

An improved technique for the determination of oxidised nitrogen in natural waters with a sequential injection analysis (SIA) system

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Abstract

An SIA system is proposed for the determination of oxidised nitrogen (nitrate + nitrite as N) in natural waters. A cadmium reductor, made of cadmium granules closely packed in a glass column reduces the nitrate to nitrite. The reduced nitrate and the nitrite present in the water samples is diazotised in the SIA system with sulphanilamide and coupled with N – (1-naphthyl) ethylene diammoniumdichloride to form a highly coloured azo dye which is detected at 540 nm with a UV/Vis spectrophotometer. The proposed system is fully computerised and is able to monitor total oxidised nitrogen as nitrite at a frequency of 36 samples per hour with a standard deviation of < 1.2%. The calibration curve is linear up to 5 mg/ℓ with a detection limit of 0.01 mg/ℓ.

Introduction

The oxidised nitrogen content (nitrate + nitrite) of lakes, rivers and streams usually arises from groundwater, sewage effluents or drainage and leaching from agricultural land (Foster et al., 1986). The determination of total oxidised nitrogen is a subject of interest in the routine laboratory analysis of potentially polluted waters.

Knowledge of nitrate concentrations in waters is important for many reasons; for example, their importance in eutrophication (Lund, 1972; Gauntlet, 1980) and their responsibility in methaemoglobinaemia of sucklings (Goodman, 1980; Schuster and Lee, 1987). Nitrate reduces itself to nitrite in the human body. Nitrites have also been shown to be an important precursor of N-nitrosoamines which are potential carcinogens (Lijinsky and Epstein, 1970; Wolff and Wasserman, 1972). On a better note nitrates and/or nitrites are used in the food industry in the curing of meat (West and Ramachandron, 1966; EUR Report, 1990). When nitrate is added to meat, nitrite is formed due to reduction by the organic matter in the raw meat. *Clostridium botulinum* grows only under anaerobic conditions and it is the nitrate rather than the nitrite which assures an aerobic environment. Meat cured with nitrate is a bright red, while nitrite imparts a darker colour to the meat; thus the latter's use is mainly to control colour, rather than the prevention of *Clostridium* infection *per se*. It is used for pigment and other colourants (Francis, 1998).

Hence, it is necessary to control the concentration of nitrate and nitrite in water. Several methods are available for the determination of nitrate in water, and may fall into the following categories:

- The reduction of nitrate to ammonia (Bremmer and Keeney, 1965; Keay and Menage, 1970)
- Photo-induced reduction of nitrate to nitrite (Takeda and Fujiwara, 1995)
- Direct spectrophotometry (Rennie et al., 1979; Huiro et al., 1991)

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- Potentiometric methods using ion-selective electrodes (Keeney et al., 1970)
- Reduction of nitrate to nitrite (Lambert and Dubois, 1971; Davison and Woof, 1978; Gine et al., 1980).

The determination of nitrate is difficult because of the relatively complex procedures required, the probability that interfering constituents will be high and the limited concentration ranges of the various techniques. Consequently, the actual determination of total oxidised nitrogen as nitrate is not recommended for waters, but rather as nitrite where the nitrate is reduced to nitrite.

Many colorimetric methods have been proposed for the determination of micro-amounts of nitrite. The most pronounced method seems to be a modified version (Watson, 1980; Chaube et al., 1982; Sunita and Gupta, 1984; Bashir and Flamez, 1981; Dasgupta, 1984; Norwitz and Kelliher, 1985; Tarafder and Rathore, 1988; Rathore and Tiwari, 1991) of the Shinn (1941) reaction which is based on a well-known Griess reaction. This is a reaction of nitrite with a primary aromatic amine to form a diazonium salt which is coupled with another aromatic compound to form the azo dye of which the absorbance is measured.

The strategy commonly adopted is based on the reduction of nitrate to nitrite which is then spectrometrically determined after diazotation and coupling reaction. Either homogeneous or heterogeneous reductors are used. The nitrite so produced is determined through formation of a reddish purple azo dye produced at pH 2.0 to 2.5.

When using the cadmium reducing method, nitrate is reduced almost completely to nitrite. The applicable range of the method is 0.01 to 1 mg NO₃⁻-N/ℓ. The technique is especially recommended for nitrate levels below 0.1 mg/ℓ where other methods lack adequate sensitivity. A number of flow injection systems (Zagato et al., 1980; Anderson, 1980; Van Staden, 1982; McCormack et al., 1994; Van Staden and Makhafola, 1996; 1999) have been developed using this modification.

