analytical chemistry

Technical Note

Cite This: *Anal. Chem.* 2018, 90, 6431−6435

pubs.acs.org/ac

Development of an Integrated Syringe-Pump-Based Environmental-Water Analyzer (*i*SEA) and Application of It for Fully Automated Real-Time Determination of Ammonium in Fresh Water

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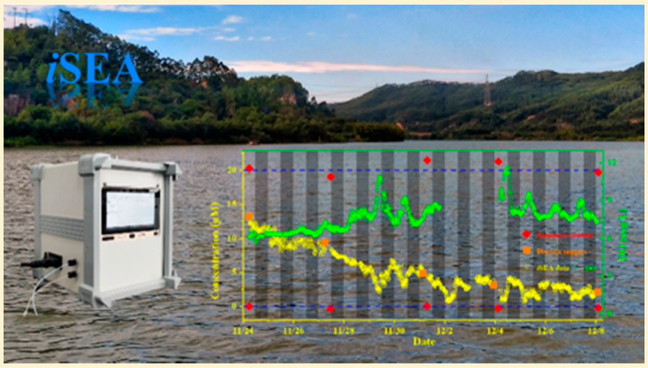
Supporting Information

ABSTRACT: The development of a multipurpose integrated syringe-pump-based environmental-water analyzer (*i*SEA) and its application for spectrophotometric determination of ammonium is presented. The *i*SEA consists of a mini-syringe pump equipped with a selection valve and laboratory-programmed software written by LabVIEW. The chemistry is based on a modified indophenol method using *o*-phenylphenol. The effect of reagent concentrations and sample temperatures was evaluated. This fully automated analyzer had a detection limit of 0.12 μM with sample throughput of 12 h^{-1}. Relative standard deviations at different concentrations (0−20 μM) were 0.23−3.36% ($n = 3−11$) and 1.0% ($n = 144$, in 24 h of continuous measurement, ∼5 μM). Calibration curves were linear ($R^2 = 0.9998$) over the range of 0−20 and 0−70 μM for the detection at 700 and 600 nm, respectively. The *i*SEA was applied in continuous real-time monitoring of ammonium variations in a river for 24 h and 14 days. A total of 1802 samples were measured, and only 0.4% was outlier data (≥3 sigma residuals). Measurements of reference materials and different aqueous samples ($n = 26$) showed no significant difference between results obtained by reference and present methods. The system is compact (18 cm × 22 cm × 24 cm), portable (4.8 kg), and robust (high-resolution real-time monitoring in harsh environments) and consumes a small amount of chemicals (20−30 μL/run) and sample/standards (2.9 mL/run).



Because of the characteristics of flow analysis (e.g., precision, robustness, simplicity, low risk of contamination, and ease of automation), analytical flow techniques present an elegant way to apply wet-chemistry procedures in environmental analysis.[1−3] Various types of flow analysis techniques have been invented and developed over the past decades. The historical perspective on developing and comparing different flow analysis techniques is provided in the literature.[1,2,4,5] A summary of 240 typical review papers about flow analysis is also tabulated in the Supporting Information (SI).

Among the unsegmented flow techniques, flow injection analysis (FIA) and sequential injection analysis (SIA) might be the most widely used methods for environmental analysis. Conventional FIA using peristaltic pumps for continuous liquid delivery is the first-generation method of flow injection analysis.[6] FIA is convenient and low cost, but its shortcomings include high reagent consumption and laborious optimization via manual manipulation of the manifold and flow rate drift.[7,8] SIA uses a bidirectional syringe pump to process samples and reagents, and the advantages are its compact instrument, robustness, simplicity, ease of operation, and low reagent and sample consumption.[9,10] Both FIA and SIA are based on flow-through detection under nonequilibrium conditions; this can increase sample throughput but can also result in decreased sensitivity compared with similar manual methods.[11]

