An automated flow-batch analyzer based on spectrophotometry for the determination of nitrite*

DONG Chen-yang (董辰杨), **LI Wen-zhan** (李文湛), **WANG Zhi-hong** (王志宏), **ZENG Jun-xiang** (曾俊翔), **and LIANG Jin-tao** (梁晋涛)**

School of Life and Environmental Sciences, Guilin University of Electronic Technology, Guilin 541004, China

(Received 15 January 2019; Revised 18 April 2019)

An automated flow-batch analyzer (FBA) based on spectrophotometry was developed for the determination of nitrite (NO_2^-) in environmental waters. The FBA consists of a homemade flow cell, light source, detector and a mini-syringe pump, which was controlled by self-designed software written in LabVIEW. Two LEDs and a light-to-voltage converter were used as the light source and detector respectively. The chemical detection method was based on spectrophotometry and modified Griess reaction. With the optimized conditions, the limit of detection (LOD) was 0.018 μmol·L^{-1} and the relative standard deviations (RSDs) at different concentrations (0—12 μmol·L^{-1}) were 0.54%—3.63% (n=3). Measurements of different aqueous samples (n=21) showed no significant difference between results obtained by developed FBA and a commercial spectrophotometer.

Document code: A **Article ID:** 1673-1905(2019)05-0339-4

DOI https://doi.org/10.1007/s11801-019-9007-3

Nitrite refers to the intermediate product during the process of nitrogenous organic compounds further oxidized to nitrate in water. The high concentration of nitrite indicates that the inorganic process of organic matter in water is very strong, indicating that the risk of pollution still exists[1-3]. In recent years, researchers have developed many methods for the determination of nitrite in seawater[4], such as spectrophotometry, chemiluminescence, electrochemical methods, spectrofluorometry, etc. Spectrophotometry, which is low cost, short-time consuming and cause no damage to the environment[5,6], is a recommended method. With the continuous improvement of water environmental monitoring technology, the flow analysis technology plays a vital role. In flow-batch analyzer (FBA) systems, sampling steps and transport of solution are in the same way as flow analyzers, while mixing and reacting are done in a mixing chamber, as in a batch system[7]. Therefore, FBA provides a good precision and accuracy, as well as high sample throughput and low consumption of reagents and samples[8].

At present, researchers at some environmental monitoring stations have used commercial continuous flow analyzer products for monitoring in estuaries and offshore waters, such as QuAAtro (SEAL Analytical, Germany) and San++ (Skalar, Netherlands). These instruments are mainly based on the segmented continuous flow analysis (SCFA) technology, and therefore have a higher degree of automation and faster analysis speed. However, high prices of these instruments are likely to increase the cost of the experiment, and these instruments are vulnerable to environmental factors in the actual operation process, so they are not suitable for on-site testing. Recently, some researchers have developed integrated analyzers based on spectrophotometry and flow analysis to determine different chemicals in environmental water[9-11]. These analyzers used commercial spectrophotometers as the detector, and data processing relied on commercial software provided by the company. Therefore, the cost of experiment was higher, and the systems were not smart enough. In this study, the data processing system including data acquisition (DAQ) board USB-6343 and software written in LabVIEW realized a high precision and a good controllability. The real-time absorbance values of two LEDs and other experimental data could be displayed on the graphical user interface (GUI). Programmable bidirectional syringe pump and a homemade "Z" shape flow cell as the experimental part made the developed FBA easy to construct and have a good stability. At the same time, the setting of two LEDs lighting alternately also reduced the influence of dark current.

The schematic diagram of developed FBA is presented in Fig.1. The core hardware of system was a programmable bidirectional syringe pump (Tecan, USA), which incorporated a gastight 2.5 mL syringe and a 6-port selection valve. According to the commands received from the software written in LabVIEW through RS-485 interface, the syringe pump switched the valve ports in turn

* This work has been supported by the Guangxi Natural Science Foundation (No.2018GXNSFAA281231), the MEL Visiting Fellowship Program (No.MELRS1715), and the Innovation Project of GUET Graduate Education (No.2018YJCX69).