Sequential injection analysis (SIA) launched in 1990 (Ruzicka et al., 1990a; b) is a technique that has tremendous potential especially for on-line process measurements and in monitoring of the environment. It is simple and convenient to operate. This technique considerably decreases sample and reagent consumption

A

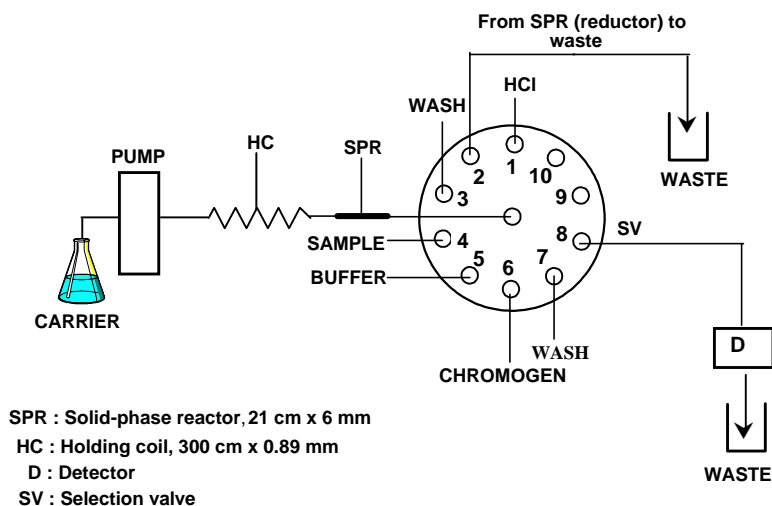


Figure 1

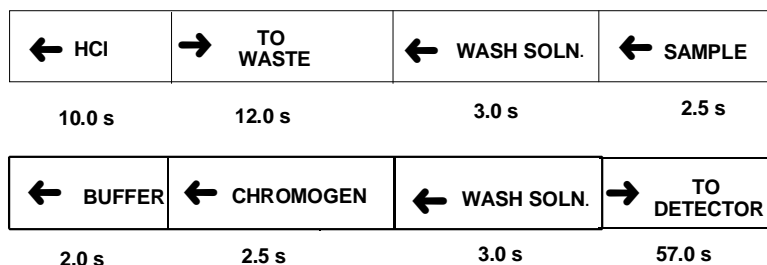
Sequential injection system for determination of iron. Hydrochloric acid solution is aspirated through the sequential valve (SV) through the solid phase reductor (SPR) into the holding coil (HC). It is flushed out via the SPR, regenerating it, then to waste. Wash, sample, buffer and chromogen solutions are now aspirated through the SV into the HC. The well-stacked penetrated zones are flushed by the carrier solution propelled by the peristaltic pump via SPR towards the detector (D) and waste.

(A) Schematic flow diagram.

(B) Sequence of hydrochloric acid, wash, sample, buffer and chromogen solutions.

B

DEVICE SEQUENCE FOR ONE SIA CYCLE



and, thus, the waste generated. In addition, devices based on SIA yield robust and stable systems that are suitable for routine monitoring.

Oms et al. (1995) proposed a semi-automated sequential injection system for the determination of nitrate and nitrite in wastewaters and aqueous extracts of atmospheric aerosols. Homogeneous reduction with hydrazinium sulphate was used and the individual quantification of both ions was achieved by two injections of the sample.

Van Staden and Van der Merwe (1998) used an SIA system concept to develop an on-line process analyser for the monitoring of nitrite in fertilizer process streams, natural and wastewater effluents. The nitrite was diazotised in the SIA system with sulphanic acid and coupled with N-(1-naphthyl) ethylenediamine dichloride to form a highly coloured azo dye which was measured at 525 nm.

The SIA system described herein allows for the determination of oxidised nitrogen (nitrate + nitrite) in water samples from different sources as nitrite. The nitrate present is reduced on-line with a solid-phase reactor, diazotised and coupled to produce a reddish azo dye which is spectrometrically detected at 540 nm. An automated pump is used in the place of a semi-automated burette and an acidified cadmium reactor instead of a copperised column. The reactor was regenerated on-line for consistency.

Experimental

Reagents

All reagents were prepared from analytical grade chemicals unless specified otherwise. All aqueous solutions were prepared from

doubly distilled, deionised water. Deionised water from Modulab system (Continental Water System, Sant Antonio, TX, USA) was used throughout. The solutions were all degassed before introduction into the system and stored in an oxygen-free environment.

