

A SIMPLE METHOD FOR THE DETERMINATION OF NITRATE IN ACIDIC SOLUTIONS WITH HIGH IRON CONTENT BASED ON THE TRADITIONAL CADMIUM REDUCTION TECHNIQUE

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Abstract— A simple and unexpensive method based on traditional cadmium column technique is proposed for determination of nitrate in acidic aqueous mixtures with high iron load. Solutions are alkalized and centrifuged, subsequently the supernatant is withdrawn for nitrate analysis. By this procedure two effects are accomplished, on the one hand, pH is adjusted to a suitable value and, on the other hand, iron interference is removed by precipitation. The obtained mixture is passed through a copperized cadmium column to reduce nitrate to nitrite which is quantified spectrophotometrically. This method is suitable to measure nitrate concentrations even lower than the maximum accepted limit, in artificial solution.

Keywords— Acid water, nitrate, iron, cadmium reduction column.

I. INTRODUCTION

Dissolved inorganic nitrogen species are important nutrients that control the biomass in natural waters. Concentrations of these compounds are, in general, extremely low in natural conditions, but higher concentrations might be found in natural water bodies as a result of anthropogenic inputs and, in lower extent, atmospheric processes (Zuo *et al.*, 2006). Particularly, the nitrate content in natural waters arises by agricultural (Zhang *et al.*, 2013; Almasri and Kaluarachchi, 2007), domestic and industrial wastewaters discharges (Wakida and Lerner, 2005). High nitrate concentrations have known undesired consequences on the environment. Since the increase on NO₃⁻ load on surface waters is associated to the phenomena of eutrophication (Istvánovics, 2009) and several human diseases (Gulis *et al.*, 2002; Zhang *et al.*, 2013), national and international thresholds have been established and it is important to monitor nitrate concentration in all waterbodies with methods suitable to detect the limits of established.

The World Health Organization (WHO) drinking water criterion states that the NO₃⁻ concentration should be less than 50 mg.L⁻¹ (i.e. NO₃⁻-N of 11.3 mg.L⁻¹) (WHO, 2011), while the US Environmental Protection Agency (US EPA) maximum permissible concentration for drinking water is 10 mg.L⁻¹ of NO₃⁻-N (EPA, 2011). In Argentina the permissible NO₃⁻ concentration in drinking water established by Código Alimentario Argentino (CAA) is 45 mg.L⁻¹ (i.e. NO₃⁻-N of 10.1 mg.L⁻¹) (CAA, 2012).

Spectrophotometric (Yao *et al.*, 1998), chromatographic (Zhang *et al.*, 2013; Pfaff, 1993) and electrochemical methods (Tang *et al.* 2012) have been reported for nitrate analysis in aqueous solutions. Among these techniques spectrophotometry is widely used, due to instrumental simplicity, relatively low investment and operational costs, and because spectrophotometric methods for nitrate determination are considered in several official standard methods (APHA, 1985; EPA, 2012). One of the most used techniques is the traditional copperized cadmium column method (Gal *et al.*, 2004; Lapa *et al.*, 2000), which relies on the pre reduction of nitrate to nitrite and the determination of the latter by the Griess reaction (Zuo *et al.*, 2006), in which NO₂⁻ is diazotized with sulfanilamide and undergoes a coupling reaction in the presence of *N*-(1-naphthyl) ethylenediamine, yielding a colored product that can be spectrophotometrically detected at 540 nm.

The reduction of nitrate to nitrite (reaction 1) is a very critical step of this method since it is significantly affected by chemical conditions of the solution and preparation of the reductant.



According to literature, a suitable pH value for this reaction appears to be around 8.5 (APHA, 1985; Gal *et al.*, 2004). At higher pHs reduction occurs (Reis Lima *et al.*, 2006) but kinetics are slow and, moreover, the possibility of precipitation of Cd²⁺ and Cu²⁺ as hydroxides increases. At lower pH values, reaction (1) is expected to proceed faster, but further reduction to hydroxylamine and ammonia is possible (Grasshoff *et al.*, 1983; Gal *et al.*, 2004). Furthermore, in acidic solutions, significant concentrations of metal cations (that are interferences of the spectrophotometric determination) may be present, due to the high solubility of salts at low pH. Usually, the pH of the mixture is regulated by using a buffer, but, in the case of extreme pH values, the buffer capacity is exceeded and the acidity cannot be adjusted to the required value. This method, thus, is not suitable for extremely acidic matrixes.

