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A colorimetric method for use within portable test kits for nitrate determination in various water matrices†

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A method using zinc powder in conjunction with the common Griess assay was developed for the detection of nitrate in water. This method is applicable to portable water test kits and allows for the accurate determination of nitrate in freshwater. The linear range for the method was shown to be 0.5–45 mg L⁻¹ NO₃⁻ and the limit of detection (LOD) was 0.5 mg L⁻¹ NO₃⁻. The proposed method was validated over a five-day period and acceptable recovery and uncertainties were achieved when analysing freshwater matrices. The performance of the developed method was compared to an ISO-accredited ion chromatographic (IC) method by carrying out blind sample analysis. A good agreement between the two methods was achieved as comparable concentrations were determined using each method. In addition, the Zn method was compared to the performance of a novel solid-phase reagent method, previously developed within the group. The most accurate performance was demonstrated by the Zn powder method when analysing freshwater samples. The novel solid-phase reagent method demonstrated the greater accuracy when analysing seawater samples.

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Introduction

Nitrate concentrations vary widely within natural and waste water samples. Nitrate concentrations can range from below 0.20 mg L⁻¹ NO₃⁻ in deep seawater to 85 mg L⁻¹ NO₃⁻ in shallow groundwater and surface streams depending on soil type and land use practices.¹ Natural sources of nitrate in the environment include gaseous nitrogen fixation through micro-organisms such as *Azotobacter* and cyanobacteria, soil degradation and the deposition of animal and plant residues. Although nitrate is found within a multiplicity of natural processes, contamination in water systems is most typically associated with anthropogenic activities.

Anthropogenic sources of nitrate include fertilisation of agricultural crops using chemical nitrogenous fertilisers, plant and animal waste, municipal and industrial wastewater discharges, sewage disposal systems, and the food industry.² In addition, atmospheric deposition of nitrogen-containing compounds also plays a role in contributing to nitrate contamination within water systems.³

Elevated concentrations of nitrate in water systems pose a significant risk to both the environment and to human health when considering the utilisation of water for drinking purposes. High nitrate levels in water systems contribute significantly to eutrophication, especially within lakes and saline waters.⁴ Eutrophication leads to the overproduction of aquatic plants and algae which in turn results in dissolved oxygen depletion; odorous waters and the stimulation of bacteria proliferation as algae and macrophytes die.⁵ When freshwater is used for drinking, nitrate contamination can negatively impact human health. The most important health effect associated with nitrate ingestion arises through the reduction of nitrate to nitrite in the digestive system. Nitrite oxidizes iron in the haemoglobin of red blood cells forming a molecule called methemoglobin. This molecule hinders oxygen transport and can result in a condition called methemoglobinemia or 'blue baby syndrome'.⁶ Nitrate which has been reduced to nitrite has also been shown to react with nitrosatable compounds in the human stomach to form carcinogenic *N*-nitroso compounds.⁷

Under EU regulation, nitrate concentrations within all fresh and marine water bodies must be monitored. According to legislation, nitrate levels must not exceed 50 mg L⁻¹ NO₃⁻ in surface waters.⁸ Many analytical methods are available for the determination of nitrate in water matrices. Suppressed ion chromatography (IC) is regarded as the standard for the analysis of nitrate in water, and is in fact the proposed method by the environmental protection agency.⁹ However, sample matrix complexity, in particular high salinity waters, can have

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a significant impact on IC performance. In addition, samples must be transported to a lab to be analysed using IC. Due to good limits of detection and simple assay-type protocols, colorimetric methods are an effective alternative to IC and are readily employed for nitrate determination.

When considering colorimetric detection of NO_3^- , the simplest and most frequently applied assay involves the reduction of nitrate to nitrite and its subsequent detection using the Griess reaction.¹⁰ A range of methods can be used to reduce NO_3^- to NO_2^- . Enzymatic reduction using nitrate reductase or photochemical reduction through the use of UV light can be used, however these methods typically offer poor reproducibility.¹² Most commonly copperised cadmium is used for nitrate reduction to nitrite and reduction efficiencies of over 90% are possible.¹² Despite this fact, the use of cadmium may be seen as undesirable due to its highly toxic nature. Therefore, the use of a less toxic reductant is desirable.

