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Automated determination of nitrite in aqueous samples with an improved integrated flow loop analyzer

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ABSTRACT

A compact loop flow analyzer was developed for automated determination of nitrite in aqueous samples based on Griess reaction and spectrophotometric detection. The integrated system employed a customized single chip microcomputer based hardware and laboratory-programmed software written by LabVIEW to control the injection valve, pumps and optical detection module. Experimental parameters related to nitrite analysis, including the reagent volume, sample temperature and salinity were evaluated and optimized. This fully automated analyzer showed a limit of detection as low as 0.02 μM with sample throughput of 15 h^{-1}. The calibration curves prepared in pure water and low nutrient seawater were consistent over the linear range of 0–10 μM (R^2 = 0.999), showing insignificant interference from the common ions in seawater. Different aqueous samples were successfully analyzed using both the present analyzer and the standard benchtop method, and the results from the two techniques showed no significant difference according to the statistical *t*-test (*P* = 0.95). This compact automatic analyzer is simple, fully integrated, and user-friendly, making it highly suitable for field applications, such as on-line environmental monitoring and underway analysis.

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1. Introduction

The nitrite plays an important role in various fields. For example, environmental scientists care about its toxicity to the environment and human health, therefore the US Environmental Protection Agency has set the maximum contaminant levels of nitrite in drinking water at 1 mg/L (21.7 μM) [1]; in the seawater, nitrite is an useful indicator for understanding the nitrification and denitrification processes in the marine nitrogen cycle, therefore nitrite concentration is considered as one of the basic nutrient data [2]; for analytical chemists, nitrite is a favorable testing analyte for newly developed analytical platforms, such as microfluidic paper-based analytical devices [3,4]. Therefore, the determination of nitrite is of importance from environmental, oceanographical and analytical chemistry perspectives. When we searched the keywords "nitrite determination" in the database "Web of Science"; we found that the average number of annual publications on this topic was 37 ± 6 from the year 2000 to 2015 (data shown as Fig. S1 in Supplementary Materials); which confirms that the determination of nitrite has received consistent attention from the scientific community over the years.

Reviews about nitrite analysis have been published [5,6], and the authors have summarized the different detection techniques, and discussed the advantages and limitations of the various methodologies. Very recently, several new techniques have been reported for nitrite detection, such as new electrodes [7], electrochemical sensors [8], capillary zone electrophoresis [9], high-resolution continuum source electrothermal molecular absorption spectrometry [10], fluorescent probes [11], quadruple isotope dilution mass spectrometry [12], etc. The most popular method for nitrite analysis is the spectrophotometric Griess assay, which is based

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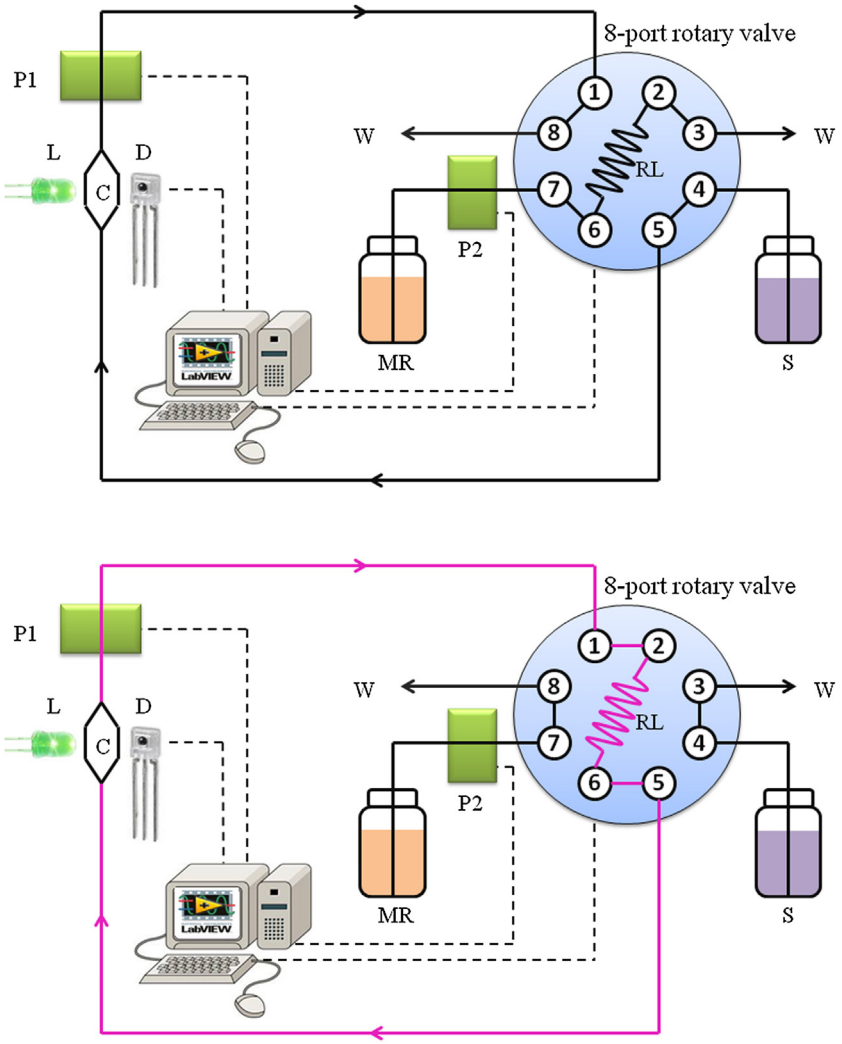