There are several acidic water bodies worldwide. Most of them are streams or lakes affected by acid mine drainage (Nixdorf *et al.*, 2005; Geller *et al.*, 1998), but there are some scarce systems acidified by natural processes (Nixdorf *et al.*, 2005; Stumm and Morgan, 1981). An example of the latter is Cavihue Lake (Pedrozo *et al.*, 2001; Varekamp 2008), a natural acidic lake with high iron content (pH=2.56, [Fe]=19.5 mg.L⁻¹). Very

few papers study the determination of nitrate in acidic waters with high Fe concentration. An automatic photometrical procedure has been proposed for the analysis of nitrate in iron rich sediment pore waters of mine lakes (Herzprung *et al.*, 2005). This is a very sensitive method, but it needs sophisticated setup and equipment. The aim of the present work is to propose a simple and unexpensive method, based on cadmium copperized reduction column that allows determining nitrate concentration in acidic solutions with high iron content. In this study, the method was applied for the analysis of artificial acidic mixtures with high iron concentration as a first approach to further develop a suitable method to measure nitrate in more complex acidic matrixes such as Caviahue Lake water.

II. METHODS

A. Reagents

Freshly prepared ultra high purity water was used throughout. Analytical-grade reagents were used. 0.3–1.6 mesh size cadmium granules (Fluka), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (98%, Anedra) and HCl 36.5–38% (Cicarelli) were used in the preparation of Cu-Cd reduction column. NH_4Cl 99.5% (Cicarelli) was used in preparation of buffer solutions.

The colour reagent for nitrate determination consists on two solutions: reagent 1 and reagent 2. For the preparation of reagent 1 10 g of sulphanilamide (98% Anedra) were weighed and added to about 500 mL of water. Then 100 mL of concentrated hydrochloric acid were added and, after dissolution of the solid, the flask was filled up to 1 L with water. Reagent 2 was prepared by dissolving 2 g of N-(1-naphthyl)- ethylenediamino dihydrochloride (ACS Anedra) into the volume of water required to obtain 1L of final solution. These solutions were stored in amber glass bottles. Reagents for ammonia determination (Uremia, Wiener lab kit) were purchased from Wiener Lab S.A.I.C., Argentina. KSCN (ACS, Cicarelli) was used in Fe(III) determination

In experiments where ionic strength was adjusted, Na_2SO_4 (>99% Merck) was used without further purification, NaOH (>99% Merck) and H_2SO_4 (95–97% Merck) were used to prepared the solutions to adjust the pH (NaOH 0.1 M and H_2SO_4 0.1 M). NaNO_3 (995 mg $\text{NO}_3^- \cdot \text{L}^{-1}$ Merck), $\text{Fe}(\text{ClO}_4)_3 \cdot \text{H}_2\text{O}$ (chloride <0.10% Aldrich), $\text{Fe}(\text{ClO}_4)_2 \cdot \text{H}_2\text{O}$ (98% Aldrich) and metallic iron (hereafter Fe^0) chips (99.98% Sigma-Aldrich) were used as received. Nitrogen was supplied by AGA.

B. Analytical Methods and equipment

The pH of the solutions was monitored using an Orion pH meter (model 920 A).

Barnstead Thermolyne Cartridge HN ultrapure (Mixed Bed DI) was used for provision of ultra pure water. In order to facilitate iron precipitation as ferric oxyhydroxides, a Rolco centrifuge without temperature control (Model 3070) was used to centrifuge the alkalized solutions.

Copperized-Cadmium reduction column for nitrate determination was constructed and prepared as described in Standard Methods for examination of Water

and Wastewater (APHA 1985). 2 mL of NH_4Cl 0.075M was added to 50 mL of each mixture. The mixed solutions were poured into the column and passed through it at a rate of 30–33 $\text{mL} \cdot \text{min}^{-1}$, 15 mL of the reduced solution were collected in a flask and 1 mL of reagent 1 (sulfanilamide solution) was added. After 4.5 minutes, 1 mL of reagent 2 (N-(1-naphthyl)-ethylenediamine solution) was added to the studied solution flask, and after 15.5 minutes at room temperature, the emerging red complex intensity was measured at 540 nm using a Metrolab 1700 spectrophotometer in a quartz cell of 1.0 cm of optical path.