Zinc represents an example of a less toxic solid state reductant which can be used to reduce nitrate to nitrite. Metallic zinc has previously been used in other studies as a reducing agent, but is rarely used in favour of cadmium due to lower reduction efficiencies.¹³ Merino (2009) successfully employed zinc reduction for nitrate determination in foodstuffs and water, achieving an analytical range of 0–1.62 mg L⁻¹ NO_3^- .¹⁴ Ellis *et al.* (2011) then successfully developed a simple spectrophotometric flow analysis method using granular Zn for NO_3^- determination in water. This flow analysis method demonstrated an analytical range of 0.01–3.1 mg L⁻¹ NO_3^- .¹⁵ In addition, other successful nitrate colorimetric determination methods which move away from the use of cadmium have also recently been developed. However, these methods employ harsh corrosive reagents and are not well suited for use in test kits.^{16,17} Despite these recent developments, the cadmium reduction method is still the leading nitrate determination methodology.

Within this study, an optimised zinc reduction method in combination with the Griess assay for water analysis is validated and assessed. Through blind sample analysis, the performance of the developed method is compared to that of an accredited IC according to ISO/IEC 17025:2005. For the blind sample analysis, a range of freshwater samples were analysed along with a selection of various effluents. The performance of the Zn powder method was also compared to that of a solid-based colorimetric nitrate determination method. The solid-based method was a novel non-toxic solid phase colorimetric method, developed by Nesterenko *et al.* (2016), which uses azo and diazo components, solid organic acid acidifier, catalyst, masking agent and zinc as the reducing agent.¹¹ The performance of the two methods was assessed following blind sample analysis of various water matrices.

Experimental

Materials and reagents

All chemicals used within this work were of analytical grade purity. Sulphanilamide, hydrochloric acid (37%) and *N*-(1-

naphthyl)-ethylenediamine dihydrochloride (NED) used to prepare Griess reagent were purchased from Sigma-Aldrich (Gillingham, UK). Zinc (99.99%) in powder form with a particle size of 150 μm was purchased from VWR International. Chromotropic acid, *p*-nitroaniline, potassium chloride, malonic acid, potassium bromide and EDTA disodium salt were all purchased from Sigma-Aldrich. All solutions and dilutions were prepared using high-purity deionised water (18 M Ω cm).

TelLab's certified 1000 mg L⁻¹ nitrate standard and 100 mg L⁻¹ nitrite standard were used as the stock solutions, from which working nitrate and nitrite standard solutions were prepared *via* serial dilutions. A range of environmental samples were provided by the Environmental Department within TelLab. Six drinking water samples from various customer wells, effluent samples from a pump manufacturing facility, a water treatment facility and a hospital, a river water sample and seawater samples from Wexford harbour were investigated.

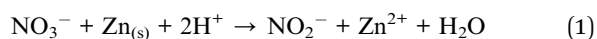
Instrumentation

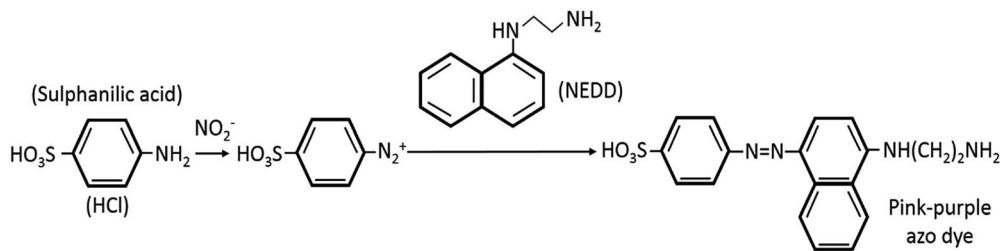
Spectroscopic measurements were performed on a Unicam UV500 spectrophotometer using a 1 cm path length Hellma far-UV quartz cuvette applicable to the range 200–2500 nm. The wavelength range of the spectrophotometer was 190–1100 nm. The bandwidth was 1.5 nm, and an integration time of 2 s was used. IC determination of nitrate was carried out using an isocratic Dionex DX-120 Ion Chromatograph (Dionex, Sunnyvale, USA), equipped with autosampler and a Dionex AERS 500 anion self-regenerating suppressor for suppressed conductivity detection.