Stock nitrate solution: 0.6070 g of oven-dried sodium nitrate (Merck, pro analysis) was dissolved and diluted to 1 l with double deionised water. A 2 ml solution of chloroform (Merck, pro analysis) was added to this solution to maintain stability and stored in a cool place. Working standards in the range of 0.25 to 50 mg/l were prepared by appropriate dilution of the stock solution with water.

Stock nitrite solution: 0.5057 g of oven dried sodium nitrite (Riedel-De Haën AG, Seelze-Hannover) was dissolved and diluted to 1 l with double deionised water. A 2 ml solution of chloroform (Merck, pro analysis) was added to this solution to maintain stability and stored in a cool place. Working standards in the range of 0.25 to 50 mg/l were prepared by appropriate dilution of the stock solution with water after it had been standardised with potassium permanganate (Protea Laboratories service (Pty) Ltd.)

Buffer solution: The buffer solution was prepared by dissolving 30 g of ammonium chloride (Merck, Darmstadt) and 0.2 g EDTA-disodium salt (GPR, Essex, England) in deionised water and diluting to 500 ml. The pH was adjusted to 6.5 using ammonia (25% NH₃, SAARCHEM). The carrier solution was prepared by dissolving 13 g ammonium chloride (Merck, Darmstadt) and 2.0 g EDTA in deionised water and diluting to 1 l. The pH of the solution was 4.75 and was not adjusted to any particular value. The appropriate dilutions were made from this solution during optimisation of the carrier concentration.

Chromogenic reagent: The chromogenic reagent was prepared by dissolving 5 g sulphanilamide (BDH, Poole, England) and 0.5

g of N-(1-naphthyl) ethylenediammonium dichloride in a mixture of 50 ml hydrochloric acid (32% HCl, Chemical suppliers) and 300 ml deionised water and diluting to 1 l. The solution was stored in an amber bottle.

Instrumentation

The sequential injection system depicted in Fig. 1 was constructed from the following components: a Gilson minipuls peristaltic pump (Model M312, Gilson, Villiers-Le Bel, France); a 10-port electrically actuated selection valve (Model ECSDIOP, Valco Instruments, Houston, Texas) and a Unicam 8625 UV-Visible spectrometer equipped with a 10-mm Hellma-type (Hellma GmbH and Co., Mulheim/Baden, Germany) flow-through cell (volume 80 µl) for absorbance measurements. The absorbance of the azo dye at 540 nm was used to study the response and precision of the samples during dispersion in the SIA manifold. The decision to use 540 nm as wavelength was based on a scan of the specified solution over the 200 to 1 100 nm range.

Data acquisition and device control were achieved using a PC30-B interface board (Eagle Electric, Cape Town) and an assembled distribution board (Mintek, Randburg). The flowTEK (Marshall and van Staden, 1992) software package (obtainable from Mintek) for computer-aided flow analysis was used throughout for device control and data acquisition. All data given (mean peak height values) are the average of 10 replicates.

Operation of the system

A schematic diagram for the SIA system is depicted in Fig. 1A. The whole procedure, from sample injection to data processing and

storage was computer-controlled via the flowTEK program. The whole SIA procedure involved designing a method which allows a single cycle of the experiment to be run. This procedure is well-illustrated in Table 1 and Fig. 1B.

When all the zones were placed in the holding coil (HC), they were then flushed with the carrier stream through the reductor to reduce the nitrate to nitrite. The oxidised nitrogen as nitrite was diazotised in the system with sulphanilamide and coupled with N-(1-naphthyl) ethylenediammonium dichloride to form a highly coloured azo dye which was detected at 540 nm with a spectrophotometer. The data obtained are converted to a response time graph on the computer screen as a peak profile. The maximum peak height was then automatically processed and stored on a computer via the flowTEK program.

The cadmium reductor

The reductor columns were made of glass with varying lengths (12 cm, 15 cm, 17 cm, 19 cm and 21 cm) but with the same internal diameter of 6 mm. The columns were then filled with cadmium granules (Merck, 0.3 - 1.5 mm). The particles were held by a glass frit at each end so that they did not block the SIA system. A vibrator was used to effect close packing of the columns. The cadmium granules prior to being packed in the glass column were prepared by washing with acetone for 10 min, adding 20 ml of 2 mol/l HCl solution, deionised water and methanol, then drying in a dessicator. An acidified cadmium reactor was chosen over a copperised one because copper has a tendency of interfering in the determination of nitrite in water. Furthermore, van Staden and Makhafola (1999), have shown that the life-span of the acidified cadmium reactor was longer than the copperised one. The cadmium reductor was regenerated by passing approximately 270 µl of 2 mol/l HCl solution at the beginning of every cycle. This was to ensure consistency in the reduction efficiency and capacity of the reductor.

