

# Micro sequential injection: environmental monitoring of nitrogen and phosphate in water using a “Lab-on-Valve” system furnished with a microcolumn

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A “Lab-on-Valve” manifold operated in the micro sequential injection ( $\mu$ SI) mode was adopted to accommodate EPA-approved methods for spectrophotometric determinations of nitrate, nitrite and orthophosphate in the ppb (N or P) concentration range. A computer programmable  $\mu$ SI protocol, utilizing stopped-flow within a copperized Cd-foil filled microcolumn was developed for nitrate reduction to nitrite with subsequent colorimetric measurement, yielding concentration ranges for nitrate of 100.0–4000.0 ppb (N) and for nitrite of 30.0–4000.0 ppb (N) and linear calibration responses of  $r^2 = 0.9999$  for nitrate and 0.9995 for nitrite. Using a stopped-flow reaction rate measurement, phosphate was determined in the range 1.0–30.0 ppb (P) with a calibration response of  $r^2 = 0.9997$ . The technical improvement of this methodology, apart from micro miniaturization, is the use of the stopped-flow technique, that resulted in improved detection limits and allowed reagent consumption to be reduced 1500-fold compared with conventional procedure while the amount of metallic cadmium was reduced 20-fold compared with the EPA-approved continuous-flow assay.

## Introduction

The assay of dissolved inorganic nitrogen (DIN) and of orthophosphate is of great interest in a wide variety of waters including river, lake, underground, potable waste, fish farming, hydroponics cultivation and also sea and brackish waters. The classical flow injection (FI) technique for DIN using a copperized cadmium granule-filled reactor, for reduction of nitrate to nitrite, with subsequent reaction of nitrite with sulfanilamide has become an EPA certified method for sub-ppm range nitrate/nitrite determinations. There are numerous assay protocols based on this principle, all using spectrophotometric detection of the final product, monitoring the azo dye at 540 nm.<sup>1–5</sup> Another example, the spectrophotometric determination of ammonium, is based on formation of Indophenol Blue<sup>6,7</sup> that is formed by reaction of hypochlorite with ammonium, followed by addition of salicylate in alkaline buffer.<sup>8,9</sup> The EPA certified method for orthophosphate is based on the reduction of Molybdenum Yellow to Molybdenum Blue by ascorbic acid in the presence of antimony tartrate serving as a catalyst.<sup>6,8</sup>

Therefore, the novelty of this work is not in the chemistry of reagent-based assays, but in the way in which they are automated and microminiaturized. In contrast to the traditional FI continuous-flow technique, where changing the assay parameters necessitates reconfiguration of manifold components, sequential injection (SI) stacks the sample and reagent zones in a required sequence into the holding coil (Fig. 1). The mixing is accomplished by selecting the amplitude of the flow reversal and the reaction time is gained by stopping the flow. In a microSI ( $\mu$ SI) system, the “Lab-on-Valve” manifold includes the flow-through detector, allowing different assays to be performed within the same unit, by charging ports 3 and 4 of the multiposition valve with appropriate reagents, while changing the assay parameters by means of a software protocol (Table 1) that controls the movement of the syringe pump and of the valve.<sup>9,10</sup>

## Experimental

### Instrumentation

The sequential injection analysis system (FI/Analab-3000, FI/Analab Instruments, Medina, WA, USA; <http://www.flowinjection.com>) consists of a syringe pump (500  $\mu$ L volume) driven by a stepper motor (24000-step full stroke), a six-port selector valve and a single-speed unidirectional peristaltic mini pump [Fig. 1(a)]. A tungsten-halogen lamp (LS-1, Ocean Optics, El Dorado Hills, CA, USA; <http://www.oceanoptics.com>) was used as the VIS light source for a UV-VIS spectrophotometer (S2000, Ocean Optics, Dunedin, FL, USA). Fiber-optic cables, furnished with a stainless-steel tip (400  $\mu$ m, tip 0.0625 in od), were used to connect the flow cell to the light source and the spectrophotometer. The flow cell was configured as shown in Fig. 1; the light pathlength was adjusted to 5 mm (10  $\mu$ L volume). Experimental data were collected by FI/Analab for Windows (version 5.7.8) installed on a PC (Pentium II, 300 MHz, 64MB RAM) with the Microsoft Windows 98 (Microsoft, Edmond, WA, USA; <http://www.microsoft.com>) operating system. The Lab-on-Valve manifold was fabricated in-house and interfaced to match a six-port selector valve as described previously.<sup>9,10</sup>

