RESEARCH ARTICLE

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Online zinc reduction-sequential injection analysis for the determination of nitrogen species in extracts of riverine sediment

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Abstract

Background: The substitution of hazardous analytical reagents such as Cd with benign alternatives for chemical determination is one of the principles of green chemistry. An eco-friendly Zn reduction method for nitrate determination was developed to achieve this end.

Methods: Online zinc reduction-sequential injection analysis (ZnR-SIA) protocol was developed for the determination of nitrogen (N) species in the Lagos Lagoon sediments by modification of some standard methods.

Results: The novel application of boric acid-borate buffer employed in borate-buffered granular Zn reduction suppressed gas evolution from the Zn micro-column, making the Zn reduction adaptable to online use, unlike the frequently used ammonia-ammonium buffer. Further results showed that the sampling rate for the two-zone stack was 65 h^{-1}. The boric acid-borate buffer offered 5.9-fold and 1.3-fold of the instrument response given by the ammonia-ammonium buffer at 0.50 and 5.0 mg N L^{-1} respectively. The method detection limits of the ZnR-SIA for the three matrices studied were 24.6 μg N L^{-1} for reagent water, 0.383 mg N kg^{-1} for KCl-extractable nitrate, and 3.18 mg N kg^{-1} for total nitrogen. The average recovery of nitrate-N in matrix samples was 94.7%, and its precision was 4.52%. The average recoveries of total N in blanks and matrix samples were 97.9 and 93.2% respectively.

Conclusions: The method accuracies demonstrated the suitability of the ZnR-SIA for sediment N determination. Zn reduction is recommended where sustainable development is desired and is a potential substitute for the standard Cd reduction owing to Cd toxicity.

Keywords: Green chemistry, Sediment nitrogen, Online zinc reduction, Borate buffer, Sequential injection

Background

One of the tenets of green chemistry is the use of substances having little or no toxic effect on human health and the environment (Sanghi et al. 2012). Therefore, the switch from Cd reduction for nitrate determination to Zn reduction will accomplish a green chemical objective (Merino 2009) because the World Health Organization's maximum admissible concentrations for Cd and Zn in potable water are 0.005 and 5.0 mg L^{-1}, respectively (Olajire and Imeokparia 2000), thus making Zn the more

attractive green option than Cd. And exposure to Zn is three orders of magnitude apparently incapable of causing appreciable harm in relation to Cd. Zn reductor is, therefore, environmentally friendly, and environmentally friendly chemical analysis is geared towards achieving sustainable development (Grudpan et al. 2011). Zinc reduction coupled to sequential injection analysis is no doubt a green chemical analytical approach for sustainable development in view of the reduced reagent input and reduced waste generation. The programmable flow derived from sequential injection analysis (SIA) in the lab-on-valve (LOV) format is a fast-growing technology in chemical process disciplines as micro-SIA-LOV minimizes resource consumption and waste generation much more than flow

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injection analysis (FIA) and also offers a stopflow mode for slow reactions (Solich et al. 2003).

Nitrate can be extracted from soil by water or a dilute or concentrated salt solution to bring it into solution prior to quantitative analysis. However, a concentrated salt solution such as 2 M KCl with high ionic strength is effective in suppressing the dispersion of some substances from the soil into the water phase (Gelderman and Beegle 2012; Dorich and Nelson 1984). Alkaline digestion is recommended for the conversion of ammonia and organic N materials to nitrate in view of the alkaline hydrolysis furnished by the method (Worsfold et al. 2005), and alkaline persulfate oxidation is particularly recommended for marine samples in order to prevent chloride oxidation by persulfate, an inherent defect of acid persulfate digestion leading to undesirable consumption of potassium persulfate (Worsfold et al. 2005).

A standard route for determination of nitrate is through reduction of nitrate to nitrite by copperized cadmium granules in an alkaline medium. Nitrite in an acid medium is diazotized with sulfanilamide and coupled with *N*-(1-naphthyl) ethylenediamine dihydrochloride (NINE) to form a red azo dye which is measured spectrophotometrically (Sun et al. 2014; APHA 2005). Tetrasodium salt of ethylenediaminetetraacetic acid (EDTA) is used in the conditioning of cadmium column to increase the column life by preventing the formation and coating of Cd $(OH)_2$. The EDTA also prevents the surface coating of cadmium by iron, copper, and other metals in environmental samples by keeping the metals in solution (APHA 2005). Zinc had been used for nitrate reduction to nitrite, but the method was not widely adopted (Ellis et al. 2011; Mir 2007). Devarda's alloy and granular zinc have been used to reduce nitrate to ammonia for eventual detection of ammonia through physical methods. In the two methods, nitrate is obtained as the difference between a sample run with reduction step and another sample run without the reduction step so as to account for the ammonium originally present in the sample (Ellis et al. 2011), and both methods become more cumbersome and time consuming when ammonium levels in samples are high relative to nitrate in view of the preliminary ammonia removal required to avoid interference, though the ammonia detection of their methods use a few reagents making them simple and economical.