Fig. 1. Schematic diagram of the integrated loop flow analyzer (up: "Fill" position; down: "Inject" position). PP1/2: peristaltic pump 1/2; L: light source; C: cell; D: detector; W: waste; RL: reagent loop; MR: mixed reagent; S, sample. The volumes of reaction loop and sample loop are 75 μL and ~3 mL, respectively.

on the diazotization of nitrite with sulfanilamide (SAM) and N-1-naphthylethylenediamine dihydrochloride (NED) under acidic conditions. The resulting pink-color azo dye can be detected at 540 nm [13]. The Griess reaction has been combined with different kinds of techniques to widen its application, such as microfluidic chip-based system [14,15], liquid core waveguide [16], solid phase extraction [17], etc. It should be noted that the flow analysis method combined with Griess reaction is still the mainstream or approved method for nitrite analysis [13].

The current flow based method suffers from problems caused by differences in refractive index of samples with various levels of salinity and the presence of incidentally formed bubbles, which can be major issues for the optical detection. Recently, we developed a loop flow analysis method for the determination of phosphate in aqueous samples using a novel cross-shaped flow cell, which was able to overcome these problems [18]. The principle of this method is that the optical path of the cell is perpendicular to the flow path (i.e. "cross-shaped"), therefore when the cell is positioned with horizontal light path and vertical liquid flow path, the bubbles in the cell can float away and be removed from the optical path [19]. Moreover, the reagent consumption of loop flow based system is less than some other flow analysis, and the steady state chemical condition is more reproducible under different circumstance. However, the previously described system utilized several individual commercial instruments, and thus it was only suitable for bench-top use and too complicated for field application (data not shown). Therefore, we demonstrate here an integrated loop flow analyzer for nutrient monitoring, which is suitable for field applications. The developed analyzer has been optimized and different parameters were evaluated. This analyzer has been successfully applied for the determination of nitrite in different kinds of aqueous samples.

2. Experimental

2.1. Reagents

All chemicals used in this study were of reagent grade or higher quality, purchased from Sinopharm Chemical Reagent Co., China (www.reagent.com.cn) and used without further purification. Nitrite stock solutions of 100 mM were prepared by dissolving pre-dried solid $NaNO_2$ in water. Working solutions of 400 μM was prepared by stepwise dilution of the stock solutions with water. The SAM solution of 10 g/L was prepared by dissolving SAM in 10% HCl (v/v) solution, and the NED solution of 1 g/L was prepared by dissolving NED in water. The working reagent solution was a 1:1 mixture of SAM and NED solutions. All the standard and reagent solutions were stored at 4 °C in a refrigerator while not in use.

Table 1
The descriptions of the proposed loop flow analysis program.