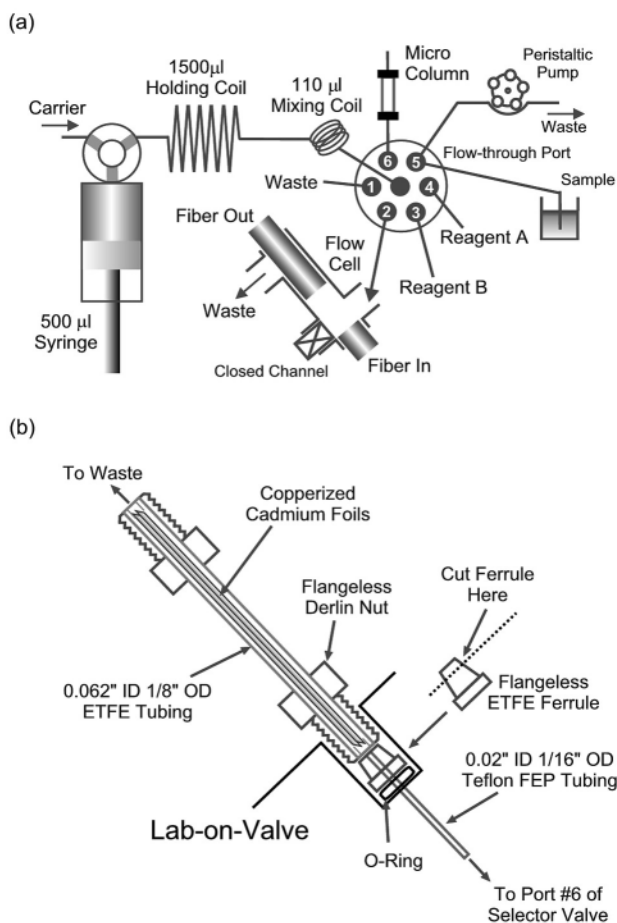
### Nitrate/nitrite assay reagents and standards

Ammonium chloride buffer (1.60 M) was prepared by dissolving 21.500 g of ammonium chloride (ACS certified, Fisher Scientific, Fair Lawn, NJ, USA; <http://www.fishersci.com>), 0.250 g of disodium(ethylenedinitrilo)tetraacetate dihydrate crystals (J. T. Baker, Phillipsburg, NJ, USA; <http://www.jtbaker.com>) and 0.500 g of sodium hydroxide pellets (J. T. Baker) in 150 mL of de-ionized (DI) water mixing and diluting to 250

mL with DI water. Sulfanilamide reagent was prepared by adding 2.50 mL of 86% w/v concentrated phosphoric acid (J. T. Baker) to 20 ml of DI water, then 1.00 g of sulfanilamide (Aldrich, Milwaukee, WI, USA; <http://www.sigma-aldrich.com>) and 0.025 g of 98% *N*-(1-naphthyl)ethylenediamine dihydrochloride (Aldrich), mixing and diluting to 25 mL with DI water.

Nitrate stock standard solution [100.00 ppm (N)] was prepared by dissolving 0.607 g of sodium nitrate (BandA Chemical, Morristown, NJ, USA) in DI water and diluting to 100 mL. Nitrite stock standard solution [100.00 ppm (N)] was prepared by dissolving 0.493 g of sodium nitrite (Aldrich) in DI water and diluting to 100 mL. Working standard solutions were prepared by serial dilutions of the stock standard solutions. All reagent and standard solutions were freshly prepared and de-gassed prior to use.

To automate this assay using the Lab-on-Valve system [Fig. 1(a)], port 6 was selected to accommodate a Cd/Cu micro-column [Fig. 1(b)]. The void volume of the column was 100.0  $\mu\text{L}$  and the volume of the connector between port 6 and the Cd filling was 2.6  $\mu\text{L}$ . The weight of Cd foil in the microreactor was 235 mg. Sulfanilamide reagent was supplied through port 4 and the sample by means of flow-through port 5, and port 2 was connected to a multi-purpose flow cell.



**Fig. 1** (a) Schematic diagram of the  $\mu\text{SI}$  system. A precision micro syringe pump is connected through holding and mixing coils to a six-way selector valve. The flow-through sampling port 5 allows quick sample exchange by aspirating sample solution by means of peristaltic pump. The flow cell connected to port 2 is configured in the absorbance mode with a light path of 5 mm. Ports 3 and 4 are assigned to reagents and port 6 is shown furnished with a microcolumn (for nitrate assay). (b) Micro-reduction column (internal void volume 100.0  $\mu\text{L}$ , amount of Cd foil 236.4 mg). Connection between the column and the selector was made with a modified ferrule and a 13.0 mm length of 0.020 in id Teflon tubing (volume 2.6  $\mu\text{L}$ ).