Published SIA and other flow-based studies for nitrate determination are not as widely reported as for phosphate and are always reported for water matrix, e.g., Patton and Kryskalla (2003) and APHA (2005), and where metal reductor is used, it is always Cd reduction in ammonia-ammonium buffer, e.g., Morales et al. (2001) and APHA (2005). Reagent concentrations and time for the Griess-Illosvay reaction were optimized in

the study. Conditions for 2 M KCl extraction of sediment nitrate and alkaline persulfate digestion for total nitrogen (TN) were also optimized. Online Zn reduction in a micro-column and SIA-LOV script were developed for determining nitrate-N. The zinc reduction-SIA (ZnR-SIA) was validated for nitrate-N and TN by recovery studies.

Methods

Materials and micro-SIA-LOV manifold

All solid analytical-grade colorimetric reagents as well as calibration standards and sample-preparation reagents were supplied by Sinopharm (China) and Sigma-Aldrich (China). Reference compounds for recovery studies were supplied by Aladdin (China), while HCl (35–38%) and NH_3 (25–28%) were supplied by Enox (China). Granular zinc was procured from Xin Hua (China), while granular cadmium was procured from Sinopharm. FIAlab-3500 (USA) was the micro-SIA-LOV used for this study, and the spectrometer was Ocean Optics USB 2000 (USA) with a halogen light source (HL-2000). The light source was connected to the spectrometer through two fiber optics which were separated by a 7-mm path length to form the flowcell. The FIAlab included an inbuilt four-channel two-way peristaltic pump, and all tubings were Upchurch products (Germany).

Procedure for online nitrate reduction

Zn granules (20–30 mesh) were washed with 3.0 M HCl and copperized with 2.0% $CuSO_4 \cdot 5H_2O$ before loading the homemade glass micro-column. The column (3.0 mm i.d.) was loaded with 3.0 g of Zn to produce a 4-cm length. One milliliter of boric acid-borate buffer (pH 8.1) composed of 73.4 mM borax, 452.9 mM boric acid, and 1.1 mM Na_2EDTA was added to 4 mL of sample. The buffer pH was adjusted with 0.3 M NaOH during its preparation. According to the SIA script implemented in the optimized manifold shown in Fig. 1a, nitrate reduction occurred as buffered sample was propelled through the micro-column for 25 s by a peristaltic pump revolving at 50% speed. For Cd reduction-sequential injection analysis (CdR-SIA), a Cd column was prepared exactly as the Zn column but 2.0 g of 40–60 mesh granules was used, and 1 mL of ammonia-ammonium buffer (pH 8.5) composed of 243.0 mM NH_4Cl and 95.9 mM Na_2EDTA was added to 7 mL of sample. The buffer pH was adjusted with *ammonia solution* (25–28%).

SI protocol for nitrate-N

Analysis of nitrate-N in stopflow mode was performed as pictured in the micro-SIA-LOV manifold (Fig. 1a). The protocol collected data at 540 nm (maximum absorption wavelength) and 700 nm (reference wavelength). The SI

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Fig. 1 a Optimized manifold for SI analysis of nitrate-N in stopflow mode. **b** Stacking of sample and reagent zones in the holding coil. *FC* flowcell, *FOC* fiber optic cable

analysis was looped four times per sample, and for each run, 10 μL of the single reagent composed of 2.48 M HCl, 3.04 mM *N*-(1-naphthyl) ethylenediamine dihydrochloride, and 0.12 M sulfanilamide was aspirated at 25 μL s^{-1}. This was followed by aspirating 40 μL of reduced sample using port 3 for reagent and flow-through inlet port 5 for sample. Thirty-five microliters of the combined zones was dispensed to the flowcell at 25 μL s^{-1} and delayed for 45 s for color development and absorbance reading. The selected flow rates were guided by FIAlab operation manual (2005). The sampling rate was 65 h^{-1}. The first peak of the four was discarded.

Optimization of manifold variables

Stacking of sample and reagent zones in the holding coil was optimized to determine whether the two-zone stack (reagent/sample combined zones) or the three-zone stack (reagent/sample/reagent sandwich) would be more sensitive. In both tests, 40-μL sample aliquots of 2.0 mg N L^{-1} standard were used. For the two-zone stack, a 10-μL reagent zone was aspirated followed by a 40-μL sample zone. For the three-zone sandwiching, the 40-μL sample zone was sequentially piled in between two 10-μL reagent lines in the holding coil. Reagent volume was varied in search of the optimum using 40 μL of 2.0 mg N L^{-1} standard. The volumes investigated were 3, 5, 7, 10, and 15 μL of the single reagent. Optimization of volume dispensed to the flowcell (delay position) for the stopflow mode of reagent/

sample-combined zones was performed. For this test, 40 μL of 2.0 mg N L^{-1} standard and 10 μL reagent were sequentially aspirated. The volumes dispensed to the flowcell in separate runs were 1, 5, 10, 15, 20 25, 27, 31, 33, 35, 37, 39, and 50 μL and delayed for absorbance reading. Cd reduction was employed for all the experiments in optimizing manifold configuration.