Sample preparation

The samples were obtained from the Institute for Water Quality Studies (Department of Water Affairs and Forestry). The samples were all collected from different localities (streams, rivers, dams, hydro plants, tunnels and effluent streams) at a depth of half a metre. The samples were then preserved in mercury (II) chloride. The samples that were received were ready for direct analysis. Samples selected were in the pH range 6.8 to 8.2 and at a concentration of between 0.01 and 2 mg/l (nitrate + nitrite as N). The buffer solution allowed the adjustment of the pH of the samples.

Results and discussions

Method optimisation

The method was optimised with regard to the following parameters: nitrite concentration, carrier concentration, flow rate, sample, reagent and buffer volume, reactor

TABLE 1
Device sequence for one cycle of the SIA system

Time (s)	Pump	Valve	Description
0	Off	Position 1	Pump off. Select HCl stream.
1	Reverse		Draw HCl solution for regeneration
10	Off		Pump stop.
11	Off	Position 2	Select waste stream
12	Forward		Pump solution to waste
23	Off		Pump stop
24	Off	Position 3	Select wash stream
25	Reverse		Draw wash solution
27	Off		Pump stop
28	Off	Position 4	Select sample stream
29	Reverse		Draw sample solution
30.5	Off		Pump stop
31.5	Off	Position 5	Select buffer stream
32.5	Reverse		Draw buffer solution
33.5	Off		Pump stop
34.5	Off	Position 6	Select chromogen stream
35.5	Reverse		Draw chromogen solution
37	Off		Pump stop
38	Off	Position 7	Select wash stream
39	Reverse		Draw wash solution
41	Off		Pump stop
42	Off	Position 8	Select detector stream
43	Forward		Pump zones through reductor to detector
100	Off	Position 1	Pump stop. Valve return home.

Conc (mg/l)	0.5	1	2.5	5	10	25	50
Relative peak height	0.402	0.412	0.618	0.736	1.708	2.325	5.367
%RSD	1.5	1	0.8	1.2	1.8	2.5	5

Conc (mg/l)	0	0.7	1.3	2.6	6.5	13
Relative peak height	0.683	0.731	0.73	0.715	0.692	0.677
%RSD	2.1	1.3	1	1.4	1.9	1.4

Volume (μl)	27.5	55	82.5	110	137.5
Relative peak height	0.377	0.486	0.629	0.765	0.877
%RSD	1.2	1.7	0.5	1.2	0.8

Volume (μl)	27.5	55	82.5	110	137.5
Relative peak height	0.504	0.507	0.513	0.528	0.558
%RSD	3.6	1.5	0.8	1.6	1

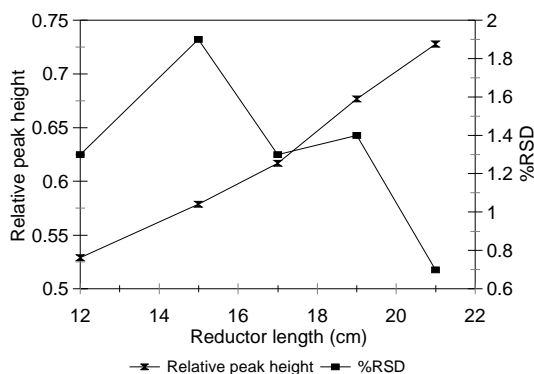


Figure 2
Effect of reductor length on response and precision

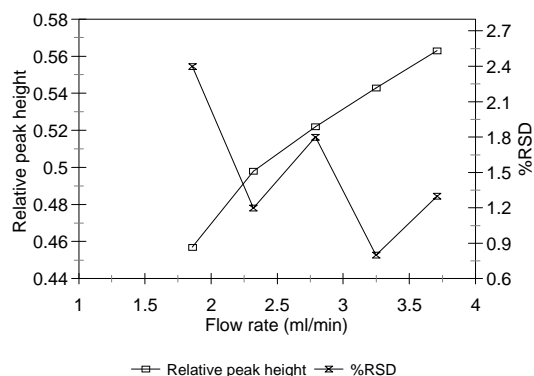


Figure 3
Effect of flow rate on response and precision

length and reduction efficiency. Both the relative peak height and %RSD were used as criteria for establishing the most appropriate optimum value in each case.