Step	Time, s	P1 flow rate, mL/min	P1 direction	P2 flow rate, mL/min	P2 direction	Valve position	Description
1	50	15	Clockwise	2	Clockwise	Fill	Adding new sample into sample loop and flushing out the residual of last sample; filling the reagent loop, spectrophotometer setting zero (100%T)
2	30	9.0	Counter Clockwise	0	–	Inject	Sample and reagent mixing
3	150	0	–	0	–	Inject	Waiting for formation of azo dye, data recording

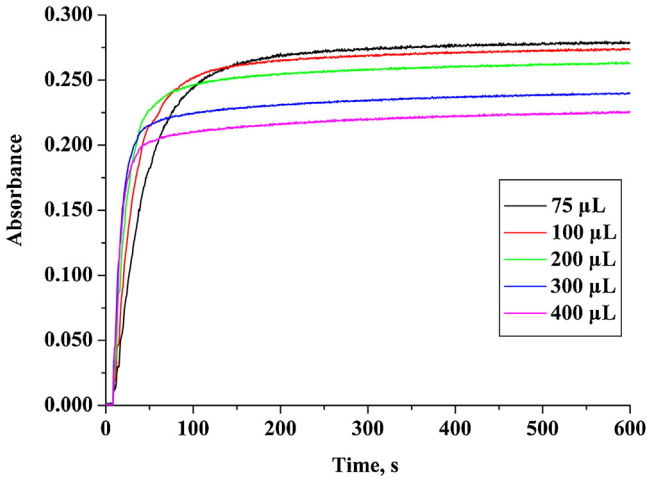

Fig. 2. Effect of reagent volume on signal with tested sample concentration of 10 μM.

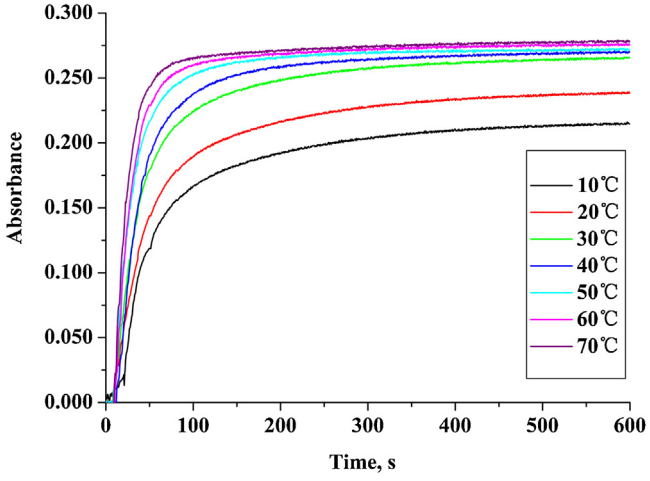

Fig. 3. Effect of sample temperature on signal with tested sample concentration of 10 μM.

Pure water (18.2 MΩ × cm) obtained from a Millipore Purification System (www.millipore.com) was used throughout.

2.2. Apparatus

The loop flow manifold in this study is similar to that previously described for phosphate [18] and total alkalinity [19] analysis, however this new analyzer is more integrated and user-friendly as a portable instrument. As shown in Fig. 1, the loop flow analyzer utilized one eight-port injection valve (C22-3188EH, www.vici.com), two low cost peristaltic pumps (BQ50-1J) equipped with 1 mm/2 mm i.d PVC tubing (www.longerpump.com) for delivering reagent/sample, and an integrated laboratory-made detection and control system. The detection system included a 5 mm green light-emitting diode (LED, λ_{max} = 532 nm, www.cree.com), a light to voltage converter (TSL 257, www.taosinc.com) and a customized cross-shaped flow cell (path length of 1.2 cm, volume of 1.5 mL). The emission spectra of LED and the absorption spectra of the formed Griess reaction product showed excellent agreement (Fig. S2). All the pump and valve switching processes and data acquisition were performed using a laboratory-programmed software written by LabVIEW (www.ni.com) and single chip microcomputer (STM32F103VBT6, www.st.com) based hardware. The analyzer size is 45 cm × 25 cm × 12 cm, and the power consumption is ~12 W. The detection system of LED-photodiode is very stable during the analysis procedure (absorbance variation of ~0.0002). The detailed circuits of main board, devices driving and controlling are shown in the Supplemental Materials (Fig. S3–S6). PTFE tubing of 0.8 mm i.d. (www.vici.com) was used as the fluid conduits throughout.