Optimization of reduction chemical variables

Sample-to-buffer ratio for Zn reduction was optimized using ammonium buffer (pH 8.5). For this test, 2.0 mg N L^{-1} standard was used, and eight ratios in a total fixed volume of 4.0 mL composed of simultaneously increasing sample volume and decreasing buffer volume were tested. The eight sample/buffer combinations were 1/3, 2/2, 3/1, 3.5/0.5, 3.7/0.3, 3.8/0.2, 3.9/0.1, and 4/0 mL. Ammonium buffer pH was varied in search of the optimum for quantitative reduction of NO$_3^-$ to NO$_2^-$ by Zn reduction using 10.0 mg N L^{-1} standard. The pH values investigated were 8.1, 8.5, 8.75, 9.0, 9.5, and 10.5.

The Na$_2$EDTA concentration for conditioning the Zn reduction micro-column was optimized using ammonium buffer (pH 8.1) and 10.0 mg N L^{-1} standard. The Na$_2$EDTA concentrations tested were 0.0, 1.15, 2.30, 4.60, and 96.6 M. To eliminate gas interference in the Zn reduction column, boric acid-borate buffer (pH 8.1) was tested for zinc reduction of nitrate and was compared with the ammonia-ammonium buffer (pH 8.1) using 0.50 and 5.0 mg N L^{-1} standards.

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Optimization of colorimetric variables

Simplex optimization method was used to simultaneously optimize sulfanilamide concentration, NINE concentration, and delay time (Mendham et al. 2000; Walters et al. 1991) using Cd reduction. All the reagents were combined in a single solution. Delay time was allowed to vary between 30 and 60 s. Sulfanilamide concentration was allowed to vary between 0.0232 and 0.1394 M, and NINE concentration was allowed to vary between 0.154 and 9.26 mM (APHA, 2005). An $[H^+]$/[sulfanilamide] ratio of 20.5 was used for the study. Therefore, for every sulfanilamide concentration prepared, its corresponding hydrogen ion concentration was also prepared together, and the resulting range of $[H^+]$ in this case was 0.480 to 2.86 M HCl. The reagent concentrations and delay times were tested using 1.0 mg N L^{-1} standard.

Optimization of nitrate extraction variables

Simplex algorithm was used for the simultaneous optimization of extractant volume, extraction speed, and extraction time using Cd reduction. Extraction time was varied between 5 and 40 min. Extractant volume was varied between 10 and 40 mL, and extraction speed was varied between 160 and 240 rpm. In every experiment, 1.0 g of the same homogenized sediment was extracted with 2 M KCl and was shaken on a thermostated shaker (Jiangsu Taicang Lab, SHZ-88, China) at 25 °C (Dorich and Nelson 1984). The extracts were filtered through a 0.45-μm filter and analyzed for N. Three replicate extractions were performed for each experiment. In order to standardize the peak height, it was calculated as absorbance times extractant volume divided by mass of sediment because the variation of extractant volume led to a different sediment-to-solution ratio for each experiment.

Optimization of alkaline persulfate oxidation for TN

Optimization of sediment digestion to achieve quantitative dissolution of TN and a colorless sediment was carried out by the method of alkaline persulfate digestion (Worsfold et al. 2005) using Cd reduction. The mass of $K_2S_2O_8$ used for digesting a constant mass of the same homogenized sediment was increased from 0.3 to 2.1 g in 0.3-g increments. For the first experiment, 0.5 g of sediment was weighed into a 100-mL digestion flask, and subsequently, 2 mL of 0.3 M NaOH solution and 0.3 g of $K_2S_2O_8$ were added to it. An Allihn condenser was fixed to the digestion flask, and the upper end of the condenser was corked and the coolant water flowing through the condenser was at approximately 2–3 °C and pumped by a water-circulation pump (Zhengzhou Greatwall, SHB-III, China). The digestion was carried out on a preheated magnetic stirrer (Jincheng Guosheng, 78–1, China) at 110 °C for 2 h. Each successive experiment had 0.3 g more $K_2S_2O_8$. After digestion, 1 drop of

phenolphthalein indicator was added and the solution was carefully neutralized to a faint pink with 1.5 M NaOH. It was diluted to 100 mL in a volumetric flask and filtered with Whatman no. 540 filter paper. The TN absorbance of each solution was measured.