Flow batch analysis (FBA) combines the characteristics of both flow (continuous) and batch (discrete) systems through the use of programmed multicommutation.[12] In an FBA system, the sampling step and solution transportation are done the same way as in a flow analyzer, whereas mixing and reaction are performed inside a mixing chamber, as done in a batch system.[13] Therefore, FBA has the favorable advantages of both flow analysis (e.g., low sample and reagent consumption and high sampling rate) and batch analysis (e.g., high sensitivity and wide application range), which is suitable for field analysis.[14] For instance, Amornthammarong et al.[15] developed a simple mixing chamber made of a pipet to mix the sample and reagents and used it in an autonomous batch analyzer for ammonium

Received: April 3, 2018
Accepted: May 6, 2018
Published: May 6, 2018

DOI: 10.1021/acs.analchem.8b01490
Anal. Chem. 2018, 90, 6431−6435

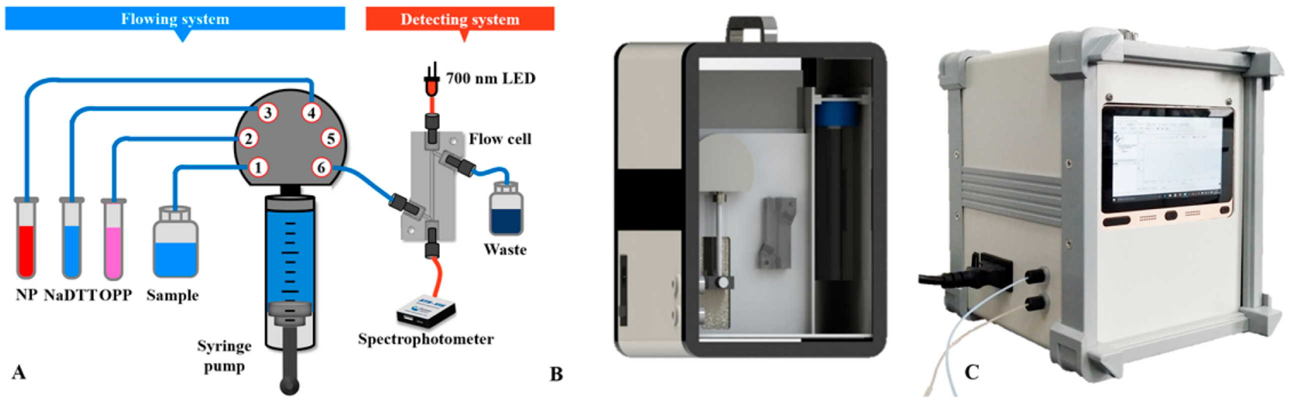


Figure 1. (A) Schematic diagram (OPP, *o*-phenylphenol; NaDTT, sodium dichloroisocyanurate; NP, sodium nitroprusside), (B) 3D design, and (C) photo of the *i*SEA.

measurements in coastal areas.[16,17] Similar FBA manifold has been modified and commercialized for near-site/at-site analysis of nutrients in natural water (e.g., serious products from Green Eyes Environmental Observing Systems, http://gescience.com/). The application and performance of these analyzers during short- and long-term deployments have been demonstrated (e.g. ref 18).

Nutrients are essential for organisms; however, excess concentrations of nutrients in aquatic systems can lead to eutrophication, followed by algal growth, depletion of dissolved oxygen, and degradation of aquatic ecosystems.[19,20] One of these nutrients is ammonium, which is an important water-quality indicator because it is the most reduced form of inorganic nitrogen.[21] Accurate, sensitive, and robust determination of ammonium has received ongoing attention from the scientific community,[22] and several comprehensive reviews have been published regarding ammonium determination.[23−25] Very recently (publications since 2017), many methods have been reported about measuring ammonium in different matrices (detailed information shown in the SI). Various advanced methods have been reported, and the most popular technique for routine analysis of ammonium is based on indophenol blue (IPB) (i.e., Berthelot's reaction).[26,27] Compared with other techniques, this spectrophotometric method is very selective, less expensive, and easily coupled to flow techniques to quantify ammonium in a variety of real samples.[23]