** E-mail: dxljt@163.com

and delivered the pure water, reagents and samples. The mixed solution in the syringe was discharged from the syringe into the flow cell for color reaction. A DAQ board USB-6343 (National Instruments, USA) was used to light two LEDs alternately, acquire data from TSL-251 (TAOS, USA) and transmit data to software through data cable. The polytetrafluoroethylene (PTFE) tubes (Valco Instruments, USA) of 0.8 mm and standard 1/4−28 flangeless PEEK fittings were used in the fluidic manifold to deliver variable amounts of samples and reagents.

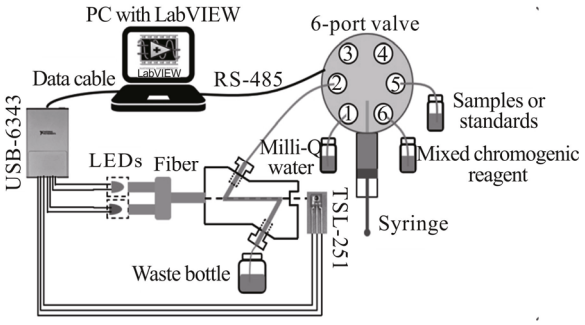

Fig.1 Diagram of automated FBA

Fig.2(a) shows the homemade "Z" shape flow cell, which has a 15-mm-long optical path length and an inner diameter of 1 mm, is connected to a "Y" shape fiber with two LEDs and sensor TSL-251. The experimental process was controlled by modifying the parameter values (e.g. the time of calculating absorbance, total number of measurements, valve switch and speed setting for syringe pump, etc) in the panel. As shown in Fig.2(b), the intensity data of two LEDs received by TSL-251 was plotted as a curve, and dual-wavelength blank signal, current signal, absorbance displayed in the panel.

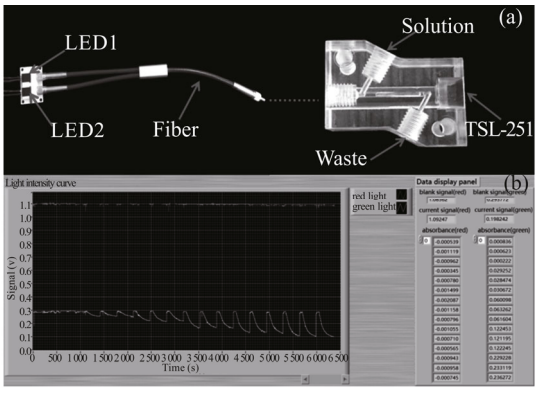

Fig.2 (a) Design of flow cell; (b) Light intensity curve and experimental data shown in LabVIEW

Without specification, all chemicals used within this work were of analytical grade purity and purchased from Sinopharm Chemical Reagent (Shanghai, CN). The experimental water was ultrapure water made by Milli-Q Integral Water Purification System (MilliporeSigma, USA). Mixed chromogenic reagent was prepared by 2 g sulfonamide (SAM) and 0.2 g N-(1-Naphthyl) ethylenediamine dihydrochloride (NED) dissolving in 450 mL 5% (V/V) HCl (guaranteed reagent), and the volume was made up to 500 mL with 5% (V/V) HCl. 50 μmol·L^{-1} nitrite standard solution was used as the stock solution, from which working standard solutions ranging from 0.5 μmol·L^{-1} to 12 μmol·L^{-1} of nitrite were prepared via serial dilutions. The nitrite solutions have a diazotization-coupling reaction with mixed chromogenic reagent and the product is a red azo dye. Fig.3 shows the UV-Vis absorption spectra of azo dye produced by 8 μmol·L^{-1} nitrite and emission spectrum of two LEDs used. The absorbance of azo dye produced by 8 μmol·L^{-1} nitrite is max at 540 nm and nearly 0 at 730 nm. Therefore, the absorbance at 730 nm is used for baseline correction and the final absorbance is a difference value according

