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# Determination of total nitrogen in water samples by means of high-pressure bombs and ion chromatography

Hilda Ledo de Medina*, Marinela Colina de Vargas, Julio Marin, Dannis Pirela

*Universidad del Zulia, Facultad Experimental de Ciencias, Laboratorio de Quimica Ambiental, Maracaibo 4011, Zulia, Venezuela*

**Abstract**

The standard method for the determination of organic nitrogen in water samples in the past has been based on the Kjeldahl digestion technique that converts organic nitrogen into ammonia, which can then be determined along with any ammonia originally present. However, this method is time consuming and the accuracy is variable because the effect of interferences causes unreliable results, especially in complex matrices. An alternative method for the determination of organic nitrogen is the alkaline peroxodisulphate digestion technique. This oxidizes all nitrogen in the sample using potassium peroxodisulphate in a strongly alkaline environment under high pressure and temperature. Nitrate is the sole product and can easily be determined by ion chromatography or by other methods (*e.g.*, cadmium reduction method). A method for total nitrogen determination was developed using 23-ml high-pressure bombs with potassium peroxodisulphate and sodium hydroxide to oxidize the organic nitrogen to nitrate. Urea and ammonium chloride were used as nitrogen compounds for calibration. The method was checked with the Kjeldahl method, showing good agreement (R.S.D. = 4.62%). Studies of digestion time were carried out to determine the optimum time in the pressure vessel. The results were checked with the cadmium reduction method (R.S.D. = 3.62%) for natural water samples. The recoveries with urea and ammonium chloride reagents were higher than 90%.

## 1. Introduction

The determination of total nitrogen (TN = inorganic plus organic fixed nitrogen) has largely been based on acid Kjeldahl digestion of nitrogenous organic compounds [1]. This procedure is tedious to perform and yields a total Kjeldahl nitrogen (TKN) value that includes only organic N and $NH_4^+$-N (not $NO_2^-$- and $NO_3^-$-N) [2]. This method involves the determination of the total concentration of ammonia nitrogen and organic bound nitrogen according to the Kjeldahl method. The organic bound nitrogen is digested with a mixture of concentrated sulphuric acid and potassium sulphate with selenium or mercury as catalyst [3].

Manual distillation is still the primary method for the determination of nitrogen as ammonia from Kjeldahl digests of soils, plants, cereals, food and fertilizer products. It is also the standard procedure for the determination of protein in meat and meat products and for alcohol and other volatiles in the wine and beverage industry [4].

One of the limiting factors of the Kjeldahl nitrogen determination is that it normally re-

* Corresponding author.

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quires more than 2 h, or about 1 h using a heating block, whereas the measurement itself takes only a few minutes with an automated distillation apparatus [5].

An alternative method for the determination of organic nitrogen modified by D'Elia *et al.* [2] is the alkaline peroxodisulphate digestion technique. This oxidizes all nitrogen in the sample using potassium peroxodisulphate in a strongly alkaline environment under high temperature and pressure.

A modified peroxodisulphate digestion technique for TN determination has also been developed [6]. The oxidation, under alkaline conditions, reduces nitrogenous compounds to $NO_3^-$ for measurement as the sole product under high temperature using a microwave digestion unit in place of an autoclave system. Nitrate can be determined by reduction to nitrite using a cadmium reduction column. Separate, rather than combined, nitrate–nitrite values are readily obtained by carrying out the procedure first with, and then without, the Cu–Cd reduction step [7].

However, this procedure leads to many problems with the measurement. The copperized cadmium column needs washing and preconditioning with buffer and/or EDTA solutions to sustain a high reduction activity for nitrate. Also, the presence of air in the solution removes the activity from the column [8].

In the digestion–chromatographic method proposed here, we used the peroxodisulphate digestion solution of Johnes and Heathwaite [6] and developed a modified technique for digestion and determination of TN. The method utilizes a high-pressure bomb in the peroxodisulphate digestion step. This technique converts all nitrogen in the sample into nitrate, which is determined using ion chromatography, giving a convenient and more accurate and rapid method of TN determination. By comparison, the chromatographic approach is simple, versatile and has the added advantage of being applicable over a wide range of concentrations.

A comparison was made between the proposed digestion–chromatographic method and the conventional Kjeldahl and nitrate–nitrite methods as prescribed in a standard text [7]. Known nitrogen-containing substances such as urea and ammonium chloride and a large number of samples of natural waters were investigated.

## 2. Experimental

Samples were taken from the rivers Motatán, Carache and Boconó, which are inflows of Lake Maracaibo. Water samples from Lake Maracaibo were also taken. The samples were preserved with concentrated sulphuric acid (2 ml/l) and refrigerated.