Herein, we describe a new automated system based on a flow-bath, and our system is termed *i*SEA (integrated syringe-pump-based environmental-water analyzer). This analyzer takes advantage of using a syringe pump to overcome drawbacks of current batch and continuous flow analyzers. The chemistry is based on a modified IPB method using *o*-phenylphenol (OPP) instead of toxic and odorous phenol, which is also more suitable for saline sample analysis compared with salicylate color reagent assays.[28−30] The novelty and applicability of *i*SEA was demonstrated in the application of fully automated real-time determination of ammonium in fresh water for 14 days. These high-resolution field measurements can potentially lead to significant advances in understanding nutrient biogeochemical cycles and complex dynamics of nitrification.

■ EXPERIMENTAL SECTION

Reagents. The preparation of the chemical solutions of OPP, sodium dichloroisocyanurate (NaDTT), and sodium nitroprusside (NP) is described in the SI and our previous work.[27] Certified materials (GSBZ50005-88, batch no. 200577) were purchased from the Institute for Environmental Reference Materials, Ministry of Environmental Protection, China.

Description of the Apparatus. The manifold, 3D design, and a photo of the *i*SEA are shown in Figure 1. The core hardware was a programmable bidirectional syringe pump (XCalibur, Tecan, USA) that was used to deliver variable amounts of sample and reagents to various components of the system. The syringe pump incorporated a gastight 1.0 mL syringe (Hamilton, USA) and a precise stepper motor with volumetric precision better than ±0.05%. The pump was connected to a six-port distribution valve, which was connected to samples, different reagents, the flow cell, and waste. Spectrophotometric detection included laboratory-made fiber optics and a Z-flow cell (3 cm path length), an STS mini-CCD spectrophotometer (OceanOptics, USA) was used as the detector, and a 700 nm LED driven at 20 mA was used as the light source. All of the pump and valve switching processes were controlled using laboratory-programmed software written by LabVIEW (NI, USA). The fluidic manifold was constructed using PTFE tubing (0.8 mm i.d.) and standard 1/4−28 flangeless PEEK fittings (IDEX Health & Science LLC, USA). All components of the instrument were housed in a plastic case (18 cm × 22 cm × 24 cm), as shown in Figure 1, and the total weight was 4.8 kg.

Analytical Procedure. Generally speaking, the syringe pump is used to aspirate sample and reagents sequentially through the selection valve, and mixing and reaction occur inside of the syringe. The mixture is then delivered to the flow cell for spectrophotometric detection. First, the valve was positioned in Port 1, and a new sample was aspirated into the syringe and injected into the flow cell through Port 6. Cleaning steps reduce contamination from previous samples and minimize carryover effects. After cleaning four times, the cell was filled with fresh sample, and the light intensity of the detector was set as 100% transmittance. In Steps 3−7, 50 μL of sample (Port 1), 20 μL of OPP (Port 2), 20 μL of NaDTT (Port 3), 30 μL of NP (Port 4), and 450 μL of sample (Port 1) were sequentially aspirated into the syringe for mixing. Mixed solution was then dispensed into the flow cell (step 8) and held in the flow cell for 290 s, during which a spectrum of the sample/reagents mixture was recorded. All of the steps were repeated until the operator manually stopped the procedure. The detailed procedures of analysis (Table S1), sampling, and method comparison are shown in the SI.

Experimental Safety. Although the OPP used here is less toxic and odorous than phenol, it is recommended to be careful

DOI: 10.1021/acs.analchem.8b01490
Anal. Chem. 2018, 90, 6431−6435

with the used chemicals in this experiment. Avoid direct contact with eyes, skin, and respiratory system during experiments. The waste generated during field application should be brought back to the laboratory for discharge.