## Preparation of Cd/Cu micro-reduction column

To prepare the microcolumn shown in Fig. 1(b), 0.1 mm thick cadmium foil (Aldrich) was cut into 0.7–0.9 mm wide, 50.0 mm long strips and inserted into 55.0 mm long, 1/8 in od, 0.062 in id Tefzel PTFE tubing (Type 1530, Upchurch Scientific, Oak

**Table 1** Operation protocols used for nitrate/nitrite assay

Syringe Pump Command (?) KOR	
Variable Define New	Syringe Pump Flow Rate ( $\mu\text{L s}^{-1}$ )
NumberOfRuns	150
Variable Define New	Syringe Pump Valve In
SampleVolume	Syringe Pump Aspirate ( $\mu\text{L}$ ) 200
Variable Define New	ReactionTime
Variable Define New	Syringe Pump Delay Until Done
ContactPosition	Syringe Pump Delay Until Done
Variable Define New	Syringe Pump Delay Until Done
ReagentVolume	Syringe Pump Valve Out
Variable Define New	Syringe Pump Flow rate ( $\mu\text{L s}^{-1}$ )
MeasureFlowRate	30
NumberOfRuns = 5	Multiposition Valve Sample
SampleVolume = 80	Syringe Pump Aspirate ( $\mu\text{L}$ )
ReactionTime = 30	SampleVolume
ContactPosition = 55	Syringe Pump Delay Until Done
ReagentVolume = 10	Multiposition Valve Column
MeasureFlowRate = 2	Syringe Pump Dispense ( $\mu\text{L}$ )
Peristaltic Pump On	SampleVolume
Delay (s) 3	Syringe Pump Delay Until Done
Peristaltic Pump Off	Multiposition Valve Column
Loop Start (#) NumberOfRuns	Syringe Pump Dispense ( $\mu\text{L}$ )
Syringe Pump Valve In	SampleVolume
Syringe Pump Flow Rate ( $\mu\text{L s}^{-1}$ )	Syringe Pump Delay Until Done
150	Multiposition Valve Reagent
Syringe Pump Aspirate ( $\mu\text{L}$ ) 300	Syringe Pump Aspirate ( $\mu\text{L}$ )Reagent
Syringe Pump Delay Until Done	Volume
Multiposition Valve Flow Cell	Syringe Pump Delay Until Done
Syringe Pump Flow Rate ( $\mu\text{L s}^{-1}$ )	Spectrometer Reference Scan
85	Spectrometer Absorbance Scanning
Syringe Pump Valve Out	Multiposition Valve Flow Cell
Syringe Pump Dispense ( $\mu\text{L}$ ) 50	Delay (s) 0.2
Syringe Pump Delay Until Done	Syringe Pump Flow Rate ( $\mu\text{L s}^{-1}$ )
Multiposition Valve STD	Measure Flow Rate
Syringe Pump Aspirate ( $\mu\text{L}$ ) 20	Syringe Pump Dispense ( $\mu\text{L}$ ) 18
Syringe Pump Delay Until Done	Syringe Pump Delay Until Done
Multiposition Valve Reagent	Spectrometer Stop Scanning
Syringe Pump Aspirate ( $\mu\text{L}$ ) 20	Syringe Pump Flow Rate ( $\mu\text{L s}^{-1}$ )
Syringe Pump Delay Until Done	150
Multiposition Valve Sample	Syringe Pump Empty
Syringe Pump Aspirate ( $\mu\text{L}$ ) 20	Syringe Pump Delay Until Done
Syringe Pump Delay Until Done	Syringe Pump Delay Until Done
Multiposition Valve Waste	Loop End
Delay (s) 0.3	Syringe Pump Flow Rate ( $\mu\text{L s}^{-1}$ )
Syringe Pump Flow Rate ( $\mu\text{L s}^{-1}$ )	150
Syringe Pump Empty	Syringe Pump Flow Rate ( $\mu\text{L s}^{-1}$ )
Syringe Pump Delay Until Done	150
Syringe Pump Valve In	Syringe Pump Valve In
Syringe Pump Valve In	Syringe Pump Aspirate ( $\mu\text{L}$ ) 200
Syringe Pump Aspirate ( $\mu\text{L}$ ) 200	Syringe Pump Delay Until Done
Syringe Pump Delay Until Done	Multiposition Valve Column
Multiposition Valve Column	Syringe Pump Valve Out
Syringe Pump Valve Out	Syringe Pump Flow Rate ( $\mu\text{L s}^{-1}$ )
Syringe Pump Flow Rate ( $\mu\text{L s}^{-1}$ )	85
Syringe Pump Valve Out	Syringe Pump Empty
Syringe Pump Flow Rate ( $\mu\text{L s}^{-1}$ )	Syringe Pump Delay Until Done
85	Syringe Pump Empty
Syringe Pump Empty	Syringe Pump Delay Until Done
Syringe Pump Empty	Syringe Pump Delay Until Done

Harbor, WA, USA; <http://www.upchurch.com>). Two inner diameter-enlarged (final id 0.06 in) 1/4-28 Derlin flangeless nuts (Type P-203x, Upchurch Scientific) were press-fitted to the ends of the cadmium foil filled ETFE tubing. A 1.0 mL plastic syringe was used to flush solution through and regenerate the cadmium foil in the ETFE tubing. The regeneration of the cadmium surface was done by flushing solutions in the order 2.0 mL of acetone (J. T. Baker), 2.0 mL DI water, 2.0 mL of 1.0 M hydrochloric acid (Fisher Scientific), 4.0 mL of DI water, 2.0 mL of 2.0% w/v cupric sulfate solution (J. T. Baker) and 6.0 mL of DI water. A modified flangeless ferrule (P-200Nx, Upchurch Scientific) and 15.0 mm long, 1/16 in od, 0.020 in id Teflon FEP tubing (Type 1548, Upchurch Scientific) were used between the Cd/Cu micro reduction column and the rotation plate of the selector valve [Fig. 1(b)] as an interface for reducing the dead volume and avoiding the double-peak phenomenon.<sup>11</sup> The constructed micro reduction column had an internal volume of 100.0  $\mu\text{L}$ , 236.4 mg of Cd foil and 2.6  $\mu\text{L}$  dead volume.