Cadmium reductor parameters

The solid phase cadmium reductor forms the heart of the manifold of the proposed system. The performance of the SIA system depends on the reduction efficiency of the reductor at the interface between the solid and the liquid phases of the reductor. In addition, the reductor packing had to be thorough and its length and efficiency had to be optimised.

The response and precision of the system were studied by varying the reductor length between 12 and 21 cm with the internal diameter fixed at 6 mm. The five reductors (12 cm, 15 cm, 17 cm, 19 cm and 21 cm) were compared for reductor efficiency. It was found that the last three reductors did not show a significant difference in response. There was, however, a significant difference for the shorter reactor lengths (Fig. 2). The 21 cm reactor length was chosen as optimum because of its good precision.

Chemical parameters

The nitrate concentration was evaluated between 0.5 and 50 mg/l (Table 2) in order to find the best conditions for both the response and precision and the 2.5 mg/l concentration that gave the best precision was chosen as the optimum concentration. The carrier was evaluated between pure deionised water and a solution of 13 g ammonium chloride and 2 g EDTA made up to a litre. However, it was a concentration corresponding to 1.3 g ammonium chloride and 0.2 g EDTA per litre of solution that was chosen as the optimum, because it gave the best precision with overall combined best response as shown in Table 3.

Physical parameters

The contact time between the sample and the reductor is of utmost importance. The 21 cm reductor length was found to be optimum and effective with 270 μl , 2 mol/l HCl solution passing through the reductor for every SIA cycle. The flow rate was evaluated between 1.86 and 3.71 ml/min. The 3.25 ml/min flow rate (Fig. 3) gave the best precision and was chosen as optimum for further work. The sample (Table 4), reagent (Table 5) and buffer volumes (Table 6) were all evaluated between 25.0 and 140.0 μl . The sample and reagent volumes were both found to be optimum at 82.5 μl and the buffer volume at 55 μl .

Method evaluation

The linearity of the system was evaluated for analyte concentration between 0.25 and 50 mg/l. The response was, however, found to be linear in the range 0.25 to 5 mg/l. The relationship between the response and the concentration is given by the equation: $H = 0.0877x + 0.2060$; $r^2 = 0.9994$, where H is the relative peak height and x the analyte concentration in mg/l.

Water samples from different sources were analysed with the proposed system. The results obtained are a mean of 10 repetitive analysis of each sample (Table 7). The accuracy was compared to

the standard method results (Table 8).

The precision of the method was determined by 10 repetitive analysis of the standard solutions as well as 10 repetitive analysis of the water samples. All these were carried out under optimum conditions. The standard deviation for the standards was <1.0% and the samples < 1.3%.

The detection limit was calculated using the formula:

$$\text{Detection limit} = \frac{[(3\sigma + k) - c]}{m}$$

where:

σ (0.00455) is the standard deviation of the baseline

k (0.1932) is the average response of the baseline

c (0.0877) is the intercept and m is the slope of the calibration graph.

The detection limit was found to be 0.01 mg/l.

The recovery of the proposed system was determined by comparing the expected results with those obtained with the proposed system as follows:

$$\text{Recovery (\%)} = \frac{\text{obtained}}{\text{expected}} \times 100$$

The results ranged between 91.4% and 112.8%.

The sample interaction carryover between consecutive samples was determined by analysing samples with low analyte concentration followed with a high analyte concentration which was again followed by the sample with a low analyte concentration. The sample interaction was then calculated using the following formula:

$$\text{Sample interaction} = \frac{A_3 - A_1}{A_2} \times 100\%$$

where A_1 is the peak height (0.2937) of a sample containing 1 mg/l, A_2 is a peak height (0.7361) of a sample containing 5 mg/l and A_3 is a peak height (0.3033) of a sample containing 1 mg/l injected after the sample containing 5 mg/l. The sample interaction was 1.3% which may be considered to be negligible.