The NH_4^+ concentration was measured by an enzymatic-colorimetric method employing a commercial kit from Wiener for urea in blood quantification. 1 mL of phenol-nitroprusside reagent and 1 mL of NaClO-NaOH reagent were added to 20 mL of studied solution. After 30 minutes of incubation at 37 °C in a thermostatic bath (Viking S.R.L, Model Masson), absorbance at 630 nm was recorded.

Fe(III) concentration was determined spectrophotometrically at 480 nm as a complex with thiocyanate (SCN^-) (Vione *et al.*, 2006)

C. Experimental Procedures and Setup

C.1. Preliminary studies. Influence of pH and the ionic strength.

Preliminary tests were performed in order to characterize the performance of the column under the studied conditions.

In order to ascertain the influence of initial pH and the ionic strength of the mixtures on the determination of nitrate, two sets of experiences varying these parameters were carried out. Solutions containing 0.1 mg $\text{N-NO}_3^- \cdot \text{L}^{-1}$ were prepared by dilution of a 995 mg $\text{NO}_3^- \cdot \text{L}^{-1}$ standard solution in deionized water. A first series of solutions was prepared adjusting the pH at different values (5.65–9.06) with H_2SO_4 or NaOH. To establish the effect of ionic strength, a second series of solutions was prepared without pH modification and adding Na_2SO_4 , the concentration of the salt varied from 0 to 0.15M. NO_3^- concentration was determined as discussed in section B. Measures were run by duplicate.

C.2. Determination of nitrate concentration in acid solutions in the presence of Fe(III).

In order to assess the recovery degree of nitrate in artificial acid mixtures with high content of Fe(III), solutions containing different concentration of nitrate were prepared as follows: Fe(III) concentration was 20–24 mg $\cdot \text{L}^{-1}$, the concentration of nitrate varied from 0.01 to 0.5 mg $\cdot \text{L}^{-1}$ and initial pH was adjusted to 2.5–2.9 with H_2SO_4 . Subsequently, the pH was adjusted to 6.1–6.6 with NaOH 0.1M and the obtained mixtures were centrifuged 1 hour at 3600 rpm. The supernatant was collected and passed through the column after addition of NH_4Cl (50 mL solution/2 mL) and nitrate concentration was determined colourimetrically as explained in section B. Since the solution containing the highest concentration of nitrate, reached an absorbance value above linearity limit (i.e. Abs = 1.5), a 1:2 dilution was made

with this mixture before adding the color reagents. In all cases, Fe(III) concentration was quantified before and after alkalization.

C.3. Determination of nitrate concentration in a NO_3^- to NH_4^+ reduction process under acidic conditions.

In order to evaluate the applicability of the technique, a nitrate reduction experience was performed. The experiment was conducted in a 500 mL batch reactor. The pH of the solution was adjusted to 3.0 with H_2SO_4 0.1 M. The concentrations of NO_3^- and Fe(II) were 0.21 mg.L^{-1} and 13.24 mg.L^{-1} respectively. 1.0153 g of Fe^0 was added to the solution. The reactor was stoppered with parafilm and, the dissolved oxygen was removed from the solution by purging with nitrogen during 15 minutes through a syringe. Subsequently, the reactor was kept at room temperature and darkness to avoid the eventual ambient light effects. Samples were withdrawn at different elapsed times through the syringe and, after this, anoxic conditions were reestablished as described above. Each sample were divided in two aliquots. The first aliquot was used to determine NH_4^+ concentration, whereas the second was subjected to the alkalization and centrifugation process described in section C.2 and passed through Cd-copperized column to determinate nitrate concentration. With the aim of assessing the possible effect of using N_2 to remove oxygen on nitrate concentration, N-NO_3^- was determined in two solutions containing 44 mg.L^{-1} . The first solution was processed immediately after preparation, whereas the second one was purged with nitrogen during 15 minutes before nitrate determination. The results for both measurements were the same, indicating, as expected, that purging studied solutions with N_2 has no influence on nitrate concentration.