The reagents urea and ammonium chloride were used to confirm the validity of the method.

### 2.1. Determination of nitrate and nitrite

*Apparatus*

A Milton Roy 21D spectrophotometer was used.

*Reagents*

Cadmium powder (150 $\mu$m) was obtained from Merck. To prepare copper–cadmium, the cadmium particles (new or used) were cleaned with dilute HCl (1.2 *M*) and copperized with a 2% solution of copper sulphate in the following manner. Cadmium was washed with 1.2 *M* HCl and rinsed with distilled water, then 2 g of the cadmium were swirled in 100 ml of 2% copper sulphate solution until the blue colour partially faded. The copper sulphate solution was decanted and the procedure was repeated with fresh copper sulphate until a brown colloidal precipitated was formed. The cadmium–copper was washed with distilled water (approximately ten times) to remove all the precipitated copper.

To prepare the colour reagent 100 ml of concentrated phosphoric acid, 40 g of sulphanilamide and 2 g of N-1-naphthylethylenediamine dihydrochloride were added to approximately 800 ml of deionized water while stirring. Stirring was continued until dissolution was complete, then the solution was diluted to 1 l with water. The solution was stored in a brown bottle and kept in the dark when not in use.

Ammonium chloride–EDTA solution was prepared by dissolving 85 g of analytical-reagent grade ammonium chloride and 0.1 g of disodium ethylenediaminetetracetate in 900 ml of distilled water. The pH was adjusted to 8.5 with concentrated ammonia solution and the mixture was diluted to 1 l with water.

Stock standard nitrate and nitrite solutions (1000 mg/l $NO_3$-N and $NO_2$-N, respectively) were prepared in 100-ml volumetric flasks.

*Procedure*

To construct calibration graphs, working standard solutions were freshly prepared from the stock standard nitrate and nitrite solutions.

The efficiency of the reduction column was verified with a standard nitrite solution and comparison with a standard nitrate solution at the same concentration.

A calibration graph was prepared with standards of $KNO_3$ in the range 0.05–1.00 mg/l $NO_3^-$-N by dilution of the stock standard nitrate solution. The concentrations were measured spectrophotometrically at 543 nm. After the digestion procedure, the absorbance was related to the total amount of nitrogen in the sample.

### 2.2. Determination of total Kjeldahl nitrogen

*Apparatus*

A Milton Roy 21D spectrophotometer, micro-Kjeldahl digestion and distillation units and a Metrohm E678 Titroprocessor were used.

*Reagents*

All the reagents were prepared following the procedure described in a standard text [7].

*Procedure*

A 100-ml volume of sample was mixed carefully with 50 ml of digestion reagent (10 ml of concentrated $H_2SO_4$, 6.7 g of $K_2SO_4$ and 1.25 ml of $HgSO_4$ solution) and added to a distillation flask. A few glass beads was added and, after mixing, the mixture was heated under a hood to remove acid fumes. The mixture boiled briskly until the volume was greatly reduced and copious white fumes were observed. Digestion was continued for an additional 30 min. As digestion continued, the sample turned clear. After digestion, the contents (cooled) were diluted to 300 ml with water and mixed. The flask was tilted and 50 ml of hydroxide–thiosulphate reagent were carefully added to form an alkaline layer at the bottom of the flask. The flask was connected to a steam distillation apparatus and shaken to ensure complete mixing. The mixture was distilled and 200 ml of the distillate were collected below the surface of 50 ml of absorbent solution. Boric acid was added as an indicator. Ammonia in the distillate was titrated with standard 0.02 *M* $H_2SO_4$ until the indicator turned a pale lavender.

### 2.3. Determination of total nitrogen by proposed digestion and ion chromatographic methods

*Oxidizing reagent*

A 15-ml volume of 3.75 *M* NaOH solution was added to 500 ml of deionized water, 50 g of $K_2S_2O_8$ were dissolved in the solution and the mixture was diluted to 1 l with water. This oxidizing reagent must be prepared freshly as required.

*Digestion procedure*

A 6-ml volume of oxidizing reagent was added to 4 ml of sample and placed in a PTFE crucible and capped. The crucible was placed in the stainless-steel body of a Parr-type bomb and closed by tightening the stainless-steel screw-cap. The system was placed in a preheated 105°C oven and kept at this temperature for 4 h. The bomb was opened after cooling to ambient temperature. Under the influence of pressure, temperature and pH, the organic and inorganic nitrogen compounds were converted into nitrate. The nitrate formed was measured by ion chromatography.