$$A=A_{530\ \mathrm{nm}}-A_{730\ \mathrm{nm}}. \quad (1)$$

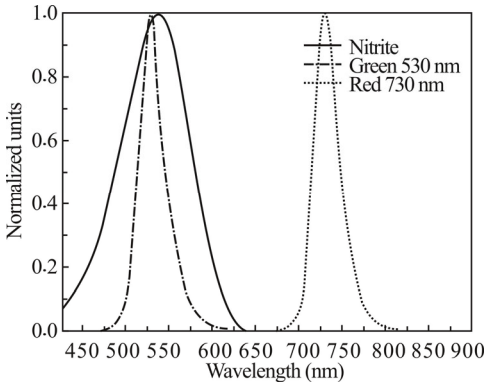

Fig.3 UV-Vis absorption spectrum of azo dye produced by 8 μmol·L^{-1} nitrite and emission spectra of two LEDs

The effects of reagent addition, reaction kinetics and aspiration rate were evaluated, and the detailed description are shown below. The optimized experiments used 2 mL nitrite standard solution of 8 μmol·L^{-1} as the test sample, and all samples were measured at least 3 times. Effect of mixed chromogenic reagent addition was studied over the range of 0.05—0.25 mL. As seen in Fig.4(a), it is obvious that absorbance was higher with higher addition, and the curve was almost identical when the addition was higher than 0.2 mL. Therefore, the optimal addition of reagent was chosen as 0.20 mL. To measure nitrite in fresh water, the time required for complete chemical reaction is at least 20 min at room temperature. As demonstrated in Fig.4(b), the absorbance increased sharply when the time was between 0—600 s, and the curve was gradually identical when the time was higher than 600 s. Although waiting for complete reaction would yield higher sensitivity, sufficient sensitivity was obtained in the application at a reaction time of 600 s. In this study, the flow rate of aspiration influenced the degree of mixing and dispersion. Fig.4(c) shows the effect of different aspiration rate. The absorbance was lower than others at the aspiration rate of 5 mL/min, and the curve was identical when the aspiration rate was between 10 mL/min and 60 mL/min.

Under optimized experimental conditions, the descriptions of analytical procedure for the determination of nitrite is shown in Tab.1. Steps 1—4 were cleaning procedures, in which steps 1—2 were cleaning tubes and flow cell with ultra-pure water, and repeated four times to remove the residual solution in the last experiment. Steps 3—4 were to clean tubes and flow cell with the sample and repeat three times. Steps 5—8 were to aspirate the sample and mixed chromogenic reagent, and then dispensed the mixed solution into the flow cell for color reaction. The absorbance of the mixed solution was calculated and displayed on the GUI when the reaction time reached 600 s.

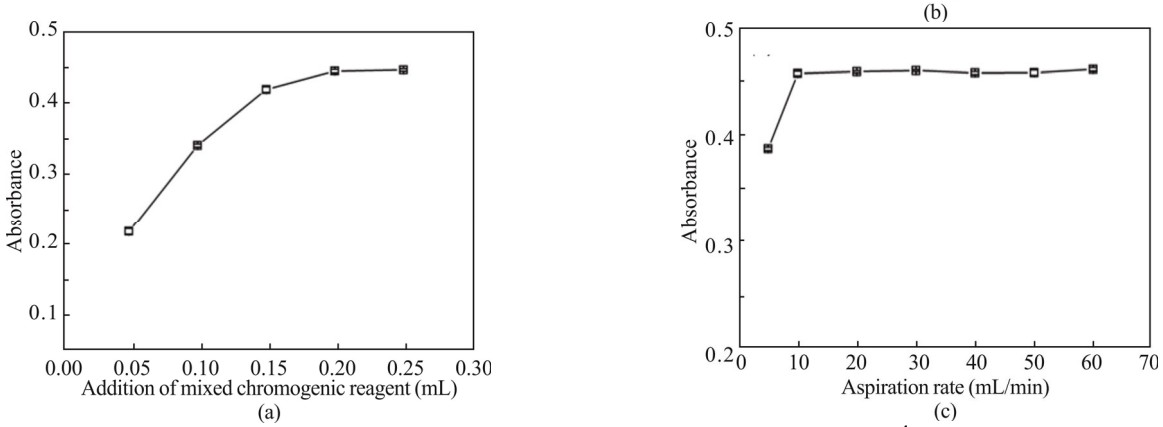

Fig.4 (a) Effect of mixed chromogenic reagent addition; (b) Kinetic study for 8 μmol·L^{-1} NO_2^- standard solution; (c) Effect of different aspiration rate