III. RESULTS AND DISCUSSION

A. Preliminary experiments.

It has been reported (Gal *et al.*, 2004; Thabano *et al.*, 2004) that the sensitivity of the method depends on pH and increases when acidity decreases. The strategy most frequently employed to guarantee alkaline conditions is the addition of a buffer to the nitrate containing sample. The buffer solution is prepared by appropriate weighing of ammonium chloride into water and adjusting the pH to the desired value by addition of ammonia solution. Although some authors indicate that the maximum yield of nitrite production is obtained at pH 9-9.5 (Nydahl, 1976; Reis Lima *et al.*, 2006), the nitrate reduction is slow, and the risk of precipitation of released cadmium ions increases with increasing pH. Determinations with buffer pH adjusted to 8.5 have proven to reach acceptable sensibility (Thabano *et al.*, 2004). Moreover, 8.5 is the pH value indicated in accepted standard methods (APHA, 1985). In the present work pH of the NH_4Cl solutions has not been adjusted, therefore, the initial pH of the nitrate containing samples may affect the sensitivity and reproducibility of the measurements. The effect of the sample pH on the analysis of nitrate concentration was assessed by measuring N-NO_3^- in four solutions containing 100 $\mu\text{g.L}^{-1}$ of N-NO_3^- and different initial pH

Table 1: influence of pH of the sample on nitrate determination

pH	$[\text{N-NO}_3^-]$ measured/ $\mu\text{g.L}^{-1}$
5.65	98.8
6.79	101.2
7.91	100.5
9.06	102.6

Table 2: influence of ionic strength, associated to Na_2SO_4 concentration, on nitrate determination

$[\text{Na}_2\text{SO}_4]/\text{M}$	$[\text{N-NO}_3^-]$ measured/ $\mu\text{g.L}^{-1}$
0	96.8
0.05	99.4
0.1	97.4
0.15	98.7

values. Table 1 shows the average concentrations obtained at each pH.

The influence of salinity on nitrate determination has also been reported (Collos *et al.*, 1992). In a previous study, some authors (Stewart and Elliott, 1996) indicated that salinity can interfere in the reaction between nutrients and colour reagents resulting in the variable formation of reaction products, and that, in the case of the determination of nitrate where a copper-cadmium column is used to reduce nitrate to nitrite, the salt effect is a function of the buffer type used in the reduction process.

With the aim of evaluating the salt effect on the quantification of nitrate in solutions with different ionic strength, NO_3^- concentration was measured as explained above in four solutions containing 100 $\mu\text{g.L}^{-1}$ of N-NO_3^- and different Na_2SO_4 concentrations. The average nitrate concentrations obtained are depicted in Table 2.

The results presented in Tables 1 and 2 suggest that neither ionic strength, nor pH of the sample exert significant effects on the determination of nitrate under the studied conditions. In all cases the measured values match the expected N-NO_3^- concentration value within the experimental error (differences lower than 4%).

B. Determination of nitrate concentration in acid solutions in the presence of Fe(III).

Different methods for the analysis of nitrate in aqueous media have been described (Tang *et al.*, 2012; Zuo *et al.*, 2006; Yao *et al.*, 1998; APHA, 1985), each method being suitable depending on sample characteristics. One of the most frequently employed is the reduction of nitrate using copperized cadmium and the subsequent determination of nitrite by formation of a highly colored azo dye that is measured colorimetrically. This technique is often used for natural water determinations, where normal pH is around neutrality. The range of pH of most open lakes is between 6 and 9 (Wetzel, 2001), whereas, the rivers studied by the United Kingdom Land Ocean Interaction Study (LOIS) program, showed a pH variation of about 6.6 to 10.4 (Neal *et al.*, 1998).

Natural waterbodies at low pH are scarce. Nearly all natural, unpolluted waters with pH values less than 4, occur in volcanic regions that receive strong mineral acids, particularly, sulphuric acid. An example of these systems is Cavihue lake, a natural acidic lake (pH

=2.56) with high concentrations of iron and other metals (Pedrozo *et al.*, 2001). The column method for nitrate quantification is not directly applicable on acidic samples, such as Cavihue lake water. Solutions that are too acidic result in nitrate being reduced further than the nitrite step (Grasshoff *et al.*, 1983). In this case the analysis will result in nitrate values lower than the real value. On the other hand, at low pH, solubility (and therefore, concentration) of metal salts increases. Several of these metal cations, as iron and copper, are known interferences of the method (APHA, 1985). In order to minimize both over-reduction reactions of nitrate and the effect of interferences, we propose a simple pre-treatment of acidic solutions: alkalization of the solutions and subsequent centrifugation.