Methods

Proposed nitrate determination method using Zn reduction.

The Griess reagent which was used was prepared as described by Robledo *et al.* (2014).¹⁰ Sulphanilamide reagent was prepared by adding 10 mL of concentrated hydrochloric acid (37%) to 60 mL of deionised water and 1.0 g of sulphanilamide was then added to the solution. This solution was then diluted to 100 mL with deionised water. The NED reagent was prepared by dissolving 0.1 g of NED in 100 mL of deionised water. Both reagents were then mixed in equal proportions to produce Griess reagent. To a 10 mL volume of sample solution, 1 mL of Griess reagent was added. A 25 mg quantity of Zn powder, particle size 150 μm , was then added. The sample container was shaken 20 times in an up down motion and the solution was allowed to stand for 10 min. Following this, a 2 mL portion of this solution was immediately transferred into a cuvette and analysed spectrophotometrically using UV-Vis at a wavelength of 540 nm. The reaction mechanisms for the reduction of nitrate to nitrite using zinc, and the subsequent detection of nitrite employing the Griess assay are highlighted in eqn (1) and (2) respectively.





Ion-chromatographic analysis. The IC system which was used was accredited by the Irish National Accreditation Board (INAB) according to ISO 17025:2005. Prior to analysis all samples were filtered using a $0.2\ \mu\text{m}$ pore size membrane filter to remove debris. An eluent comprised of $3.5\ \text{mM}\ \text{Na}_2\text{CO}_3/1.0\ \text{mM}\ \text{NaHCO}_3$ solution was used at a flowrate of $1.2\ \text{mL min}^{-1}$. An IonPac AS14 ($250 \times 4\ \text{mm I.D.}$) anion exchange column along with an AERS 500 anionic suppressor (Dionex, Sunnyvale, USA) was used.

Solid-phase test reagent. The solid-phase reagent was comprised of *p*-nitroaniline (2 mass%), chromotropic acid (2 mass%), potassium chloride (4 mass%), potassium bromide (12 mass%), EDTA disodium salt (0.4 mass%) and malonic acid (79.6 mass%). To this 1.5% Zn powder was added relative to the amount of solid reagent. For the determination of nitrate, a 5 mL volume of sample was added to a $100 \pm 1\ \text{mg}$ portion of the powdered reagent and allowed to react. When a sample containing nitrate was added to the powder, the reagent powder dissolved within 10–15 s and colour formation began within 1 min. At low nitrate concentrations ($0.5\ \text{mg L}^{-1}$), the colour change which was observed was from pale hay-yellow to ochroid-yellow. At higher concentrations of nitrate (up to $100\ \text{mg L}^{-1}$), the colour transitioned to a ripe cherry red. The intermediate hues which were observed were orange-yellow, orange, orange-red and red. A wavelength of $515\ \text{nm}$ was determined to be the λ_{max} and was used to determine sample nitrate concentrations.

reagent, $10\ \text{mL}$ of $10\ \text{mg L}^{-1}\ \text{NO}_3^-$ standard and $25\ \text{mg}$ of Zn powder was shaken a specific number of times (1–60 times) in an up-down motion at a rate of 1 shake per second. Each solution was allowed to stand for 5 min and the absorbance was measured. As illustrated in Fig. 1B, by shaking the solution 20 times the greatest reduction efficiency is achieved as an absorbance of 0.699 was obtained. The decrease in absorbance resulted due to the fact, the more the sample is shaken, the greater the contact time between the reductant and the sample. Thus, an over reduction of nitrogen to lower oxidation states most likely occurs.