Tab.1 The descriptions of analytical procedure for the determination of nitrite

Step	Syringe pump	Valve position	Flow rate (mL·min^{-1})	Operation	Description
1	In	1	30	Aspirate 1 mL water	Washing the syringe and flow cell with fresh water, repeat 4 times[a]
2	Out	6	60	Dispense 1 mL water	
3	In	2	30	Aspirate 0.5 mL sample	Washing the syringe and flow cell with fresh sample, repeat 3 times[b]
4	Out	6	30	Dispense 0.5 mL sample	
5	In	2	10	Aspirate 0.2 mL sample	
6	In	3	10	Aspirate 0.2 mL mixed chromogenic reagent	Aspiration of the sample and reagents, mixing in the syringe[c]
7	In	2	30	Aspirate 1.8 mL sample	
8	Out	6	30	Dispense 2.2 mL mixture	Deliver the sample and reagents mixture to the flow cell, waiting for chemical reaction in the cell.

a: step 1, 2; b: step 3, 4; c: step 5—7

As shown in Fig.5, high similarities in the method is demonstrated and three calibration curves have an excellent linear (R^2>0.999 4) in a concentration range of 0—12 μmol·L^{-1}. The RSDs (n=3) of automated FBA were 0.54%—3.63% on three days in the range of 0.5—12 μmol·L^{-1}. The LOD was calculated as three times the standard deviations for measurements of the blank (n=11) divided by the slope of the calibration curve, and the values of the LOD using automated FBA was 0.018 μmol·L^{-1}. Therefore, it can be concluded that developed method has excellent linear fit, high precision, low detection limit and good repeatability.

The samples used in application study were collected from Jiulong River estuary and Xiamen coastal area, and were processed through 0.45 μm filters at collection sites and preserved in 250 mL polyethylene bottles at 4°C after collection. All samples were determined by FBA and monitored by a commercial spectrophotometer V-1100D (Mapada, CN) at 540 nm. As shown in Fig.6, the bars around sampling stations in two colors represent the concentrations of nitrite determined by two methods. In terms of the specific repeatability of the study, the RSDs (n=3) for the determination of samples using automated FBA was 0.26%— 1.1%, indicating a good repeatability of the method. After the data using FBA do the paired-samples t test with the data using V-1100D, there are no statistically significant differences at the 95% confidence level. The results indicates that the developed method can replace the UV-Vis V-1100D in the determination of nitrite. Compared with the chemiluminescence, spectrofluorometry and

electrochemical method previously reported for nitrite detection, the results are shown in Tab.2.

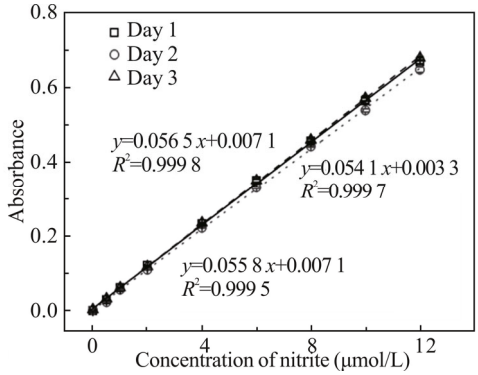

Fig.5 Three calibration curves for a series of standard nitrite solutions on three days using FBA