With the aim of evaluating the validity of the suggested procedure, artificial acidic mixtures with high iron load and different nitrate concentrations were prepared. The solutions were processed as described in section C.2. and nitrate concentration was measured by Copperized-Cd column method. Iron was quantified before and after alkalization. The results obtained are presented in Table 3, and show that, in all cases, the treatment removes more than 99% of the iron. The final load of Fe(III) lies below the concentration that interferes the method (APHA, 1985).

The values of recovered nitrate are in good agreement with expected results. At high concentrations (from 0.05 to 0.494 mg.L⁻¹), the differences between the measured and the expected values are $\leq 6\%$. At low nitrate amounts (from 0.01 to 0.02 mg.L⁻¹), the differences between the expected and the obtained concentrations are around 5 $\mu\text{g.L}^{-1}$. This value, although stands for a small concentration of nitrate, represents a significant percentage of the expected value. Taking into account these results, it can be considered that the proposed method is applicable for nitrate concentrations $\geq 0.05 \text{ mg.L}^{-1}$.

C. Determination of nitrate concentration during a NO_3^- to NH_4^+ reduction process under acidic conditions.

Several authors have studied the reduction of nitrate to ammonia in the presence of iron and in acidic conditions (Cheng *et al.*, 1997). Particularly, Huang *et al.* (1998), reported that under anoxic conditions, nitrate reduction rate is enhanced when pH decreases, being the reaction products NH_4^+ and Fe(II). Although these authors used ion selective electrode and ion chromatography, respectively, to quantify nitrate, under the predominant conditions of these systems (low pH and high iron concentration), the method proposed in this work should be suitable to determine nitrate concentration.

In order to assess the applicability of the modified method on a practical case, a nitrate reduction experience was carried out. The removal of oxygen to reach the condition of anoxia was obtained as previously explained in section II. Methods. C. Experimental Procedures and Setup. C3. The evolution of nitrate and ammonia concentrations was followed for 7 days. N-NO_3^-

Table 3: quantification of nitrate by Copperized-Cd column method in acidic solutions with high iron content.

$[\text{N-NO}_3^-]$ added ^a mg L ⁻¹	pH ₀	Fe(III) ₀ mg L ⁻¹	pH _f	Fe(III) _f mg L ⁻¹	$[\text{N-NO}_3^-]$ measured ^b mg L ⁻¹
0.010	2.93	21.28	6.57	0.10	0.014
0.020	2.92	19.99	6.56	0.15	0.026
0.049	2.61	24.79	6.25	0.11	0.052
0.252	2.97	23.21	6.61	0.17	0.256
0.494	2.85	22.88	6.31	0.17	0.476

^aAdded concentration of nitrate was calculated from the concentration indicated in the commercial standard.

The subscript "0" stands for the condition before alkalization.

The subscript "f" stands for the condition after alkalization and centrifugation.

^bMeasured concentration was obtained after alkalization and centrifugation of artificial solutions.

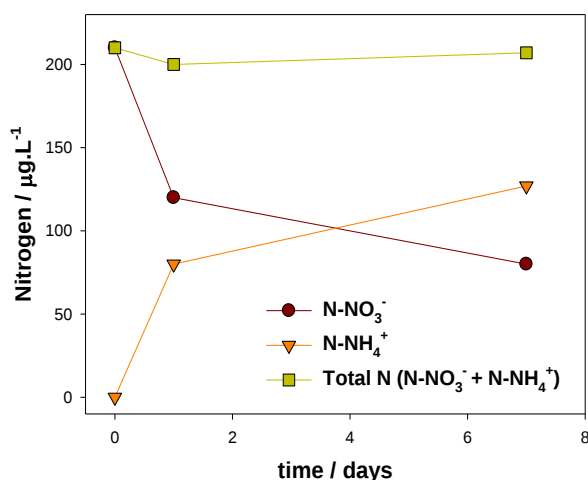


Figure 1. Temporal evolution of nitrated species under acidic and anoxic conditions. $[\text{NO}_3^-]=0.21 \text{ mg.L}^{-1}$, $[\text{Fe(II)}]=13.24 \text{ mg.L}^{-1}$, $\text{Fe}^0=1.0153 \text{ g}$, N_2 atmosphere.

and N-NH_4^+ were quantified as described in section B. Total Nitrogen was calculated by adding, at each time, the measured concentrations of ammonia and nitrate. The obtained results are depicted in Fig 1.