Kinetic studies were then carried out. In order to establish whether or not different kinetic profiles are observed for different nitrate concentrations, both a $5\ \text{mg L}^{-1}\ \text{NO}_3^-$ solution and a $45\ \text{mg L}^{-1}\ \text{NO}_3^-$ solution were assessed. As demonstrated in Fig. 1C, the absorbance readings increased for both solutions until 5 min and then declined. Although the highest absorbance was observed at 5 min, a considerable standard deviation was also present. As the lowest standard deviation was observed at 10 min, a standing time of 10 min was selected as optimal. According to Nollet *et al.* (2013), reduction using metallic zinc requires a strict control of standing time in order to avoid reduction of nitrogen to lower oxidation states.¹² The results obtained in this experiment are in agreement with this statement as absorbance readings fluctuate with varying standing times.

Results and discussion

Determination of optimum working conditions for the Zn powder method

In order to determine the optimum quantity of Zn powder to be used, quantities of Zn powder ranging from 10–250 mg were added to a sample container containing $10\ \text{mL}$ of $10\ \text{mg L}^{-1}\ \text{NO}_3^-$ standard solution and $1\ \text{mL}$ Griess reagent. Each sample container was shaken 10 times and allowed to stand for 5 min. The average absorbance ($n = 3$) for each quantity was calculated and plotted against the quantity of Zn powder added as shown in Fig. 1A. The highest absorbance was routinely observed when $25\ \text{mg}$ of Zn powder was added. Thus, the quantity of $25\ \text{mg}$ of Zn was determined to be optimal. The decrease in absorbance readings when higher quantities of Zn is used is likely attributed to the over reduction of nitrogen to lower oxidation states such as ammonia. Furthermore, when higher quantities of Zn powder are added, this leads to an increased turbidity and consequently a drop in absorbance values.

The effect of mixing on NO_3^- reduction using Zn powder was then established. A sample container containing $1\ \text{mL}$ of Griess

Method validation

Calibration curves. Firstly, a calibration curve for nitrite (Fig. 2A) was generated by analysing fresh nitrite standards using the standard Griess assay ($n = 5$). The linear range was determined to be between 0.025 – $4.0\ \text{mg L}^{-1}\ \text{NO}_2^-$. Following this, nitrate standards were analysed using the optimised Zn powder method and a nitrate calibration curve was generated ($n = 5$). This calibration curve is shown in Fig. 2B. The linear range was 0.5 – $45\ \text{mg L}^{-1}\ \text{NO}_3^-$. Due to the low standard deviation which was observed, error bars are not clearly visible despite being present on both graphs. The upper detection limit of the method was determined to be $45\ \text{mg L}^{-1}\ \text{NO}_3^-$. At concentrations above this point, the detectable difference in colour and absorbance values decreased.

Sample analysis. All water samples were analysed in triplicate over a five day validation period (*i.e.* $n = 15$). The four sample matrices were firstly analysed using Griess reagent to establish whether or not nitrite was present. An average absorbance of 0.058 AU was observed for the seawater sample, which correlated to a nitrite concentration of $0.0895\ \text{mg L}^{-1}\ \text{NO}_2^-$.