### Phosphate assay reagents and standards

Ammonium molybdate(vi) reagent (6.0 mM) was prepared by dissolving 0.800 g of ammonium molybdate tetrahydrate (Aldrich) and 0.020 g of antimony potassium tartrate half-hydrate (J. T. Baker) in 80 mL of DI water and diluting to 100 mL. Ascorbic acid solution (600 mM) was prepared by dissolving 12.0 g of ascorbic acid (99%) (Aldrich) in 80 mL of DI water, adding 0.4 mL of acetone (reagent grade) (J. T. Baker) and diluting to 100 mL using DI water. Phosphate stock standard solution [100.0 ppm (P)] was prepared by dissolving 4.392 g of monobasic potassium phosphate crystals (J. T. Baker) in 100 mL of DI water. Working standard solutions were prepared by serial dilution of the stock standard solution. All reagent and standard solutions were freshly prepared and degassed prior to use.

### Surfactant effect on phosphate assay

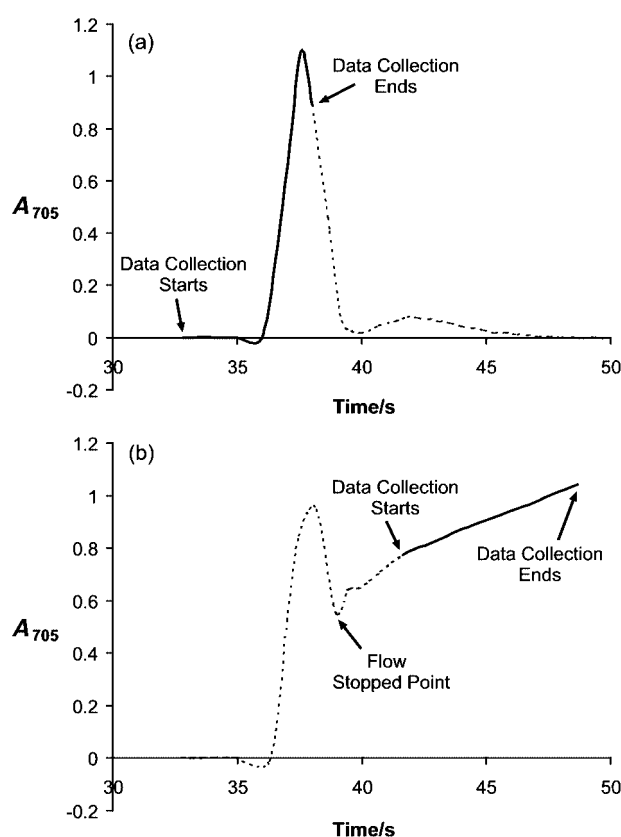
The commercial detergent Joy (Procter and Gamble, Cincinnati, OH, USA; <http://www.pg.com>), Triton X (*tert*-octylphenoxypolyethoxyethanol) (Sigma, St. Louis, MO, USA) and glycerol (J. T. Baker) were used as carrier stream surfactants in the phosphate assay to study the effect of surfactants.

## Results and discussion

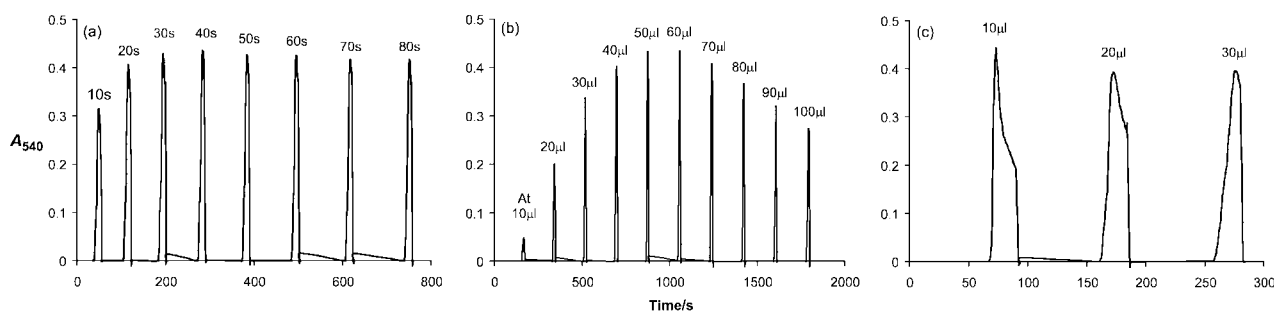
### Optimization of assay protocols and data collection