RESULTS AND DISCUSSION

General Considerations of the *i*SEA Design. The system described here is designed for automated measurement of discrete samples in the laboratory and real-time field monitoring in a river or for shipboard underway analysis. Therefore, the criteria for the design are reliability, robustness, reasonably fast sample throughput, sensitivity, and accuracy. Reagent consumption and instrument size and weight are also concerns for long-term deployment of a field portable instrument.

For continuous flow (e.g., FIA), the manifold relies on mixing of reagents and samples under laminar flow conditions, and different integrated mixing coils were designed to enhance radial mixing (secondary flow).[31] In contrast, the *i*SEA was designed as a stopped-flow manifold. As described in the Analytical Procedure section and Table S1, samples and reagents were sequentially aspirated into the syringe, where mixing and chemical reaction occurred. Therefore, mixing efficiency relied mainly on diffusive processes, and laminar flow conditions were dominant. In previous studies that used a similar flow-batch mode, a secondary mixing device (e.g., pipet or mixing coil) was needed.[15−17] In this study, the flow rate of aspiration influenced the degree of dispersion and mixing. A relatively thorough mixing was obtained with a suitable flow rate. Visual mixing conditions at different aspiration rates are shown in Figure S1 in the SI.

Optimizing the Chemistry. The mechanism of the Berthelot reaction is very complicated, and thus reagent compositions and aspiration order of the reagents and sample into the syringe are key factors for the formation of the chemical product.[32] Using our previous comprehensive study on indophenol chemistry,[28,33] the effects of different reagents on reaction kinetics were evaluated, and the detailed description and results are shown in the SI. The optimized values are tabulated in Table 1.

Table 1. Recommended Analytical Parameters

parameters	range of tested values	selected value
OPP concentration (g/L)	0.36−1.79	1.43
NaDTT concentration (g/L)	0.09−0.71	0.36
NaOH concentration (g/L)	0.36−1.79	1.79
NP concentration (g/L)	0.09−0.42	0.26
temperature (°C)	4−70	20−30

Effects of Sample Temperature. Field analysis is a potential application of this analyzer, where the analyzer would be operated at room temperature. However, collected samples may have various temperatures because of different sampling locations or seasons. Therefore, the effects of sample temperature on the reaction were evaluated using a sample bottle immersed in a water bath with various temperatures ranging from 4 to 70 °C. As seen in Figure S3, higher temperature can accelerate the rate of reaction and shorten the analysis time, which is in accordance of our previous study.[28] Temperature effects vary for different reaction times (inset of Figure S3). At the current analytical frequency (5 min/sample), temperature effects were not significant when sample temperature was >20 °C. Therefore, room temperature was selected to simplify the system and to avoid potential risks in using high temperature for reaction. However, it is recommended that cold samples (e.g., refrigerated stored samples) be warmed to room temperature before analysis or that reaction time be increased when needed.

Analytical Figures of Merit. Using the optimized conditions (Table 1), a series of ammonium standard solutions were prepared and analyzed with *i*SEA. Calibration curves at two wavelengths are $A = (0.0493 \pm 0.0003)*C$ (μM) + (0.076 ± 0.003) ($R^2 = 0.9998$, $n = 7$, range of 0−20 μM, wavelength of 700 nm for sensitive detection) and $A = (0.0147 \pm 0.0001)*C$ (μM) + (0.063 ± 0.003) ($R^2 = 0.9998$, $n = 7$, range of 0−70 μM, wavelength of 600 nm for wide range). Sample throughput with the optimized conditions was 12 h^{-1}. A higher frequency could be obtained if needed. Figure S4 shows the typical detector output for sample concentrations between 0 and 20 μM at a wavelength of 700 nm.