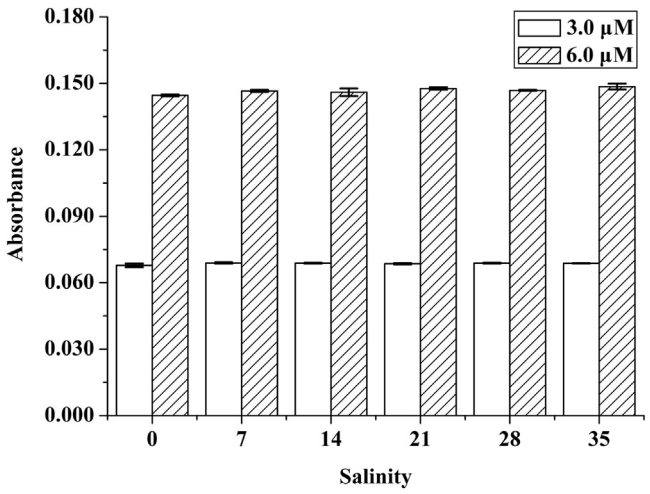

Fig. 4. Effect of sample salinity on the determination of samples.

2.3. Analytical procedure

The analytical procedure has three steps as shown in Table 1. During step 1, new sample and fresh reagent were introduced to the sample and reagent loops, respectively. When the cell was filled with fresh sample, the detector was set to zero (100% transmittance). In the next step, the valve was switched from "Fill" to "Inject" position, which resulted in these two loops forming a whole loop, where sample and reagent would be mixed for 30 s. Finally, in step 3, both pumps were stopped and the formed pink azo dye was monitored and the absorbance data were shown by the software. All the steps were repeated until the procedure was stopped manually by the operator. It should be noted that in the preliminary experiments, it was determined that 50 s and 30 s were sufficient for

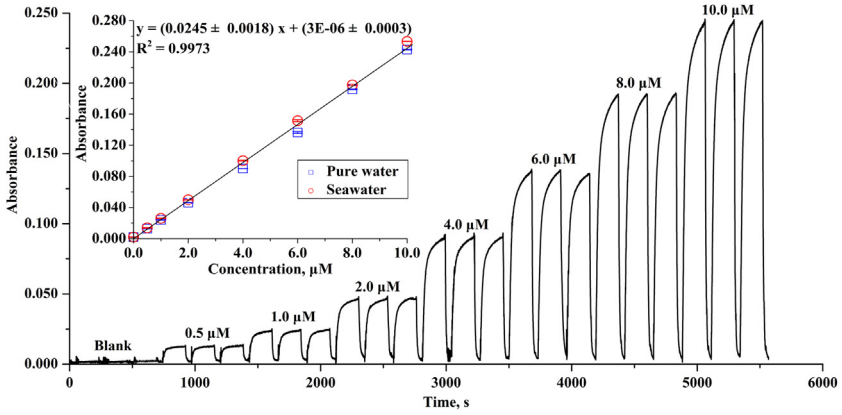

Fig. 5. Typical system signal output and calibration curves in pure water and seawater matrix (inset). Error bars are ± standard deviation for triplicate analysis.

flushing the sample/reagent loop and mixing of sample and reagent, respectively (Fig. S7).

2.4. Sampling

Low nutrient seawater was collected from the surface of the South China Sea and used as a matrix for salt-effect experiments. Lake water was collected from Furong Lake in the Xiang'an campus of Xiamen University. River water was collected from Jiulong River. Coastal water was collected from Wuyuan Bay, Xiamen. These samples were filtered through a 0.45 μm syringe type polyether sulfone filter immediately after collection. The filtered samples were kept at 4 °C and analyzed within 24 h.

3. Results and discussion

3.1. The effect of reagent loop

With a fixed reagent concentration, the reagent volume will determine the amount of reagent added to the sample. Therefore, the influence of reagent loop volume, from 50 to 400 μL, was studied to determine the optimum value. Several typical output signals are shown in Fig. 2. With less reagent, the reaction was slow and took longer time to complete. On the other hand, with more reagent, the kinetic rate increased but the final result was lower because of the dilution effect from the reagent loop. Therefore, 75 μL was chosen as the optimum volume of the reagent loop based on the balance of signal, reaction time and reagent consumption.