Figure 1 shows, in agreement with previously reported results (Cheng *et al.*, 1997; Huang *et al.*, 1998), an increase in NH_4^+ concentration concomitant with a decrease in NO_3^- concentration. Given that, using the proposed method, we obtained similar results than those described by others authors who used other techniques for comparable systems, it can be inferred that the applicability of the modified method is acceptable. Total nitrogen (calculated as the sum of N-NH_4^+ and N-NO_3^-) remained practically constant throughout the experiment. This result suggests that NH_4^+ is the only end product of NO_3^- reduction, which is consistent with that reported by Cheng *et al.* (1997) and Huang *et al.* (1998)

IV. CONCLUSIONS

A simple modification of the conventional cadmium copper column method for nitrate determination in neutral aqueous systems has been proposed with the aim of broaden the applicability of the method to lower values of pH and high iron load.

Under the studied conditions, variations on pH and ionic strength showed no influence on the method efficiency.

The modification proposed for nitrate determination method in acidic mixtures allows the determination of NO_3^- concentrations even lower than the maximum accepted level in artificial acidic solutions with high iron concentrations.

Although further investigation is necessary to establish whether the proposed method is effective to determine nitrate in natural matrixes at low pH values the results reported here may be the basis for developing suitable method for natural acidic aqueous samples.

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REFERENCES

- Almasri, M.N. and J.J. Kaluarachchi, "Modeling nitrate contamination of groundwater in agricultural watersheds," *J. Hydrol.*, **343**, 322-229 (2007).
- APHA, *Standard Methods for the Examination of water and wastewater*, 16 edn. American Public Health Association, Washington, DC (1985).
- CAA, *Código Alimentario Argentino. Bebidas Hídricas, agua y aguas gasificadas*, http://www.anmat.gov.ar/alimentos/normativas_alimentos_caa.asp. vol Ley 18284. http://www.anmat.gov.ar/alimentos/normativas_alimentos_caa.asp (2012).
- Collos, Y., K. Yin and P.J. Harrison, "A note of caution on reduction conditions when using the cadmium-copper column for nitrate determinations in aquatic environments of varying salinities," *Mar. Chem.*, **38**, 325-329 (1992).
- Cheng, F., R. Muftikian, Q. Fernando and N. Korte, "Reduction of Nitrate to ammonia by zero-valent Iron," *Chemosphere*, **35**, 2689-2695 (1997).
- EPA, *2011 Edition of the Drinking Water Standards and Health Advisories*, **EPA 820-R-11-002**, United States Environmental Protection Agency Office of Water, Washington (2011).
- EPA, *Water: Monitoring & Assessment. 5.7 Nitrates*, <http://water.epa.gov/type/rsl/monitoring/vms57.cfm>, United States Environmental Protection Agency (2012).
- Gal, C., W. Frenzel and J. Möller, "Re-Examination of the Cadmium Reduction Method and Optimisation of Conditions for the Determination of Nitrate by Flow Injection Analysis," *Microchim. Acta*, **146**, 155-164 (2004).
- Geller, W., H. Klapper and M. Schultze, "Natural and anthropogenic sulfuric acidification of lakes," In: *Acidic mining lakes: acid mine drainage, limnology and reclamation*, Springer, Berlin (1998).
- Grasshoff, K., M. Ehrhardt and K. Kremling, *Method of Seawater Analysis*, Second edn., Verlag Chemie, Weinheim (1983).
- Gulis, G., M. Czompolyova and J.R. Cerhanw, "An Ecologic Study of Nitrate in Municipal Drinking-Water and Cancer Incidence in Trnava District, Slovakia," *Environmental Research Section A*, **88**, 182-187 (2002).
- Herzsprung, P., A. Duffek, K. Friese, M. de Rechter, M. Schultze and W.V. Tümpling, "Modification of a continuous flow method for analysis of trace amounts of nitrate in iron-rich sediment porewaters of mine pit lakes," *Wat. Res.*, **39**, 1887-1895 (2005).
- Huang, C.-P., H.-W. Wang and P.-C. Chiu, "Nitrate reduction by metallic Iron," *Wat. Res.*, **32**, 2257-2264 (1998).
- Istvánovics, V. "Eutrophication of Lakes and Reservoirs," *Encyclopedia of Inland Waters*, vol. Reference Module in Earth Systems and Environmental Science, Elsevier, Budapest (2009).
- Lapa, R.A.S., J.L.F.C. Lima and I.V.O.S. Pinto, "Sequential Injection Analysis-Based System for on line Monitoring of Nitrite and Nitrate in Wastewaters," *Anal. Sci.*, **16**, 1157-1160 (2000).
- Neal, C., W.A. House and K. Down, "An assessment of excess carbon dioxide partial pressures in natural waters based on pH and alkalinity measurements," *Sci. Total Environ.*, **210-211**, 173-185 (1998).
- Nixdorf, B., D. Lessmann and R. Deneke, "Mining lakes in a disturbed landscape: Application of the EC Water Framework Directive and future management strategies," *Ecol. Eng.*, **24**, 67-73 (2005).
- Nydahl, F., "On the optimum conditions for the reduction of nitrate to nitrite by cadmium," *Talanta*, **23**, 349-357 (1976).
- Pedrozo, F., L. Kelly, M. Diaz, P. Temporetti, G. Baffico, R. Kringel, K. Friese, M. Mages, W. Geller and S. Woelfl, "First results on the water chemistry, algae and trophic status of an Andean acidic lake system of volcanic origin in Patagonia (Lake Caviahue)," *Hydrobiologia*, **452**, 129-137 (2001).
- Pfaff, J.D., *Method 300.0 Determination of inorganic anions by ion chromatography*, Environmental Monitoring Systems Laboratory Office of research and development, US Environmental Protection Agency EPA (1993).
- Reis Lima, M.J., S.M.V. Fernandes and A.O.S.S. Rangel, "Determination of nitrate and nitrite in dairy samples by sequential injection using an inline cadmium-reducing column," *Int. Dairy J.*, **16**, 1442-1447 (2006).
- Stewart, B.M. and P.A.W. Elliott, "Systematic salt effects in the automated determination of nutrients in seawater," *Wat. Res.*, **30**, 869-874 (1996).
- Stumm, W. and J.J. Morgan, *Aquatic Chemistry*, 2nd edn. Wiley-Interscience, New York (1981).
- Tang, W., J. Ping, K. Fan, Y. Wang, X. Luo, Y. Ying, J. Wu and Q. Zhou, *All-solid-state nitrate-selective electrode and its application in drinking water*, *Electrochim Acta*, **81**, 186-190 (2012).