Recovery studies of the ZnR-SIA methods

For the validation of KCl-extractable nitrate method, four 2.5-g aliquots of a sediment sample previously analyzed to contain 3.6 mg NO_3-N kg^{-1} were placed in separate 250-mL conical flasks and each of them was spiked with 0.5 mL of 10.0 mg N L^{-1} KNO_3. They were stirred, allowed to stand for 1 h, and analyzed for nitrate through the KCl extraction procedure stated above using 10 mL of 2 M KCl. KNO_3 used for recovery studies was supplied by Surechem (UK), while Sinopharm (China) supplied KNO_3 used for all calibration curves.
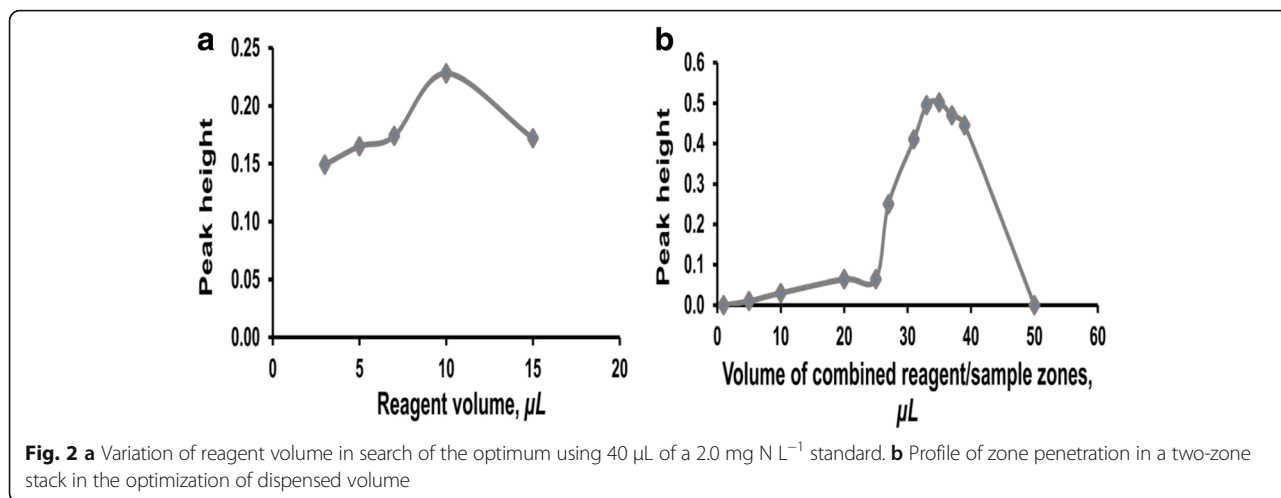
Laboratory-fortified matrix samples and laboratory-fortified blanks were prepared for recovery studies to validate the TN method. For the matrix sample set, three 1.0-g aliquots of sediment sample previously analyzed by the TN oxidation procedure to contain 1.542 g TN kg^{-1} were placed in three 10-mL glass bottles. Aqueous solutions of 4.000 g N L^{-1} glycine, 4.000 g N L^{-1} adenosine 5′-diphosphate disodium (ADP), and 2.500 g N L^{-1} KNO_3 were prepared. Then, 0.1 mL of each reference solution was added to the 1.0-g sediment. It was corked, gently stirred, allowed to stand for 1 day, and analyzed for TN through the oxidation procedure using 3.6 g of $K_2S_2O_8$ in 2 mL of 0.30 M NaOH. For the fortified blank set, three 1.0-mL aliquots of deionized water were separately spiked with 0.1 mL of 0.400 g N L^{-1} NH_4Cl, 0.1 mL of 0.400 g N L^{-1} ADP, and 0.1 mL of 0.250 g N L^{-1} KNO_3, and the three samples were analyzed for TN.

Results and discussion

All results reported for the optimization of nitrate reduction chemical variables and recovery studies were obtained using ZnR-SIA, and all other results reported in this study were obtained using CdR-SIA. The physico-chemical characteristics of the sediment sample adopted were as follows: pH, 3.98; total organic carbon, 2.26% by the Walkley-Black method (Environment Agency 2009); and mineral Fe, 2.36 g kg^{-1} by SIA-spectrophotometric method (Oladosu et al. 2016).

Manifold configuration

The optimization of zone stacking in the holding coil showed that the two-zone stack (reagent/sample combined zones) gave a higher sensitivity as depicted in Fig. 1b where its peak heights were greater than the peaks of reagent/sample/reagent sandwich for 2.0 mg N L^{-1} standard indicating that for Griess-Illosvay reaction, the greater sample-reagent mixing provided by the sandwich was not