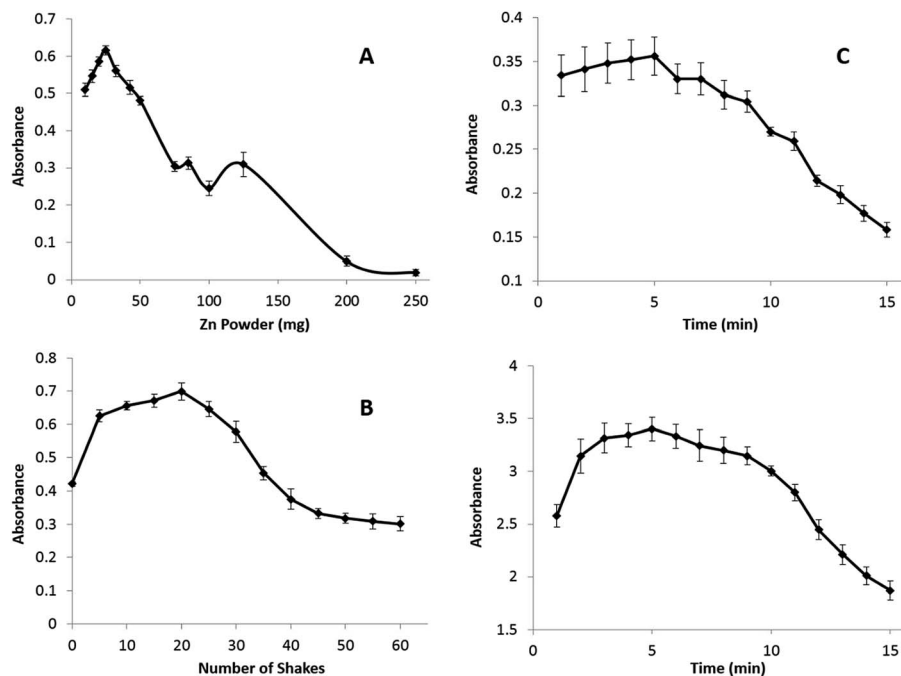


Fig. 1 (A) Quantity of Zn powder used and resulting absorbance values; (B) effect of mixing on reduction of nitrate; (C) kinetic study for NO_3^- standard solutions, 5 mg L^{-1} (top figure) and 45 mg L^{-1} (bottom figure), using Zn powder to reduce nitrate to nitrite ($n = 3$).

While no absorbance was obtained for the other matrices meaning no nitrite was present. The nitrite content (expressed as 0.027 $\text{mg L}^{-1} \text{NO}_2^- \text{--N}$) present within the seawater sample was subtracted from the concentration ($\text{mg L}^{-1} \text{NO}_3^- \text{--N}$) obtained when analysing the seawater for nitrate. Using the equation of the line from the calibration curve shown in Fig. 2B, the concentrations of nitrate in the sample solutions were determined based on the average absorbance obtained. The actual nitrate concentrations present in each of the samples determined using IC, the average nitrate concentrations determined using the Zn powder method, the standard deviation and the RSD values are shown in Table 1. The actual nitrate concentrations present in the seawater samples were determined using standard Hach spectrophotometric methods. The low range cadmium reduction method (method 8192) was

used for the unspiked sample and the high range cadmium reduction method (method 8171) was used for the spiked sample.

From the information obtained from the analysis of the spiked and unspiked samples over the five day period, the recovery, uncertainty and limit of detection of the method were calculated. The calculations were carried out using equations recommended by the Water Research Centre's (WRC) guide to analytical quality control for water analysis, document CEN/TC 230 N 180 section 8, and 'standard methods for the examination of water and wastewater'.¹⁸

Recovery. Recoveries were calculated for each of the sample matrices and are represented as a percentage. Recoveries were calculated using equations as recommended by Eaton *et al.* (2005).¹⁸

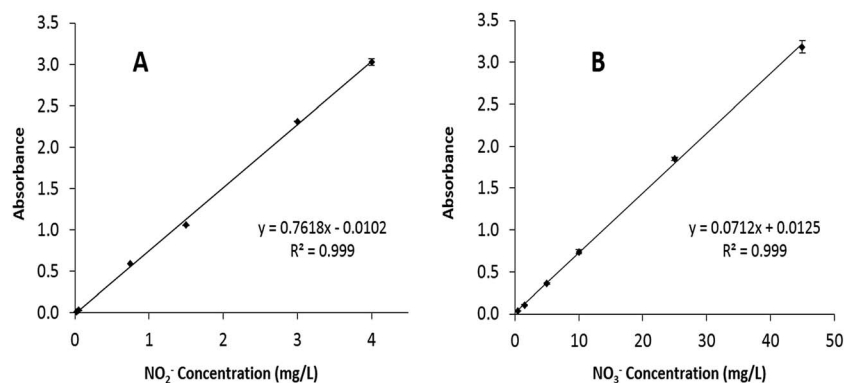


Fig. 2 (A) Nitrite calibration curve using Griess assay; (B) nitrate calibration curve using Zn powder method ($n = 5$).