The detection limit was calculated as three times the standard deviation for measurements of the blank ($n = 11$) divided by the slope of the calibration curve, and the value for the detection limit was 0.12 μM. Relative standard deviations (RSDs) for triplicate determinations of samples for the calibration curves were in the range of 0.24−3.36% (Figure S4). In terms of the specific repeatability of the study, the RSD values were 0.52% ($n = 11$, 2.5 μM), 0.27% ($n = 11$, 7.5 μM), 0.23% ($n = 11$, 15 μM), and 1.0% ($n = 144$ in 24 h continuous measurement, ∼5 μM, inset of Figure S4), indicating the good repeatability of the results at different sample concentrations for both short and long time periods. The excellent RSD data also prove the efficiency of mixing in the *i*SEA. Reagent consumption is in the range of 20−30 μL/run, and this is much less than that for conventional methods.

Carryover Effect. The carryover effect describes how analyte in a sample is "carried" by an analytical system "over" to the next sample, and this is dependent on the number and volume of flushing solutions.[34] To minimize carryover between samples, the required number of flushing steps was determined, and it was found that flushing four times was sufficient. Using the procedure specified in Table S1, the carryover effect was quantified according to the method proposed by Zhang.[34] The carryover coefficients (k_{CO}) for ammonium measurements were 0.001, and these were negligible for the samples from high concentration (10 μM) to low concentration (2.5 μM). The detailed carryover effect data is shown in Figure S5 in the SI.

Validation of the Methods. Three methods were used to evaluate the precision and accuracy of the *i*SEA, and these methods were: (1) measurement of certified materials, (2) comparison with reference methods, and (3) spiked recovery testing. Diluted certified materials (GSBZ50005-88) and 19 fresh water samples were analyzed using *i*SEA. The measured concentrations of ammonium were compared with the certified value and with results obtained using standard benchtop methods. As shown in Figure S6, the measured concentration using *i*SEA was equal to $(0.9688 \pm 0.0070) \times$ certified/measured concentration using other methods + (0.05 ± 0.09) μM, and for this relationship, $R^2 = 0.9988$ and $n = 26$, over the range of 0−50 μM. Therefore, the analytical results using *i*SEA showed no statistically significant difference compared with the label value and compared with results obtained using reference methods at the 95% confidence level.

Recovery percentages were performed for five samples to further assess accuracy. These samples were spiked at two