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Fig. 2 a Variation of reagent volume in search of the optimum using 40 μL of a 2.0 mg N L^{-1} standard. **b** Profile of zone penetration in a two-zone stack in the optimization of dispensed volume
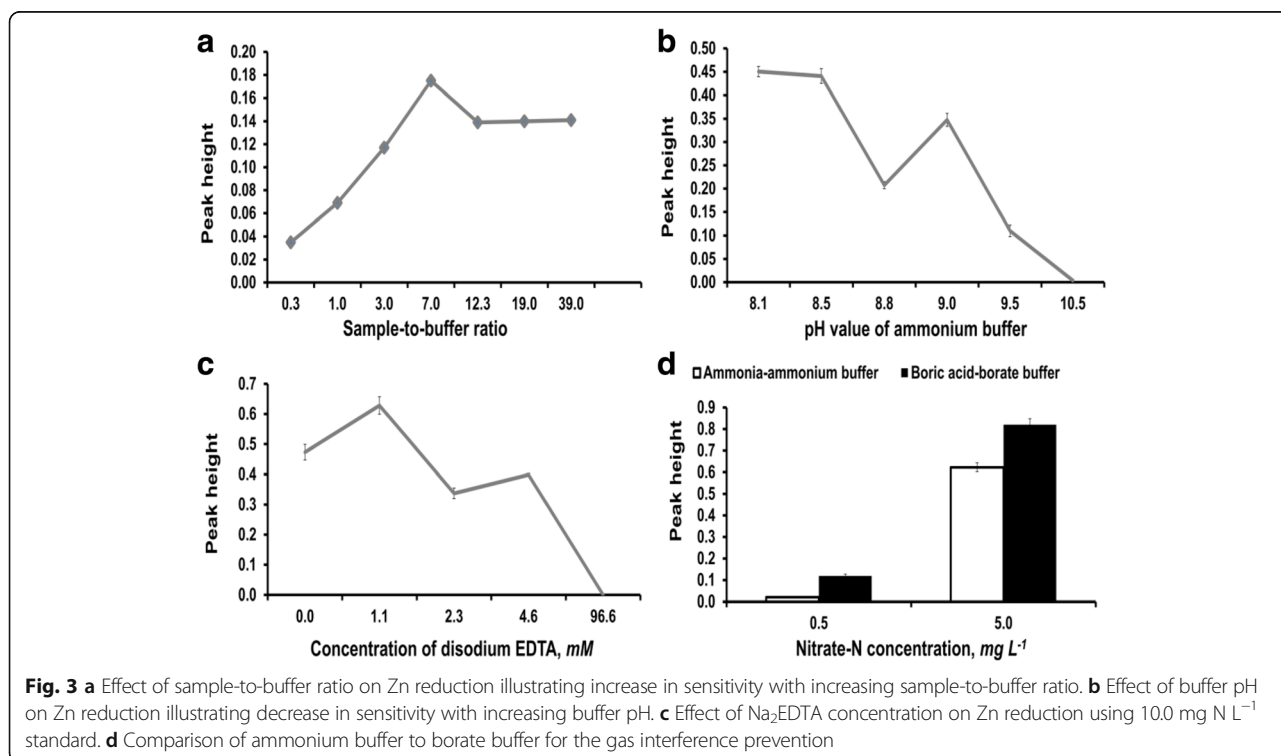
important because less mixing was derived from a sample zone flanked on one side by a reagent zone and flanked on the other side by a water carrier zone. The result indicated that the reaction requires a very small volume of reagent for optimum color intensity or else the sample will be unnecessarily diluted by the excessive reagent volume. Therefore, reagent/sample zone stack was adopted for this study.

The optimization of reagent volume at a constant sample volume (40 μL) showed that 10 μL was the optimum reagent volume as depicted by the peak maximum in Fig. 2a. From 3 to 7 μL in this figure, the peak height

was lower because the reagent volume was too small and the reagent was too diluted by the penetrating sample. Above 10 μL, specifically at 15 μL, the peak height was lower because the reagent volume was too high and the sample was too diluted. A reagent volume of 10 μL was therefore adopted for further analyses.

The variation of dispensed volume out of 10-μL reagent/40-μL sample lines showed that the optimum position was 35 μL, which was at the 70% position of the total stacked line (50 μL), as illustrated by the peak maximum in Fig. 2b. Unlike FIA, this position did not coincide with the supposed sample concentration maximum which was

Fig. 3 a Effect of sample-to-buffer ratio on Zn reduction illustrating increase in sensitivity with increasing sample-to-buffer ratio. **b** Effect of buffer pH on Zn reduction illustrating decrease in sensitivity with increasing buffer pH. **c** Effect of Na$_2$EDTA concentration on Zn reduction using 10.0 mg N L^{-1} standard. **d** Comparison of ammonium buffer to borate buffer for the gas interference prevention

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expected at the sample center (30 μL in this case). The gentle slope between 1 and 20 μL described the reagent line overlapping with the sample line, and the rising peak here was due to decreasing sample dispersion. Towards 50 μL, there was a sharp descent to zero because the sample was overlapped by a carrier water line and reagent was totally absent at this end. In further analyses, 35 μL was the volume adopted for dispensing to the flowcell.

Reduction chemical conditions

For Zn reduction, a sample-to-buffer ratio of 7:1 by volume was the optimum as illustrated by the peak maximum in Fig. 3a. At lower ratios, sample volume was too small and too diluted by buffer volume, and at higher ratios, samples were not sufficiently buffered as the reduction process is pH dependent; these reasons accounted for the low peaks of these ratios. A sample-to-buffer ratio of 4:1 was adopted for ZnR-SIA study so as to prevent low precision and accuracy observed at 7:1 for KCl and $K_2S_2O_7$ matrices.

