts, respectively. Since the assembly of the microchip and the housing is compact, it has great potential to be developed as an unmanned system for accurate, high spatial-resolution nitrite monitoring.

2. Experimental

2.1. Chemicals and reagents

All solutions were prepared using deionized (DI) water from a Milli-Q water purification system (Millipore, Milford, MA). The stock solution of 100.0 mg L^{-1} $N-NO_2^-$ was prepared by dissolving 0.493 g of sodium nitrite (Sigma–Aldrich, St. Louis, MO) in water and diluting it to 1 L. Nitrite concentrations are measured by the Griess method where through the reaction with sulfanilamide, nitrite is converted to a diazonium salt that is then coupled with N-(1-naphthyl)-ethylenediamine (NED) to form a red azo dye whose high absorbance at 540 nm is directly proportional to the concentration of nitrite [18,19]. The color-developing reagent for the Griess assay on our microchip analysis was prepared by dissolving 1.0 g of sulfanilamide (Sigma–Aldrich, St. Louis, MO) completely in 10 mL of 85% phosphoric acid, then adding 0.10 g of NED dihydrochloride (Sigma–Aldrich, St. Louis, MO) to it, and finally making the total volume of 500 mL with DI water [19].

The salinity of the nitrite standard samples was adjusted by sodium chloride or magnesium sulfate. The 10‰, 20‰, and 30‰ salinity of the standard samples were prepared by dissolving 1.0 g, 2.0 g, and 3.0 g of sodium chloride (Samchun Pure Chemical Co., Ltd., Pyeongtaek, South Korea) in 100 mL of the sample, respectively. The molar concentration of sodium chloride in the standard samples is 171 mM, 342 mM, and 513 mM, respectively. Similarly, another standard sample set with the same concentrations of salt ions was prepared by dissolving 2.06 g, 4.12 g, and 6.17 g of magnesium sulfate (Samchun Pure Chemical Co., Ltd.) in 100 mL of the sample, respectively. The salinity of all samples was measured by a salimeter (Multi 3410 SET 7, WTW GmbH, Weilheim, Germany). All real samples are filtered through 0.45 μm syringe filters (Sartorius Stedim Biotech GmbH, Göttingen, Germany).

2.2. Standard addition/absorption detection microchip

The microchip for absorbance measurements in a continuous flow mode comprises two black PDMS plates (26 mm in width and 70 mm in length). In Fig. 1A, the 3 mm thick top layer has two serpentine patterns and six holes. Four holes of these are used for the inlets of a sample, standard-addition solutions, and the Griess reagent, and the outlet of waste, respectively. The other two holes