### 2.4. Determination of nitrate by ion chromatography

*Samples and reagents*

Samples were diluted tenfold before injection into the chromatograph because the $SO_4^{2-}$ peak, derived from potassium peroxodisulphate, after the digestion procedure interferes in the detection of nitrate. A blank of the oxidizing reagent, after the digestion procedure, was injected into the chromatograph.

All reagents were of the highest purity and deionized water was used for dilutions. Calibration standards were prepared by diluting mixed stock standard solutions containing 1000 mg/l of $NO_3^-$-N using a series of dilutions.

*Apparatus*

Samples were analyzed using a Dionex Model 2000i/SP ion chromatograph equipped with an anion precolumn (Dionex AG4A), an anion separation column (Dionex AS4A), a suppressor column (Dionex AMMS-II) and a conductivity detector. The mobile phase (flow-rate 2 ml/min) was 1.7 m*M* $NaHCO_3$–1.8 m*M* $Na_2CO_3$ and the regenerant solution was 12.5 m*M* $H_2SO_4$. The injection volume, conductivity sensitivity and chart speed were 100 $\mu$l, 30 $\mu$S and 0.5 cm/s, respectively.

*Procedure*

Standards and samples were injected into the ion chromatograph with an analysis time of 10 min, which permitted the elution of the sulphate peak, which was the last to elute. External standardization was used with recalibration after every ten samples during a run. A calibration graph was plotted of peak area against concentration and used to interpolate unknown concentrations.

## 3. Results and discussion

### 3.1. Ion chromagraphy

Digested and diluted samples were injected into the ion chromatograph to detect total nitrogen concentrations as nitrate. After injection, nitrate ion was eluted from the column, and detected with a conductivity detector and recorder and quantified with an integrator (Fig. 1).

The response for $NO_3^-$ was linear in the working range 1.00–100 mg/l. An important feature of conductivity detection in ion chromatography is linearity of the response over a wide concentration range. Linearity of the response is guaranteed by the dependence of conductivity on concentration, provided that other effects or matrix effects do not intervene. The correlation coefficient for a linear least-squares fit was $r = 0.9996$. This showed that the conductivity detector gave a linear response over the whole calibration range used in this work. The relative standard deviation (R.S.D.) was 1.62% ($n = 8$).

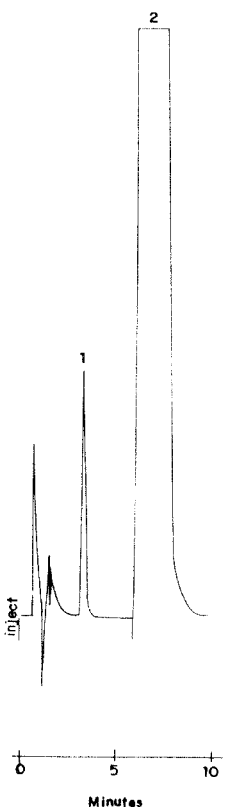


Fig. 1. Separation of nitrate in digested sample. Peaks: 1 = $NO_3^-$ (0.88 mg/l); 2 = $SO_4^{2-}$.

### 3.2. Comparison of TKN spectrophotometric and chromatographic methods

Samples of water were analyzed for TN (TKN plus inorganic N) by standard methods and the results were compared with those obtained by the proposed digestion–ion chromatographic (IC) method. The recovery of TN was determined using analytical-reagent grade 99% urea and ammonium chloride. Table 1 gives results for TN obtained by the IC and Kjeldahl methods. The results obtained by the IC method show good recovery.

In Table 2, columns 1 and 2, the concentrations of nitrate measured in river waters and the urea and ammonia reagents, after the digestion step, by ion chromatography are compared with those obtained by the conventional spectrophotometric method using a copperized cadmium column. It is clear that the results obtained by the proposed method agree with those obtained using the standard method which involves the reduction of nitrate to nitrite. The R.S.D. using ion chromatography was 1.20% and that using a copperized cadmium column was 4.55% ($n = 5$). The mean difference between the values obtained was 3.67%.

Water samples with total nitrogen concentrations between 0.040 and 5.400 mg/l were analyzed using the proposed method and the Kjeldahl–nitrate–nitrite method (columns 1 and 3, Table 2). To decide if the difference between the methods is significant, a paired $t$-test was used because the samples contained substantially different amounts of analyte. As the calculated value of $t$ is less than the tabulated value, the methods did not give significantly different values for the mean nitrogen concentration. By comparing squared standard deviations, the $F$-test shows whether the two methods show similar precision. The conclusion is that the proposed method does not differ from the Kjeldahl plus nitrate–nitrite method because no significant differences in the results were found. The proposed method generally reports more precise results (lower standard deviation). The mean difference between the values obtained by the methods used were 3.67% and 4.12%, as can be seen in Table 2.