The variation of buffer pH in search of the optimum for ZnR-SIA showed a decreasing pattern in peak height with increasing pH from 8.1 to 10.5 (Fig. 3b). Therefore, 8.1 was used in all analyses. It was likely that zinc reduced nitrate to N species other than nitrite at higher pH, and this accounted for the decreasing nitrite peak height with pH. Zn was reactive in ammonia-ammonium buffer and generated copious volumes of gas which increased with pH from 8.1 to 10.5. It was also likely that Zn dissolution to release hydrogen gas dominated the chemistry over nitrate reduction at high pH in a manner similar to Zn dissolution in alkalis to form tetrahydroxozincate ion (Lee, 2009).

In the absence of Na_2EDTA, peak height was gradually decreasing with the number of uses. In 11 analysis runs without Na_2EDTA in the conditioning buffer for the Zn column, the 11th run had only 19.1% of the first run peak height. Therefore, EDTA was necessary to protect the column. The concentration effect of Na_2EDTA on response was shown in Fig. 3c. Low concentration of Na_2EDTA, namely 1–4 mM, favored the Zn column life, but the very high concentration did not as illustrated by the fall to zero in the figure. It is likely that excessive EDTA ions adversely competed with NO_3^- for Zn surface because NO_3^- reduction to NO_2^- is a surface process or the EDTA inhibited either the diazotization or coupling or both in the Griess reaction. In further study, 1.1 mM Na_2EDTA was adopted.

The bar chart in Fig. 3d showed that boric acid-borate buffer was more efficient for Zn reduction of nitrate than ammonia-ammonium buffer with the taller peaks of the former. The boric acid-borate buffer gave 585 and 132% of the instrument response given by ammonia-ammonium buffer at 0.5 and 5.0 mg

NO_3-N L^{-1} respectively. The borate buffer also minimized greatly gas generation in the column making Zn micro-column adaptable to online use as Zn was more reactive in ammonium buffer. Borate buffer presumably suppressed hydrogen gas generation by Zn. Evolution of hydrogen from Devarda's alloy and granular Zn columns have been highlighted. The peculiar properties of boric acid-borate buffer almost entirely stopped gas evolution from the zinc column and prevented its interference, which would have been significant and disallowed the online use of zinc, because of the confined nature of sequential injection analysis-lab-on-valve manifold.

Colorimetric parameter conditions

The results of three-factor optimization of the Griess-Illosvay reaction variables are displayed in Fig. 4a. The simplex probed around a wide region comprising 22 experiments to reach the highest peak height at experiment 19. The conditions at experiment 19 were 0.12 M sulfanilamide, 2.48 M HCl, 3.04 mM NINE, and 56 s. These reagent concentrations were adopted for all sample analyses but with a delay time of 45 s. The multi-factor optimization data (Additional file 1) were further analyzed by linear regression with the instrument response as the dependent variable in order to determine the influence of the three univariate components.
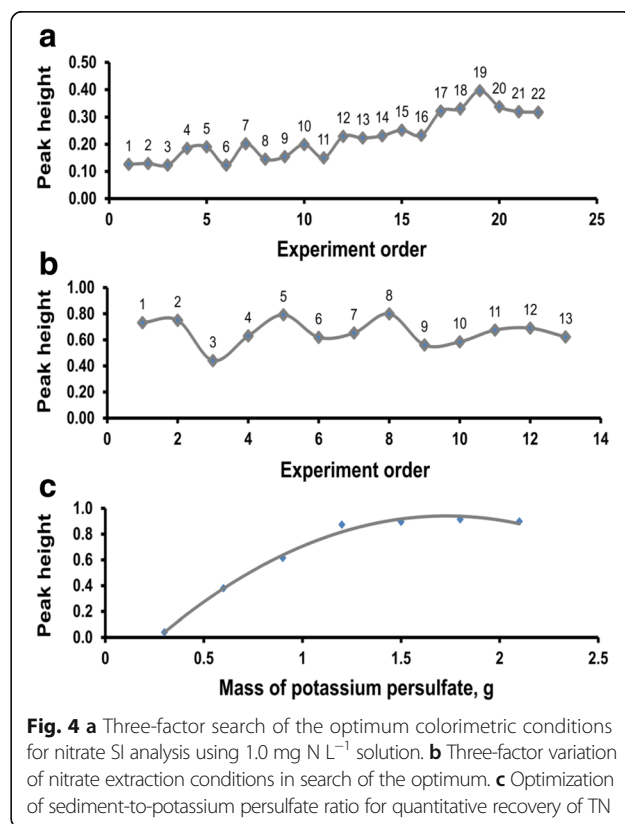

Fig. 4 a Three-factor search of the optimum colorimetric conditions for nitrate SI analysis using 1.0 mg N L^{-1} solution. **b** Three-factor variation of nitrate extraction conditions in search of the optimum. **c** Optimization of sediment-to-potassium persulfate ratio for quantitative recovery of TN