DOI: 10.1021/acs.analchem.8b01490
Anal. Chem. 2018, 90, 6431−6435

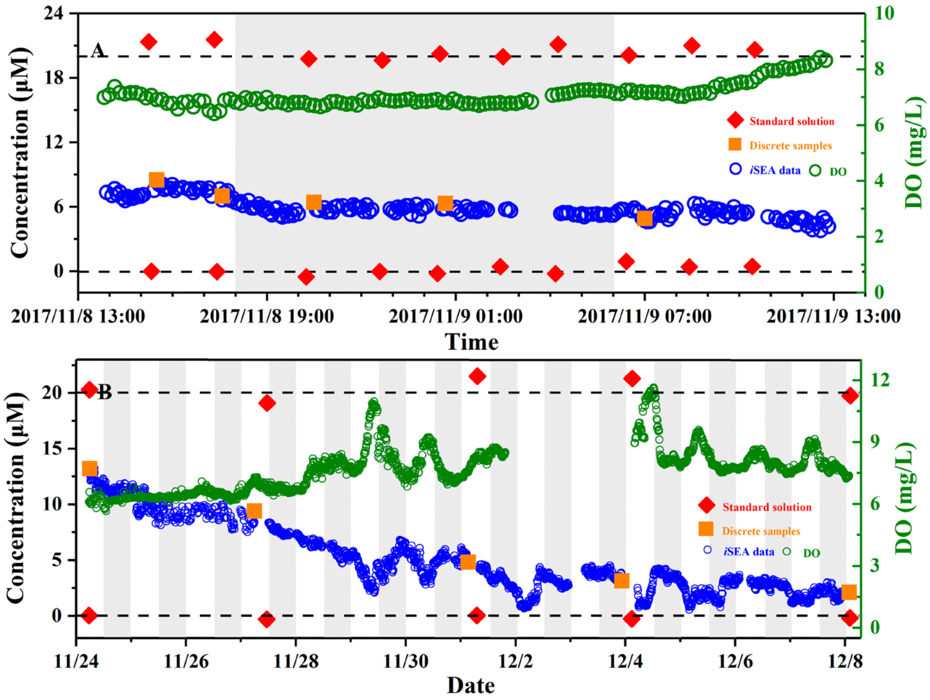


Figure 2. Continuous readings of ammonium concentrations using the *i*SEA compared with analytical results obtained using a reference method and quality-control data. (A) Test one for 24 h with dissolved oxygen (DO) data. (B) Test two for 14 days with DO data. Time-series data for ammonium using the *i*SEA (blue circles) and manually collected samples analyzed using reference methods (orange diamonds).

different levels, such as 5−20 μM (low level) and 10−40 μM (high level). The spike recoveries are presented in Table S2, and the values are in the range of 95.9−105%, indicating that there was no significant matrix interference.

Field Application. To evaluate the reliability and robustness of the developed portable analyzer, the *i*SEA was deployed twice (November 8 to 9 and November 24 to December 8, 2017) for continuous real-time monitoring of ammonium variations at a drinking water reservoir in the lower Jiulong River, Southeast China (location shown in Figure S7). The data shown in Figure 2 are real-time sample measurements and standard solutions (blank and 20 μM) plotted versus time. The measured standards are consistent with the prepared concentrations used in both tests (0.10 ± 0.42 μM and 20.53 ± 0.68 μM during test 1, $n = 10$; −0.15 ± 0.16 μM and 20.38 ± 1.02 μM during test 2, $n = 5$). Field precision was 3.33% ($n = 15$) for measurements of 20 μM ammonium standard solutions under harsh conditions for more than 2 weeks. Discrete samples were also collected and analyzed to compare the online method with a standard laboratory AutoAnalyzer method.[35] The results are shown in Figure 2, and there is excellent agreement between these two data sets ($R^2 = 0.99$, slope of 0.97 ± 0.04; intercept 0.04 ± 0.26 μM; $n = 10$). Therefore, the performance of the *i*SEA is confirmed as stable for both reagents and hardware over the whole deployment period, which was up to 2 weeks. Moreover, there were reasonable observed trends of ammonium with large temporal variation. Ammonium concentration has been shown to decline when dissolved oxygen (DO) peaked, likely due to uptake by phytoplankton during photosynthesis.[36] A detailed description of the data can be found in the SI. Because the focus of this paper is not the nitrogen cycle, further explanations of the data set will be discussed in future publications.

■ CONCLUSIONS

A robust and reliable flow analyzer system (*i*SEA) for field spectrophotometric analysis is presented. Compared with other flow analyzers, the *i*SEA has the advantages of simplicity (a single manifold composed of one pump and valve), low reagent consumption (20−30 μL/sample), high precision (RSD of 1.0% without any stoppage during repeated analysis for 24 h), portability (18 cm × 22 cm × 24 cm, 4.8 kg), ease of optimizing chemical parameters (same as routine batch mode), and wide analytical range using different detection wavelengths. Successful application of *i*SEA for real-time ammonium measurement with high-quality field data for 2 weeks proved that the analyzer is robust and reliable. The proposed analyzer is flexible and can be easily modified for other spectrophotometric detection after changing the chemicals and parameters of the control software.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.8b01490.

> 240 review papers about flow analysis (1978−2018), recent publications about ammonium measurements (since 2017), reagents, sampling, analytical procedures, method comparison, optimization of chemistry, effect of temperature, typical signal output, carryover effect data, location of monitoring station, and recovery data. (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was financially supported by the National Key Research and Development Program of China (2016YFC0502901), National Natural Science Foundation of China (41576097), Xiamen Southern Oceanographic Center (14GST68NF32), and the Program for New Century Excellent Talents in Fujian Province University (2016). Miss Junou Du and Xinjuan Gao are acknowledged for performing the method comparison. The critical comments of three reviewers greatly improved the manuscript.

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