Standard additions of different concentrations of nitrate were made to the water samples from Lake Maracaibo and the results are given in Table 3. The mean recovery of nitrate by the proposed method was 99.08%. This result indicates than suspected interferences are not significant.

A general comparison of the proposed method with the Kjeldahl and nitrate–nitrite methods to determine total nitrogen shows several advantages of the former. The tedious processes to determine TN using the Kjeldahl procedure plus the nitrate–nitrite method are not required in the proposed method. Moreover, the reduction of nitrate to nitrite is not necessary in the determination of nitrate, because it can be determined directly using ion chromatography. The proposed method eliminates the slow standard procedure which requires distillation of the whole digested sample. The Kjeldahl method produces substantial amounts of chemical waste

Table 1
Recoveries of nitrogen compounds using the Kjeldahl and IC methods

| Compound | Nitrogen added (mg/l) | Mean recovery (%)[a] | |
|---|---|---|---|
| | | Kjeldahl method | IC method |
| Urea | 0.039 | 94.87 | 101.56 |
| Ammonium chloride | 2.800 | 105.71 | 102.86 |

[a] $n = 5$.

Table 2
Comparison of TN obtained by IC and TKN and inorganic N data obtained on water samples

| Sample | Total nitrogen (N) (mg/l) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ion chromatography[a] | | Cadmium reduction[a] | | TKN plus inorganic N (standard methods [7]) | | F-test | |
| | (1) | S.D. | (2) | S.D. | (3) | S.D. | (1) – (2) | (1)–(3) |
| Urea reagent | 0.040 | 0.000 | 0.039 | 0.001 | 0.037 | 0.002 | 0.000 | 0.000 |
| Ammonia reagent | 2.880 | 0.001 | – | – | 2.960 | 0.002 | – | 4.000 |
| Carache river | 0.480 | 0.007 | 0.458 | 0.015 | 0.471 | 0.015 | 4.592 | 4.592 |
| Motatán river | 5.335 | 0.172 | 5.084 | 0.320 | 4.800 | 0.322 | 3.461 | 3.505 |
| Boconó river | 0.713 | 0.009 | 0.674 | 0.041 | 0.671 | 0.053 | 20.750 | 34.679 |

Mean difference:
(1) – (2) 3.67%.
(1) – (3) 4.12%.
Mean R.S.D., IC: 1.20% ($n = 5$).
Mean R.S.D., cadmium reduction: 4.55% ($n = 5$).
Mean R.S.D., TKN plus inorganic N: 4.57% ($n = 5$).
$F$ (theoretical): 6.39 ($P = 0.05$).
$t$-Test (95%):
(1) – (2) Theoretical: 3.18
Calculated: 1.35.
(1) – (3) Theoretical: 2.78
Calculated: 1.50.
[a] After digestion procedure.

Table 3
Results of standard additions to Lake Maracaibo water for the determination of total nitrogen by ion chromatography

| Sample No. | Total nitrogen concentration (mg/l) | | | |
|---|---|---|---|---|
| | Taken | Added as $NO_3$-N | Found | Recovery (%) |
| 1 | 1.400 | 1.680 | 2.940 | 95.5 |
| 2 | 1.120 | 0.840 | 1.960 | 100.0 |
| 3 | 2.800 | 1.400 | 4.480 | 106.7 |
| 4 | 15.400 | 14.000 | 29.820 | 101.4 |
| 5 | 35.000 | 28.000 | 62.440 | 99.1 |
| 6 | 1.400 | 0.420 | 1.778 | 97.7 |
| 7 | 0.700 | 0.140 | 0.812 | 96.7 |
| 8 | 2.100 | 0.140 | 2.142 | 95.6 |

Mean recovery: 99.08%.
Mean R.S.D. 3.75% ($n = 3$).

with high concentrations of sodium hydroxide and low concentrations of the catalyst (selenium or mercury).

## 4. Conclusions

The TKN and spectrophotometric nitrate–nitrite results showed no significant difference from those obtained by the ion chromatographic method. The determination of nitrate ion by anion-exchange ion chromatography is reliable and gives reproducible results. The technique involves minimum handling and sample preparation. An additional benefit of the technique is the possibility of the determination of total nitrogen. The peroxodisulphate digestion–ion chromatographic method for determining total nitrogen is simple, sensitive, rapid, precise and suitable for the analysis of large numbers of samples. The analysis has the advantage of achieving high precision for samples while utilizing only small amounts of sample.

## 5. Acknowledgement

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