In contrast to manual assays, when reactions are always allowed to reach equilibrium, flow injection techniques allow reliable data to be obtained under non-equilibrium conditions, since

samples and standards are processed automatically in exactly the same way. Sequential injection goes one step further, by exploiting stopped flow to gain sufficient time for chemical reactions to produce a desirable amount of detectable compound. If this amount is produced by a fast reaction already during the mixing stage, then the reacted zone flows continuously through the detector, yielding a typical peak. Since the calibration curve is based on peak height measurement, the flow can be momentarily accelerated after the peak maximum has been reached with the aim of increasing the sampling frequency [Fig. 2(a)]. There is no need to collect data after this point and therefore in this work the superimposed readouts of this type are shown without the tail sections (see Fig. 4). If, however, the chemical reaction proceeds while the zone is situated within the flow cell, it is advantageous to carry out reaction rate measurement by stopping the flow until a desirable readout is obtained and then restarting the flow at a high flow rate to speed up the washout [Fig. 2(b)]. The data collection period is selected to cover the linear range of reaction rate measurement and used for calibration [see Fig. 5(b)].



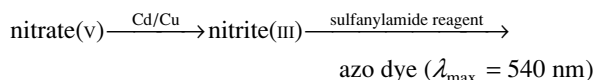
**Fig. 2** Data collection protocols. (a) Continuous-flow peak height measurement and (b) stopped-flow reaction rate measurement. Solid lines represent actual collected analyte responses.



**Fig. 3** Optimizations of experimental protocol for nitrate/nitrite assay using 5.0 ppm (N) sodium nitrate standard solutions. Flow cell used for optimization: light path 3.0 mm, volume 6.0  $\mu\text{L}$ . (a) On-column stopped-flow interval optimization: the maximum amount of nitrite was formed after 30 s residence time in the microcolumn. (b) Volume of reduced sample aspirated from the microcolumn for subsequent colorimetric reaction. (c) Influence of the volume of reagent injected on peak height (for details, see text).

## Nitrate and nitrite assays

The classical FI technique for nitrate/nitrite assays in the FIALab method was based on the following reaction sequence:



where nitrate was reduced originally by means of a 10.0 cm long, 5.0 mm id column packed with copperized Cd granules through which the sample zone is transported by a continuous-flow. This approach has several drawbacks: backpressure, degradation of reductive capacity caused by incessant flow of oxygen-containing carrier stream, high waste production caused by continuous flow and necessity to make the column large in order to achieve a sufficient contact time between the reducing surfaces and sample material flowing through.

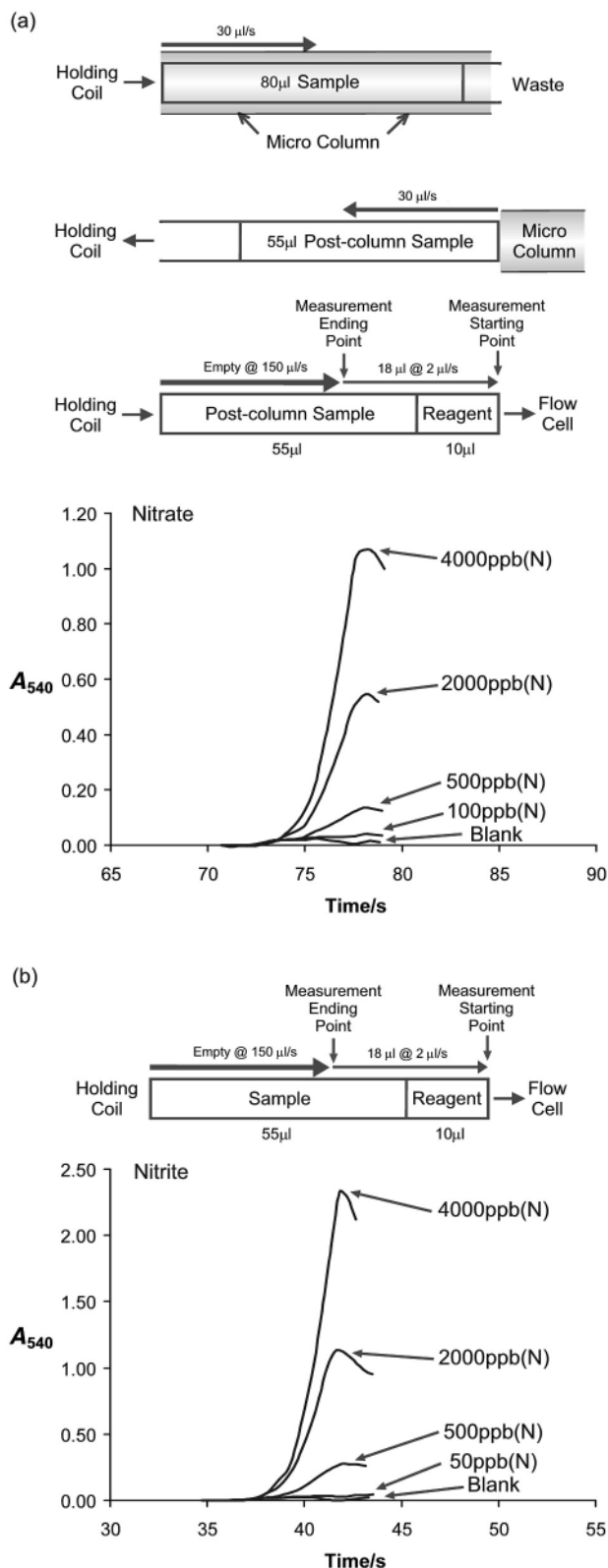
By injecting the nitrate sample into the column, that has been nested in port 6 [Fig.1(b)], and by stopping the flow, these drawbacks are eliminated and the column can be miniaturized. The contact time between nitrate sample and Cd/Cu surface is selected by the length of the stopped-flow period during which the sample solutions are held within the column. An 80.0  $\mu\text{L}$  volume of 5.0 ppm (N) nitrate sample was injected into the column and the stopped-flow period was varied from 10 to 80 s. As shown, a 30 s residence time yielded the maximum peak height. Interestingly, longer stopped-flow periods resulted in reduction of nitrite to ammonium, yielding lower responses.<sup>3</sup> Such a diminished response is well known, being influenced by pH, buffer composition and length of contact time.<sup>3,8</sup> Another important parameter is the volume of the reduced sample solution to be aspirated from the micro reduction column and transferred into the holding coil for subsequent reaction with sulfanilamide reagent. As would be expected [Fig. 3(b)], the signal increases as the volume of the aspirated sample is increased, until the point when non-reduced material (from the channel upstream of the Cd/Cu filling) starts to dilute the reduced material, which has been in contact with the Cd/Cu surface. Aspiration of 55  $\mu\text{L}$  yields the maximum response. (The void volume of the column was 100  $\mu\text{L}$  and the connector volume between the column and port was 2.5  $\mu\text{L}$ .)