The only possible interferences that may disturb the accuracy of this analysis are: iron, copper, phosphates, chromium (VI), magnesium, manganese and chloroamines. Fortunately, all these ions were found to be at acceptable levels and may not affect the results. Table 9 gives some of the ions, their ranges and tolerance levels in the water samples analysed. Oms et al. (1995) found that copper (II) interfered in the reduction step and for it not to interfere, the copper sulphate used for the copperised cadmium reductor should be less than 25 mg/l. However, in this instance, the tolerance level is 20 mg/l. An acidified reductor was used in the place of the copperised one, thus minimising any interference due to copper that may arise from copper.

Statistical comparisons

The comparison was done between the SIA and the standard method (Institute for Water Quality Studies) (Table 8). The comparison was done to establish whether the SIA system can be accepted as giving reliable results in the determination of oxidised

TABLE 6
Effect of buffer volume on response and precision

Volume (μ l)	27.5	55	82.5	110	137.5
Relative peak height	0.662	0.608	0.574	0.589	0.596
%RSD	1.4	0.8	1.7	1.7	0.8

TABLE 7
SIA results for water samples analysed

Sample Id	pH	mg/l (NO ₃ ⁻ -N + NO ₂ ⁻ -N)
Unknown	8.2	0.65
Mtafufu-Ntafufu	7.8	0.88
Tshinane	7.7	1.07
Vink	8.3	0.96
Wolwekloof Tunnel	6.9	1.2
Van Rhyreveldspass Dam	7.5	1.68
Mgwale-clackburg	7.8	0.48
Tsitsa-Tsitsa Bridge	7.3	0.47
Duiwe River	7	0.8
Mutshedi Dam	7.4	1.13

TABLE 8
Comparison of SIA and standard method results in mg/ and paired differences

Sample ID	SIA method	Standard method	$X_d \times 10^{-2}$	$(X_d)^2 \times 10^{-3}$
Unknown	0.65	0.63	2	0.4
Mtafufu-Ntafufu	0.88	0.78	10	10
Tshinane	1.07	0.97	10	10
Vink	0.96	1.05	-9	8.1
Wolwekloof Tunnel	1.2	1.16	4	1.6
Van Rhyreveldspass Dam	1.68	1.77	-9	8.1
Mgwale-Clackburg	0.48	0.45	3	0.9
Mutshedi Dam	1.13	0.99	14	19.6
Tsitsa-Tsitsa Bridge	0.47	0.46	1	0.1
Duiwe River	0.8	0.72	7.9	6.2

TABLE 9
Some of the ions present in the water samples, their ranges and tolerance levels in mg/l when added to 2 mg/l standard (nitrate + nitrite)

Ion	Tolerance level	Range in samples
Phosphate as P	1	0.008 - 0.080
Chloride	800	10 - 400
Sulphate	300	0.018 - 66
Sodium	750	4 - 67
Potassium	700	0.3 - 8.3
Calcium	350	3 - 19
Magnesium	300	2 - 19
Copper	20	0.011 - 0.057
Nickel	40	0.043 - 0.79
Chromium	5	0.084 - 0.14
Iron	20	11.4 - 14.2

nitrogen. The null hypothesis was used. The t-test with multiple samples (paired by differences) was applied to examine whether the two methods differed significantly at a 95% confidence level. The null hypothesis is $H_0: \zeta = 0$, against the alternative $H_1: \zeta \neq 0$, where ζ is the population paired difference (McCormick and Roach, 1987). The test is two tailed, as we are interested in both $x_d < 0$ and $x_d > 0$. Thus, $t_{calc} = |x_d| \times \sqrt{n/s_d}$ and from Table 10, $x_d = 0.0339$ and $s_d = 0.0877$ with $n = 10$. Substituting for t_{calc} we find $t_{calc} = 0.0339 \times \sqrt{10/0.0877} = 1.222$.

At 95% confidence level $t_{0.05,9} = 2.36$. The critical t-values are therefore ± 2.36 . Since the calculated value is less than the critical value, H_0 cannot be rejected and it follows that there is no statistically significant difference between the two techniques.

Conclusions

The determination of the oxidised nitrogen (nitrate + nitrite as N) by SIA using a solid-phase reductor incorporated into the SIA manifold is an improvement on similar techniques which used FIA and semi automated burettes. Furthermore, in this work an acidified cadmium reductor was used which eliminated all possible interferences that may have been caused by copper or/and phosphate ions. In contrast to work already done, in this work the cadmium reductor was regenerated on-line without having to disconnect the system or replace it after regeneration. Thus, once more the SIA system was found to be time and reagent saving and suitable for the determination of oxidised nitrogen in water samples to a very low level.

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