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The most important factor influencing the peak is the sulfanilamide concentration as shown by the significant variance in the peak height, which was accounted for by sulfanilamide concentration ($R^2 = 0.598$, F [1, 20] = 29.735, $p = 0.000$, Additional file 2). The next important factor affecting peak height is the NINE concentration owing to the significant variance in the response explained by NINE concentration ($R^2 = 0.147$, F [1, 18] = 11.930, $p < 0.05$). Delay time did not exert any significant influence on the instrument response ($R^2 = 0.033$, F [1, 19] = 1.687, $p > 0.05$). Sulfanilamide diazotizes nitrite in the first step of the reaction, and the availability of diazotized molecular ion determines the coupling reaction of NINE in the second step (azo dye formation), and because all diazotized molecules would not be available for coupling, the influence of the NINE concentration on Griess-Illosvay reaction is much less important than that of the sulfanilamide concentration.

The three investigated variables explained 77.8% of the variation in the azo dye (response) (model 3, Additional file 2), implying that some uninvestigated factors such as temperature might explain the remaining percentage. The optimization data—colorimetric conditions—obtained from the simplex algorithm supported the generalizability of the model because R^2 shrunk only slightly from 0.778 to 0.741 (the adjusted R^2). The coefficients (B values) for the sulfanilamide concentration and the NINE concentration in model 3 (Additional file 2) are significantly different from zero ($p = 0.001$ and $p = 0.003$ respectively) and this agrees with the F statistic, whereas B value for delay time is not different from zero ($p = 0.000$), suggesting that the delay time cannot predict or explain the outcome variable—the response.

However, if the analysis and discussion of the three univariate factors were based only on Pearson correlations (zero-order correlations) where the sulfanilamide concentration, time, and NINE concentration have the Pearson values 0.773, 0.764, and 0.429, respectively, there would be misleading conclusions such as time having a strong positive association (Pearson $r = 0.764$) with the instrument signal. In fact, model 3 indicates that delay time was negatively related to the signal (azo dye) in its B value (−0.005), though non-significant and due to chance by 14.1% (Additional file 2); this is supported by the part correlation (−0.171), the unique relationship, of time with the signal. Hence, the powerful usefulness of the linear regression was used.

Sediment nitrate extraction conditions
The results of three-factor optimization of nitrate extraction variables using 2 M KCl are pictured in Fig. 4b. In the figure, the highest maximum occurs at experiment 8 among the 13 experiments. The conditions at

experiment 8 were 19.1 min, 16.8 mL (1:16.8 sediment-to-solution), and 224 rpm. However, 20-min extraction time, 10-mL extractant volume (2.5:10 sediment-to-solution), and 220-rpm extraction speed were adopted for sample extraction. The multivariate optimization data (Additional file 1) were further analyzed by linear regression with the instrument signal as the dependent variable, and it was found that the single important factor influencing the peak height is extraction speed. It accounted for 0.302 of the variance in the instrument response (F [1, 11] = 4.771, $p = 0.051$, Additional file 2), and this was nearly significant, missing the significance criterion by 0.1% chance.

Other variables—volume of extractant and extraction time—were apparently not associated with the response (zero-order correlations −0.172 and 0.291, respectively, and their part correlations were nearly zero, Additional file 2) and lacked any predictive power on the response (F [1, 10] = 0.000 and F [1, 9] = 0.011, respectively, $p > 0.05$). However, the non-significant negative relation (−0.172) between extractant volume and peak height can be very important in some situations and is not likely due to the third-variable problem. This negative effect becomes important at low nitrate concentration of the sediment because only a small extractant volume will make the nitrate level detectable in this situation. At higher volumes, the nitrate level becomes undetectable except that sediment mass is increased relative to extractant volume. This necessitated the high 1:4 sediment-to-solution ratio adopted for further work. Literature of methods does not ascribe other factors except for those investigated in this study on nitrate extraction from soils, yet a large 69.7% of the variance in the nitrate quantitative extraction could not be explained by these factors. A reasonable property of nitrate which can account for the greater part of this unexplained variance is the ready solubility of all nitrate salts in water, thereby enhancing the extraction. Another uninvestigated variable, which can account for the variance, is the particle size of sediments, since nitrate extraction will increase with decreasing particle size. The contributions of the two foregoing factors were not captured in the regression model 3 (Additional file 2), hence the high magnitude of unexplained variation.