Table 1 Actual nitrate concentrations present in samples and concentrations determined within samples which were analysed in triplicate on separate days over a five day period

Sample	Actual NO ₃ ⁻ conc. (mg L ⁻¹)	Average NO ₃ ⁻ conc. (mg L ⁻¹) determined using Zn powder method	Standard deviation (n = 15)	RSD (% , n = 15)
QC20	20.40	21.26	1.05	4.93
LOD 0.5	0.51	0.33	0.08	24.24
LOD 1	1.02	0.76	0.08	10.52
Potable water unspiked	14.23	15.68	0.42	2.68
Potable spiked	24.15	24.02	0.76	3.16
Effluent unspiked	18.07	18.47	0.40	2.16
Effluent spiked	28.05	28.49	0.78	2.74
Stream water unspiked	25.02	26.89	0.85	3.16
Stream water spiked	35.03	36.26	1.85	5.10
Sea water unspiked ^a	0.12	<0.5	—	—
Sea water spiked ^b	10.04	6.41	0.57	8.89

^a Hach LR cadmium reduction method (method 8192) used to determine NO₃⁻ concentration. ^b Hach HR cadmium reduction method (method 8171) used to determine NO₃⁻ concentration.

Recovery = spiked sample conc. – ((unspiked sample conc./250 mL) × 247.5 mL)

$$\text{Recovery}\% = \text{recovery}/10 \text{ mg L}^{-1} \times 100$$

The nitrate concentration determined within the unspiked samples were divided by 250 mL, then multiplied by 247.5 mL to account for dilution. This was due to the fact that the spiked samples were prepared by pipetting 2.5 mL of 1000 mg L⁻¹ NO₃⁻ standard into a 250 mL volumetric flask which was filled to the mark with the specific sample matrix. The calculated recovery was then divided by 10 mg L⁻¹, as 10 mg L⁻¹ was the concentration of the spike. By multiplying this value by 100, recovery as a percentage is obtained. From the calculated recoveries, average recoveries were determined and these recoveries as percentages are illustrated in Table 2 below.

According to the WRC, a recovery value observed within the range 85–115% is seen as a suitable recovery in terms of validation.¹⁸ The average recovery obtained for drinking, effluent and surface water matrices were within this accreditation range and thus demonstrated suitable accuracy. However, the recovery observed for the seawater matrix was 64.09% and was therefore outside of the desired range. Based on this result, the developed Zn powder method would not be applicable for nitrate determination within seawater. This observation is in agreement with an interference study which was recently carried out by

Jayawardane *et al.* (2014) when analysing for nitrate using zinc in combination with the Griess assay. Following the assessment of a wide range of potential interfering ions, it was shown that Na⁺ had the greatest interfering effect. When analysing nitrate in solutions containing 1150 mg L⁻¹ Na⁺ and above, a percentage recovery of 58.9 ± 10.4% was observed.²² Ellis *et al.* (2011) overcame these interferences through the use of granular zinc and citrate buffer, as ions such as Na⁺, Mg²⁺, Ca²⁺, Fe²⁺ and Cu²⁺ had no significant interfering effect on nitrate determination.¹⁵ However, this method only achieved an analytical range up to 3.1 mg L⁻¹ NO₃⁻, well below the nitrate levels which are readily observed in freshwater samples.

Limit of detection. Two samples were analysed to determine the limit of detection of the Zn powder method. LOD 1 was a 0.5 mg L⁻¹ NO₃⁻ standard and LOD 2 was a 1 mg L⁻¹ NO₃⁻

Table 2 Recoveries observed for each sample matrix and associated standard deviation for nitrate analysis using Zn powder method

Sample	Average recovery	Standard deviation (% , n = 15)
Drinking water	85.06%	0.507
Effluent water	102.06%	0.508
Surface water	96.34%	1.453
Sea water	64.09%	0.523

Table 3 Concentrations and standard deviation observed for the samples LOD 1 and LOD 2