Finally, the influence of the volume of the reagent used to push the sample zone upstream into the holding coil was examined [Fig. 3(c)]. Since the azo dye is formed along the interface of the sample and reagent zones, the use of excessively large volumes of reagent is not desirable, as the increased displacement of the sample zone upstream into the holding coil and its subsequently longer path towards the flow cell lead to dilution and reduction of the peak height. Therefore, 10  $\mu\text{L}$  of reagent were chosen. Since the diazotization reaction is very fast, the measurement is made whilst the reaction product passes through the flow cell at a rate of 2.0  $\mu\text{L s}^{-1}$ . The flow rate was increased to 150.0  $\mu\text{L s}^{-1}$ , after peak maximum had been monitored to speed up the system washout [see tail section of peaks in Fig. 3(c)]. The optimized software protocol is shown in Table 1.

## Efficiency of the microcolumn

The performance of the micro reduction column was evaluated by injecting identical volumes and concentrations of nitrate and nitrite solutions, using the same operation protocol as used for nitrate samples [Fig. 4(a)], and by comparing the resulting peak heights. The conversion efficiency was obtained by summing triplicate injections of 80.0  $\mu\text{L}$  of 3.0 ppm (N) sodium nitrate standard solution and sodium nitrite standard solution and averaging the peak heights (nitrate 0.400, 0.400, 0.403; nitrite

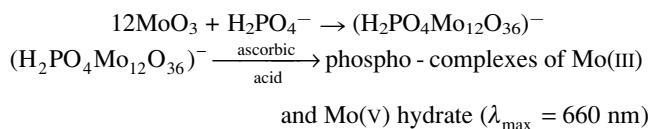
0.400, 0.403, 0.402), yielding 99.9% conversion efficiency for nitrate to nitrite.