Sediment oxidation conditions for TN
In the optimization of sediment-to-potassium persulfate ratio to obtain an efficient mass of $K_2S_2O_8$ that would give quantitative oxidation/hydrolysis of organic N to nitrate, the sediment color faded as the $K_2S_2O_8$ mass used for sediment oxidation was increased from 0.3 g to 2.1 g and a colorless sediment

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Table 1 The recoveries for N species using spiked sediments and reagent water

Recovery studies of NO_3^--N		Recovery studies of TN			
		Matrix sample			
Spike level, mg N kg^{-1}	Found concentration plus 3.6 mg N kg^{-1} in original sediment	Reference compound	Spike level, g N kg^{-1}	Found concentration plus 1.54 g N kg^{-1} in original sediment	Recovery, %
2.0	5.4	Glycine	0.400	1.98	109.7
2.0	5.4	ADP	0.400	1.87	82.4
2.0	5.6	KNO$_3$	0.250	1.76	87.4
2.0	5.5	Average recovery			93.2
Average recovery, %	94.7				
Precision, %	4.52	Fortified blank			
		Reference compound	Spike level, g N L^{-1}	Found concentration, g N L^{-1}	Recovery, %
		NH$_4$Cl	0.400	0.380	94.9
		ADP	0.400	0.394	98.4
		KNO$_3$	0.250	0.251	100.4
		Average recovery			97.9

was obtained at the sixth and seventh experiments comprising 0.5-g sediment plus 1.8-g $K_2S_2O_8$ and 0.5-g sediment plus 2.1-g $K_2S_2O_8$ respectively. In further sample oxidation for TN determination, a sediment-to-potassium persulfate ratio of 1:3.6 (that is 1.0 g sediment/3.6 g $K_2S_2O_8$) was adopted. Besides visual inspection, after each oxidation experiment, the TN absorbance of the solution was determined, and the results are displayed in Fig. 4c. The maximum of this figure is broad and occurs at approximately 0.9 (peak), and this value corresponds to 1.5 to 2.1 g $K_2S_2O_8$. Therefore, quantitative digestion of TN was obtained from 1.5 to 2.1 g $K_2S_2O_8$ used in experiments 5 to 7, and this was closely in agreement with the visual inspection.

Validation of methods
The ZnR-SIA had good accuracies for all the matrices studied as shown by the following recoveries. The average recovery of nitrate-N in matrix samples was 94.7% (Table 1), and its precision was 4.52%. The average recoveries of total N in blanks and matrix samples were 97.9 and 93.2% respectively (Table 1). The high recoveries of TN from glycine, ADP, and NH$_4$Cl indicate the quantitative oxidation/hydrolysis of organic and reduced inorganic N by the sediment-to-persulfate ratio of 1:3.6 used. The method detection limits for the three matrices studied were 24.6 μg N L^{-1} for reagent water, 0.383 mg N kg^{-1} for KCl-extractable nitrate, and 3.18 mg N kg^{-1} for TN following alkaline persulfate digestion. In reagent water, Zn-SIA had a linear calibration range of 0.06–6.00 mg N L^{-1}. In KCl solution, the matrix effect was not important as

the upper calibration limit for sediment nitrate was 24.0 mg N kg^{-1} (equal to 6.00 mg L^{-1} × solution-to-sediment ratio). The upper limit for sediment TN in $K_2S_2O_8$ matrix was 200 mg N kg^{-1}.

The Zn column could not be stored owing to rapid daily-scale diminishing in efficiency but could be used continuously for 24 h with sustained reduction. A white precipitate, possibly Zn (OH)$_2$, was formed on storage. The coating of the Zn surface by this precipitate reduced nitrate electrolytic reduction since the reduction was a surface process (Table 1).

Conclusions
The novel application of boric acid-borate buffer in the zinc reduction-sequential injection analysis virtually stopped gas evolution from the zinc micro-column and prevented gas interference, which would have been significant and disallowed the online use of zinc. This study demonstrates that zinc reduction can accurately determine nitrogen species in estuarine sediments. Zinc is less monitored in human foods, potable water, and surface waters because it is a micronutrient although it could be toxic at high levels. Therefore, zinc reduction is a green chemical process in contrast to the cadmium reduction owing to cadmium toxicity and likely to attract wide acceptability where sustainable development is desired. The automation of the technique coupled with its high turnover of 65 h^{-1} demonstrated its suitability for routine laboratory determination of nitrate in water and sediment.

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Additional files

Additional file 1: Data obtained from optimization experiments and employed for regression analyses. (XLSX 10 kb)

Additional file 2: Results of regression analyses and the individual variances explained by the investigated conditions. (DOCX 15 kb)

Abbreviations
ADP: Adenosine 5′-diphosphate disodium; CdR-SIA: Cadmium reduction-sequential injection analysis; FIA: Flow injection analysis; LOV: Lab-on-valve; N: Nitrogen; NINE: *N*-(1-naphthyl) ethylenediamine dihydrochloride; SIA: Sequential injection analysis; TN: Total nitrogen; ZnR-SIA: Zinc reduction-sequential injection analysis

Authors' contributions
ONO carried out the optimization experiments, interpreted the results, and wrote the manuscript. AAA collected the samples and contributed knowledge to the method development; ZX assisted in making the micro-column and procuring the materials. OKO, ABI, and DA supervised the optimization experiments and reviewed the manuscript. All authors agreed to the publication of the study. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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