- Thabano, J.R.E., D. Abong'o and G.M. Sawula, "Determination of nitrate by suppressed ion chromatography after copperised-cadmium column reduction," *J. Chromatogr. A*, **1045**, 153-159 (2004).
- Varekamp, J.C., "The volcanic acidification of glacial Lake Caviahue, Province of Neuquen, Argentina," *J. Volcanology Geot. Research*, **178**, 184-196 (2008).
- Vione, D., G. Falletti, V. Maurino, C. Minero, E. Pelizzetti, M. Malandrino, R. Ajassa, R.-I. Olariu and C. Arsene, "Sources and Sinks of Hydroxyl Radicals upon Irradiation of Natural Water Samples," *Environ. Sci. Technol.*, **40**, 3775-3781 (2006).
- Wakida, F.T. and D.N. Lerner, "Non-agricultural sources of groundwater nitrate: a review and case study," *Wat. Res.*, **39**, 3-16 (2005).
- Wetzel, R.G., *Limnology, Lake and River Ecosystems*, Third edn. Academic Press, San Diego (2001).
- WHO, "Nitrate and Nitrite in Drinking-water Background document for development of WHO Guidelines for Drinking-water Quality," In: *WHO Guidelines for Drinking-water Quality*, 4th edn. World Health Organization, Geneva (2011).
- Yao, W., R.H. Byrne and R.D. Waterbury, "Determination of Nanomolar Concentrations of Nitrite and Nitrate in Natural Waters Using Long Path Length Absorbance Spectroscopy," *Environ. Sci. Technol.*, **32**, 2646-2649 (1998).
- Zhang, X., Z. Xu, X. Sun, W. Dong and D. Ballantine, "Nitrate in shallow groundwater in typical agricultural and forest ecosystems in China 2004-2010," *J. Environ. Sci.*, **25**, 1007-1014 (2013).
- Zuo, Y., C. Wang and T. Van, "Simultaneous determination of nitrite and nitrate in dew, rain, snow and lake water samples by ion-pair high-performance liquid chromatography," *Talanta*, **70**, 281-285 (2006).

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