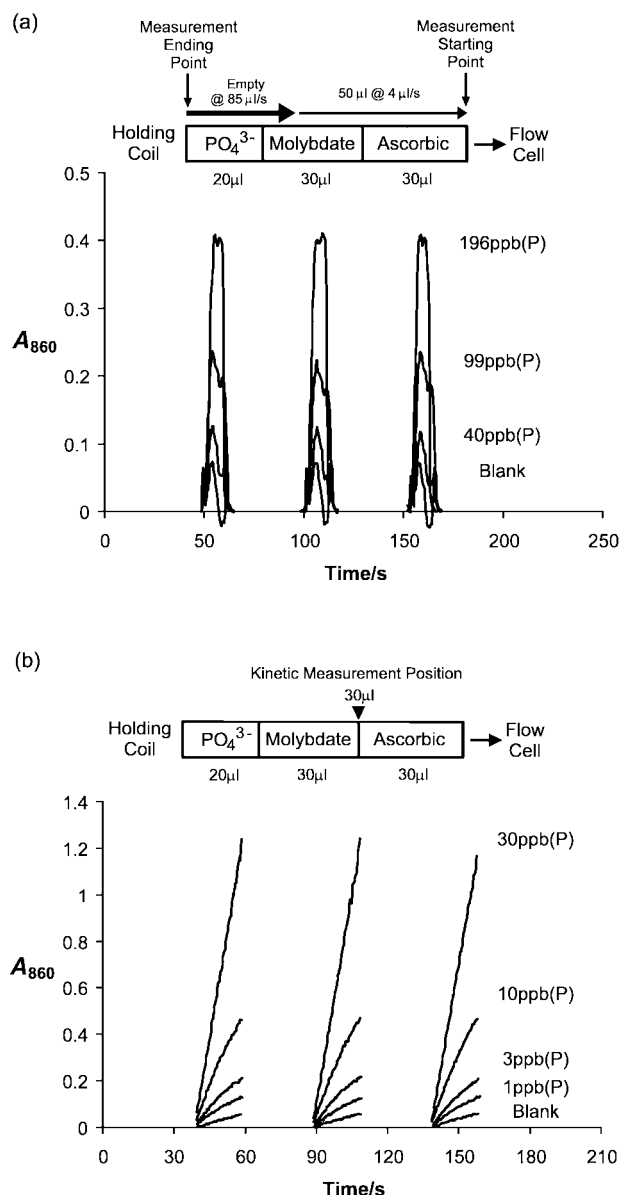
**Fig. 4** Protocols and responses for nitrate/nitrite assays. (a) Nitrate assay: a sample volume of 80.0  $\mu\text{L}$  was injected into the holding coil and transported into the microcolumn. Then 55.0  $\mu\text{L}$  of sample were aspirated from the microcolumn and mixed with 10.0  $\mu\text{L}$  of reagent. Following flow reversal at 2.0  $\mu\text{L s}^{-1}$  the reaction product is measured under continuous flow at a flow rate of 2.0  $\mu\text{L s}^{-1}$  (18.0  $\mu\text{L}$  displacement volume). (b) Nitrite assay: using the same protocol but avoiding microcolumn treatment, nitrite responses in the standard solutions are shown. Note that the responses of identical concentrations of nitrate and nitrite differ since the nitrite sample did not pass through the microcolumn (see text for details).

## Phosphate assay using continuous-flow mode and stopped-flow kinetic mode

Reaction of orthophosphate with molybdate followed by reduction to Molybdenum Blue:



is usually carried out at slightly elevated temperature (38 °C) under continuous flow. Whereas the first step, formation of Molybdenum Yellow, is very fast; the reduction to the blue heteropoly compound is the rate-determining step, which can be



**Fig. 5** (a) Phosphate assays with continuous-flow measurement. Protocol: 20.0 µL of phosphate standard, 30.0 µL of molybdate reagent and 30.0 µL of ascorbic acid were stacked into the holding coil. Then the flow was reversed and the sequenced profile was transported at a flow rate of 4.0 µL s<sup>-1</sup> into the flow cell. Data collection was stopped after 50.0 µL had been delivered and the flow rate was increased to 85.0 µL s<sup>-1</sup> to flush the system. Superimposed peaks obtained with orthophosphate standards in the range 0–196 ppm (P) are shown. Above the 200 ppb (P) concentration level the peak height was no longer linearly dependent on concentration. (b) Stopped-flow reaction rate measurement. Protocol: as above, but after delivering 30 µL of the reaction mixture into the flow cell, the flow was stopped for 20 s to collect data on reaction rate. Superimposed reaction rate curves are shown for phosphate standards in the range 0–30 ppb (P).

accelerated by increasing the temperature or by introducing a catalyst such as antimony.<sup>6,8,12</sup> In this work, all experiments were carried out at room temperature (25 °C) in presence of antimony tartrate. The Lab-on-Valve system [Fig. 1(a)], albeit without a microcolumn, was assembled and port 4 was used for molybdate reagent and port 3 was charged with ascorbic acid. Then 20.0 µL of sample, 30.0 µL of molybdate and 30.0 µL of ascorbic acid were sequentially aspirated into the holding coil and the flow was reversed to transport the reaction mixture into the flow cell at a rate of 4 µL s<sup>-1</sup>.

For *continuous-flow measurement* a typical peak was obtained and after 32.0 µL had been dispensed at a flow rate of 4 µL s<sup>-1</sup>, the flow was accelerated to 85.0 µL s<sup>-1</sup> in order to wash out the flow cell, while data were no longer collected. The heights of the resulting peaks, shown superimposed in Fig. 5(a), were proportional to phosphate concentration (Table 2); however, above the 200 ppb (P) level the calibration curve was no longer linear, owing to the depletion of reagent. Stopped-flow reaction rate measurements were successfully carried out since the formation of Molybdenum Blue is the rate-determining step. After delivering the forming complex into the flow cell at a rate of 4.0 µL s<sup>-1</sup> using a displacement volume of 30 µL, the flow was stopped for 20 s and the data were collected to obtain a reaction rate curve [Fig. 5(b)].