Day	LOD 1 (mg L ⁻¹ NO ₃ ⁻)	LOD 2 (mg L ⁻¹ NO ₃ ⁻)
1	0.33	0.68
	0.27	0.78
	0.24	0.71
2	0.22	0.63
	0.23	0.60
	0.25	0.70
3	0.45	0.78
	0.44	0.80
	0.38	0.80
4	0.32	0.76
	0.30	0.81
	0.30	0.77
5	0.39	0.84
	0.40	0.85
	0.42	0.91
Average concentration	0.33	0.76
Standard deviation	0.08	0.08

Table 4 Calculated uncertainties associated with the analysis of each sample matrix ($n = 15$)

Sample	% uncertainty	Expanded uncertainty
Drinking water	3.234	$\pm 0.647 \text{ mg L}^{-1}$
Effluent water	0.983	$\pm 0.197 \text{ mg L}^{-1}$
Surface water	2.597	$\pm 0.519 \text{ mg L}^{-1}$
Sea water	7.353	$\pm 1.471 \text{ mg L}^{-1}$

standard. The concentrations determined over the five day validation period and standard deviations for the two LOD samples are shown in Table 3 below.

According to Eaton *et al.* (2005), the average concentration obtained for the NO_3^- standard must be greater than 4 times the standard deviation of the pooled results in order for a concentration to be accepted as the limit of detection.¹⁸ In this case, results indicated that $0.5 \text{ mg L}^{-1} \text{NO}_3^-$ was an acceptable limit of detection for accreditation, as $0.33 \text{ mg L}^{-1} \text{NO}_3^-$ was >4 times the standard deviation of the pooled results.

Uncertainty. The uncertainties associated with each sample matrix were calculated using equations as recommended by Eaton *et al.* (2005).¹⁸

$$\% \text{ uncertainty} = (2(P_R)^2 + 2(W_R)^2)^{1/2}$$

$$\text{where: } P_R(\text{precision}) = \sqrt{\text{sum of square difference}/(n-1)},$$

$$W_R(\text{bias}) = \sqrt{\text{sum of square difference}/n},$$

$$\text{Difference} = \text{recovery} - 10 \text{ (as spike was } 10 \text{ mg L}^{-1} \text{NO}_3^-)$$

The expanded uncertainties associated with each matrix were also calculated. The expanded uncertainty (U) for each matrix was calculated using the following equation;

$$U = (k)(u)$$

where k was the coverage factor and u was standard uncertainty. In this case a coverage factor of 2 was used, defining an interval with a confidence level of approximately 95%.

The % uncertainty and expanded uncertainties determined for each sample matrix are illustrated in Table 4. Each sample was analysed in triplicate over the five day validation period. Uncertainty was lowest for the effluent water matrix. The effluent sample was taken from a pump manufacturing plant following treatment of the raw effluent in the complexes water treatment facility. This treatment of the effluent most likely enabled the low uncertainty value to be achieved. The uncertainty determined for the drinking and surface water matrices were also at low levels. The highest uncertainty was observed for the seawater matrix, as an expanded uncertainty of $\pm 1.471 \text{ mg L}^{-1} \text{NO}_3^-$ was obtained. This again highlights the image that this Zn powder method is not applicable for seawater analysis.

Comparison of Zn powder method against accredited IC analysis

Blind sample analysis was carried out for this comparison study. The samples were analysed using the Zn powder method and an accredited IC method. Samples were analysed for nitrite first by adding Griess reagent only to the sample solution. The nitrite concentration was determined using the calibration curve in Fig. 2A. The samples were then analysed for nitrate using the optimised Zn powder method. The nitrite concentration ($\text{mg L}^{-1} \text{NO}_2^- - \text{N}$) present in the sample was subtracted from the nitrate concentration ($\text{mg L}^{-1} \text{NO}_3^- - \text{N}$) determined using the Zn powder method. The nitrate concentrations determined within each sample using the Zn powder method in comparison to the nitrate concentrations determined using an accredited IC are shown in Table 5 below.

On evaluation of the NO_3^- concentrations determined for each sample using IC and the developed Zn method, it is evident that the NO_3^- concentrations determined are comparable. The largest percentage difference was observed for the effluent sample as a difference of 15.63% was obtained. This comparability highlights the effectiveness of the Zn powder method in terms of NO_3^- determination in various water matrices.