3.2. The effect of sample temperature

The potential application of this analyzer is for field analysis on shipboard laboratories, where the collected samples will have various temperatures at different areas (from polar region to tropical area), depths (from surface to the deep ocean) and seasons (from summer to winter). Therefore, it is necessary to evaluate the effect of sample temperature on the Griess reaction. For this purpose, the sample bottle was immersed in a water bath and the temperature was changed from 10 to 70 °C. As shown in Fig. 3, it is obvious that higher temperature accelerates the reaction and shortens the analysis time. However, there are some potential risks in using high temperature for the reaction, such as the decomposition of organic nitrogen compounds, sample evaporation, and other consequences that might change the sample composition. Therefore, it is recommended to place the sample bottle in a water bath maintained at 30 or 40 °C to keep the samples at constant temperature before analysis, especially for sample collected from cold areas (e.g. polar area).

3.3. The effect of sample salinity

In order to apply this analyzer to underway analysis in coastal and estuary areas, it is necessary to study the effect of sample salinity on the results. Samples containing 3 and 6 μM nitrite were prepared in water over a range of salinity levels (0–35) and analyzed as described in Section 2.3. As shown in Fig. 4, no significant salinity effect was found with this integrated LED-photodiode based optical detection system, which was in accordance with our previous study using commercial light source and detector [17]. The detector was set to zero (100% transmittance) when the cell was filled with a fresh sample at different salinity value, therefore the refractive index variance from the samples of different salinity values was corrected in the calculation.

3.4. Analytical figures of merit

Fig. 5 shows the typical detector output for sample concentrations between 0 and 10 μM. The sample throughput for the optimized conditions was 15 h^{-1}. Standard curves prepared in pure water and low nutrient seawater are also shown in the inset of Fig. 5. The identical curves from both samples indicate that there is

Table 2
Analytical results and recovery data of aqueous samples.

Sample type	Salinity	Concentration (n = 3, μM)		Calculated t-value	Critical t-value(P = 0.95)	Spiked (μM)	Recovery (%)
		Flow method	Standard method				
Coastal water	18	2.44 ± 0.03 (RSD = 1.2%)	2.39 ± 0.07 (RSD = 2.8%)	1.33	2.78	5	105
Lake water	0	0.81 ± 0.01 (RSD = 1.4%)	0.83 ± 0.04 (RSD = 3.5%)	0.87	2.78	2	97.3
River water	0	1.58 ± 0.01 (RSD = 0.3%)	1.62 ± 0.04 (RSD = 2.2%)	1.8	2.78	3	98.6

no significant ionic strength effect and no specific ion effect from the major components of seawater.

The detection limit of the method was 0.02 μM, which was calculated as three times the standard deviation for measurements of blank ($n = 15$) divided by the slope of the calibration curve. The relative standard deviations for triplicate determinations of samples were 0.13–2.99% (Fig. 5), showing good reproducibility of the results at different sample concentrations.

3.5. Application

To evaluate the precision and accuracy of the developed analyzer, different water samples including river water, lake water and coastal water were analyzed using both this analyzer and the standard benchtop Griess assay. The results are tabulated in Table 2, and it can be seen that there is no statistically significant difference between the proposed and reference methods with the paired Student's *t*-test at the 95% confidence level. The spike recoveries were 97.3–105.0%, indicating little matrix interference in the determination of nitrite in different samples.

4. Conclusions

Compared with other flow mode, the combination of loop flow and "cross-shaped" flow cell showed the novelty of insignificant interference from salinity, free of the bubble problem and easier operation [18]. Therefore, a compact automatic loop flow analyzer has been developed and applied for the determination of nitrite in aqueous samples. The limit of detection was found to be 0.02 μM with a short analysis time of ~4 min/sample. The precision and accuracy of the developed analyzer are more than satisfactory based on the highly reproducible recovery data and comparison results with the standard method. More importantly, the proposed analyzer can be easily modified for the determination of nutrients and other chemicals that are based on spectrophotometric detection. The more integrated design offers a simpler, lower cost and more user friendly method for nutrient analysis, and is highly suitable for field applications.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2016.07.002.

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