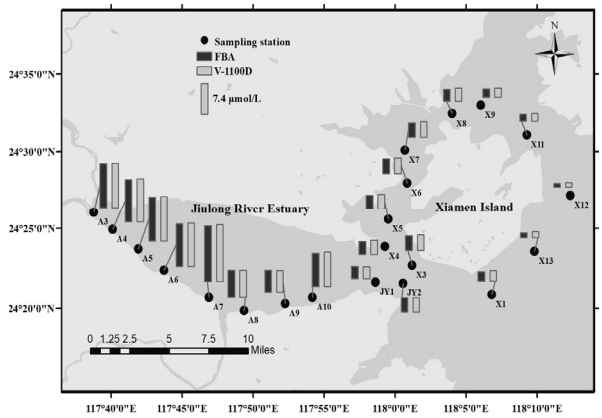

Fig.6 Distributions of nitrites in the Jiulong River estuary and Xiamen coastal areas

Tab.2 Comparison of parameters used in different methods for nitrite detection

Methods	Sample	Detection limit ($\mu mol \cdot L^{-1}$)	Detection range ($\mu mol \cdot L^{-1}$)	Reference
Chemiluminescence	Water	0.036	0.1—100	[12]
Spectrofluorometry	Water	0.02	0—8.7	[13]
Electrochemical	Water	2.4	10—5 000	[14]
Spectrophotometry	Water	0.018	0—12	This work

In conclusion, an automated FBA based on LED for the determination of nitrite has been successfully developed. The developed FBA has a simple structure, a good stability and a high degree of automation by means of self-designed software written in LabVIEW, homemade flow cell and a programmable bidirectional syringe pump. Therefore, the developed method offers an effective alternative for the applications such as laboratory analysis and on-site testing.

References

[1] White S A, Santos I R and Hessey S, Environmental Pollution **243**, 1036 (2018).

[2] Yin Guo-yu, Hou Li-jun, Liu Min, Zheng Yan-ling, Li Xiao-fei, Lin Xian-biao, Gao Juan and Jiang Xiao-fen, Environmental Pollution **214**, 265 (2016).

[3] Poulsen R, Cedergreen N, Hayes T and Hansen M, Environmental Science & Technology **52**, 3869 (2018).

[4] Wang Qiu-hua, Yu Li-ju, Liu Yang, Lin Lan, Lu Ri-gang, Zhu Jian-ping, He Lan and Lu Zhong-lin, Talanta **165**, 709 (2017).

[5] Sreekumar N V, Narayana B, Hegde P, Manjunatha B R and Sarojini B K, Microchemical Journal **74**, 27 (2003).

[6] Al-Okab R A and Syed A A, Talanta **72**, 1239 (2007).

[7] Monte-Filho S S, Lima M B, Andrade S I, Harding D P, Fagundes Y N, Santos S R, Lemos S G and Araujo M C, Talanta **86**, 208 (2011).

[8] Nascimento E C L, Araujo M C U and Galvao R K H, Journal of the Brazilian Chemical Society **22**, 1061 (2011).

[9] Ma Jian, Li Pei-cong, Chen Zhao-ying, Lin Kun-ning, Chen Neng-wang, Jiang Yi-yong, Chen Ji-xin, Huang Bang-qin and Yuan Dong-xing, Analytical Chemistry **90**, 6431 (2018).

[10] Lin Kun-ning, Ma Jian, Yuan Dong-xing, Feng Si-chao, Su Hai-tao, Huang Yong-ming and Shangguan Qi-pei, Talanta **167**, 166 (2017).

[11] Zhu Yong, Yuan Dong-xing, Huang Yong-ming, Ma Jian, Feng Si-chao and Lin Kun-ning, Marine Chemistry **162**, 114 (2014).

[12] Li Jin-ge, Li Qian-qian, Lu Chao and Zhao Li-xia, Analyst **136**, 2379 (2011).

[13] Fernández M A T, Cañabate B, Costa-Fernández J M, Pereiro R and Sanz A, Talanta **62,** 991 (2004).

[14] Cui Yan-ping, Yang Chang-zhu, Zeng Wei, Oyama M, Pu Wen-hong and Zhang Jing-dong, Analytical Sciences **23**, 1421 (2007).