### Effect of surfactants on phosphate assay

The use of surfactants in flow analysis can be traced all the way back to air-segmented AutoAnalyzer systems invented by Skeggs.<sup>13</sup> Whereas their beneficial properties such as wetting of surfaces, decreasing adsorption on conduit walls and resulting carryover are well documented, their influence on the chemical reactions has seldom been investigated. Since it was observed that various surfactants influence the rate of formation of Molybdenum Blue, the influence of surfactants present in the carrier solution was investigated. The influence of glycerol was the smallest compared with other surfactants, and the concentration range was narrower compared with the use of the commercial detergent Joy and Triton X. Charged surfactants tend to interact with Molybdenum Blue in an unpredictable manner, such as shifting the wavelength of maximum absorbance, influencing the reaction rate or forming product-surfactant colloids. Therefore, in order to avoid any complications, degassed DI water without surfactant was used as the carrier stream in all assays.

**Table 2** Performance data for each assay

Assay	Parameter	Continuous-flow mode	Stopped-flow mode
Nitrate	Concentration dynamic range [ppb (N)]	100.0–4000.0	—
	Linearity, $r^2$	0.9999	—
	Standard error (%)	0.35	—
	Detection limit [ppb (N)]	3.91	—
	Sample throughput/h <sup>-2</sup>	48	—
Nitrite	Concentration dynamic range [ppb (N)]	30.0–4000.0	—
	Linearity, $r^2$	0.9995	—
	Standard error (%)	0.87	—
	Detection limit [ppb (N)]	4.53	—
	Sample throughput/h <sup>-2</sup>	72	—
Phosphate	Concentration dynamic range [ppb (P)]	19.9–196.0	1.0–30.0
	Linearity, $r^2$	0.9995	0.9997
	Standard error (%)	1.22	0.80
	Detection limit [ppb (P)]	9.92	0.10
	Sample throughput/h <sup>-2</sup>	54	60

**Table 3** Standard addition method for water samples

Sample	Analyte	Method <sup>a</sup>	Content found [ppb (N or P)]	Standard spiked final concentration [ppb (N or P)]	Standard error (%)	r <sup>2</sup>
Green Lake water	Nitrate	CM	104.06	100, 150, 200	6.80	0.9908
	Nitrite	CM	140.82	100, 150, 200	0.92	0.9998
	Phosphate	SM	1.18	1, 2, 3	6.92	0.9905
Tap water	Nitrate	CM	91.79	100, 150, 200	3.71	0.9986
	Nitrite	CM	200.84	100, 150, 200	4.33	0.9981
	Phosphate	SM	1.59	1, 2, 3	6.76	0.9909

<sup>a</sup> CM = continuous-flow mode; SM = stopped-flow mode.

### Assay of water samples

Water sample assays were controlled by the standard additions method (Table 3) in order to eliminate any matrix effects. The DIN contents and orthophosphate in the water samples were assayed by spiking three standard solutions for each desired analyte within a predetermined concentration dynamic range (Table 2). Continuous-flow measurement was used for nitrate and nitrite assays and stopped-flow measurement for phosphate assay.

### Conclusion

The present work introduces the  $\mu$ SI technique into environmental monitoring of water quality and demonstrates the advantages of microminiaturization for real-life assays: a substantial reduction of reagent consumption, waste generation and versatility, that allows various analytes to be assayed with the same system without physical reconfiguration. The typical assay rates were 48 samples h<sup>-1</sup> for nitrate assay and 72 samples h<sup>-1</sup> for nitrite assay, and 60 samples h<sup>-1</sup> for kinetic mode phosphate assay and 54 samples h<sup>-1</sup> for continuous-flow mode phosphate determination. Since the reagent-based assays used are approved by the EPA and have been used for the assay of a wide variety of potable, lake, waste and industrial water samples, the approach presented here is expected to find numerous practical applications.

The technical improvement in this work is the use of a microcolumn, mounted on the Lab-on-Valve and operated in the stopped-flow mode. It is hoped that this approach will serve as inspiration for microminiaturization of assays that utilize, in the continuous-flow mode, packed microreactors. The use of solid reducing or oxidizing agents is closest to the application described here. In addition, the use of packed enzymatic reactors in the format presented here is likely to be successful. Also, the use of ion-exchange materials or other sorbents for analyte preconcentration can conceivably be carried out with a forward flow in a microcolumn nested in a Lab-on-Valve port, followed by a stopped-flow elution after which the accumulated analyte will be flushed out to the detector by a flow reversal. Yet another attractive feature of the presented approach is automa-

tion of protocol optimization procedures, as seen in Fig. 3. Optimization of the nitrite/nitrate assay was accomplished within less than 1 h. Finally, it is hoped that this work, by demonstrating the advantages of the Lab-on-Valve system, will serve as a guide for the development of assay protocols for various reagent-based assays as applied in environmental monitoring. This will prove beneficial since the  $\mu$ SI instrumentation is compact and robust and is suitable for both laboratory and field measurements.

### Acknowledgements

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