Table 5 Nitrate concentrations observed following nitrate analysis using Zn powder method and a comparison against an accredited IC system ($n = 5$)

Sample	Characteristics	Zn powder method ($\text{mg L}^{-1} \text{NO}_3^-$)	Accredited IC ($\text{mg L}^{-1} \text{NO}_3^-$)	Percentage difference (%)
A	Potable water	37.06	37.51	1.20
B	Stream water	19.75	19.16	3.08
C	Effluent	13.54	11.71	15.63
D	Potable water	51.51 ^a	50.93	1.14
E	Effluent	46.52 ^a	49.45	5.93
H	Blank	<0.5	0.49	—
I	10 mg L^{-1} standard	10.07	9.27	8.63

^a Concentrations which were above the upper limit of the Zn powder method.

Table 6 Nitrate concentrations of blind samples determined using IC, the developed Zn method and a novel solid-phased reagent method ($n = 5$)

Sample reference	Sample characteristics	IC ($\text{mg L}^{-1} \text{NO}_3^-$)	Zn powder method ($\text{mg L}^{-1} \text{NO}_3^-$)	Solid-phased test reagent ($\text{mg L}^{-1} \text{NO}_3^-$)
A	Drinking water	37.87	38.51 ± 0.15	38.99 ± 0.09
B	Borehole	3.95	3.44 ± 0.21	1.76 ± 1.41
C	Standard ($2 \text{ mg L}^{-1} \text{NO}_3^-$)	2.17	1.95 ± 0.07	4.59 ± 0.09
D	Process water	11.82	12.63 ± 0.11	14.43 ± 0.51
E	Standard ($4 \text{ mg L}^{-1} \text{NO}_3^-$)	3.89	3.12 ± 0.31	6.26 ± 0.18
F	Borehole	0.55	<0.5	3.48 ± 0.16
G	Seawater ^a ($10 \text{ mg L}^{-1} \text{NO}_3^-$)	9.79	6.35 ± 1.39	9.5 ± 1.05

^a Hach HR cadmium reduction method (method 8171) used to determine NO_3^- concentration in seawater sample.

Comparison against alternative colorimetric method

Blind sample analysis was again carried out using a different range of water samples containing nitrate. Samples were analysed using IC, the developed Zn powder method and a novel solid-based reagent method. The nitrate concentrations obtained using each methodology are illustrated in Table 6. Again, a good correlation is observed between the developed Zn powder method and the accredited IC when analysing freshwater samples. The solid-phased method exhibited good accuracy when analysing samples containing higher NO_3^- concentrations, but in general was less accurate compared to the Zn powder method. The solid-phased method demonstrated accurate analysis of the seawater sample, whereas the Zn powder method demonstrated poor accuracy when analysing the seawater matrix.

Conclusions

A nitrate determination method for use within portable, field water test kits based on nitrate reduction using zinc and the Griess assay was developed. When using Zn as a reducing agent for nitrate it was shown that Zn quantity, the extent of mixing and standing time had a significant effect on nitrate reduction efficiency. Following validation and investigation of performance characteristics it was shown that the developed method is capable of determining nitrate in various water matrices including drinking, river, effluent and groundwater. However, the method was not applicable to marine water. When compared to a novel non-toxic solid-based reagent method the Zn powder method demonstrated greater accuracy when analysing freshwater. The solid-based method achieved greater accuracy when analysing seawater.

The developed Zn method allows for ease of use at fast analysis times and the method is now being employed within TelLab's hydromonitrix water test kits for NO_3^- analysis.¹⁹ In comparison to leading freshwater test kits,^{20,21} the hydromonitrix NO_3^- test kit offers the potential for greater analytical accuracy than test strip kits and is less toxic than cadmium based nitrate test kits. In addition, the Zn method also offers the potential to be coupled with a portable spectrophotometer, providing the opportunity to achieve higher sensitivity and reproducibility in comparison to using visual test kits.²³

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