Indirect determination of nitrate by electrothermal atomic absorption spectrometry using an on-line cadmium microcolumn

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The proposed indirect automatic method is based on the reduction of nitrate to nitrite using a cadmium reductant microcolumn where the flow of the sample through the microcolumn oxidizes the metallic cadmium to Cd\textsuperscript{II}, which is measured by electrothermal atomic absorption spectrometry. The automatic system is achieved simply by replacing the sample tip of the autosampler arm with the reductant microcolumn. This system results in a powerful integrated system which permits fully automated operation, avoiding time-consuming manual work and enhancing the sensitivity. The manifold was evaluated with respect to accuracy, precision and sensitivity for nitrate determination in water, the detection limit achieved being 0.05 \( \mu \text{g} \text{ ml}^{-1} \) (3\( s \)) with a sampling rate of about 35 h\textsuperscript{-1}. A 2.3\% relative standard deviation was obtained in a repeatability study (\( n = 11 \) ) at the 0.2 \( \mu \text{g} \text{ ml}^{-1} \) level of nitrate and 2.6\% at the 8.0 \( \mu \text{g} \text{ ml}^{-1} \) level. Some ions which commonly occur with nitrate in water samples, such as Na\textsuperscript{+}, Ca\textsuperscript{2+} and Mg\textsuperscript{2+}, interfered to a certain extent, although these interferences were minimized by passing of the sample through an Amberlite cation-exchange resin. The method was applied to samples of bottled and spring waters with good results. The matrix effects were minimized by using the standard addition method.

**Keywords:** Electrothermal atomic absorption spectrometry; on-line cadmium reduction; nitrate; waters

In recent years there has been increasing interest in the development of automatic methods for the routine determination of nitrite and nitrate. These methods can be divided into two groups: (a) flow injection (FI) methods based on earlier methods, namely the direct determination of nitrite by the modified Griess reaction and the sum of nitrite and nitrate after passage of the sample through a column of copper-coated graphite tube as a single drop of a precisely defined volume. The maximum absorbance of the unreacted Ag\textsuperscript{+} ions was measured in an air–acetylene flame. Based on the same philosophy, Valcárce and co-workers\textsuperscript{a} developed two methods for the sequential determination of nitrate and nitrite by continuous liquid–liquid extraction with a flow injection flame atomic absorption detection system, both based on the reaction of nitrate with a Cu\textsuperscript{II} complex to form an ion pair that is extracted into isobutyl methyl ketone. The displaced copper in the extract is determined in the flow injection system by AAS detection. Anwar et al.\textsuperscript{b} described another method that involves reduction of nitrate by Hg\textsuperscript{2+} in an air–acetylene flame. No methods have been reported for the automatic determination of nitrate by electrothermal atomic absorption spectrometry (ETAAS), no doubt owing to the discrete non-flow-through nature of this technique. Off-line FI procedures involving the collection of the treated sample in a vessel followed by conventional sample introduction\textsuperscript{e,f} have the disadvantages of being laborious and time consuming. However, ETAAS is one of the most sensitive techniques for the determination of a large number of elements with detection limits in the \( \mu \text{g} \text{ l}^{-1} \) range.

Water analysis has an important place in the chemical analysis of environmental samples. The development of new methods, or the adaptation of existing methods, for the analysis of water is a major task for analytical chemists, especially since each type of water has its own specific constituents and any one procedure may not be completely amenable to different water types. Hence the aim of this investigation was to develop and evaluate a sensitive, fast and reliable method for the determination of trace amounts of nitrate in water.

Sample introduction into the graphite tube is usually performed either manually using a microlitre pipette or automatically by a special autosampler. In this work an automatic system for the indirect determination of nitrate was achieved simply by replacing the sample tip of the autosampler arm with a cadmium reductant microcolumn. In this system the sample passes through the microcolumn and oxidizes the metallic cadmium to Cd\textsuperscript{II}, which is directly deposited in the graphite tube as a single drop of a precisely defined volume. The sample passes twice through the microcolumn, during sample loading and unloading, which produces a higher concentration of oxidized cadmium. The wash step of the microcolumn is performed in a direction counter to that of sample loading because it is known that when small or medium sized grains of the reductant are used (easier column packing and higher reduction yield) reversal of flow between the washing and reduction steps is necessary to preserve the degree of column compactness and yield reproducible signals.\textsuperscript{c,d}

**Experimental**

**Reagents**

All reagents used were of the highest purity available and of at least analytical-reagent grade. Doubly de-ionized water (18 M\( \Omega \) cm\textsuperscript{-1} ) was used throughout. A nitrate stock standard solution was prepared from Mg(NO\textsubscript{3})\textsubscript{2}·6H\textsubscript{2}O ( > 99.995\%, Aldrich, Milwaukee, WI, USA). Working standard solutions were prepared daily by appropriate dilution as required. The standards were adjusted to pH 4.7 with 0.001 mol l\textsuperscript{-1} HCl before they were adjusted to volume.

**Instrumentation and procedure**

A Perkin-Elmer (Norwalk, CT, USA) Zeeman/4100Zl atomic absorption spectrometer equipped with an AS-70 furnace...
autosampler was used throughout. Pyrolytic graphite coated tubes with pyrolytic graphite platforms were used in all experiments. The light source was a cadmium hollow cathode lamp operated at 4 mA; the wavelength was set to 228.8 nm with a spectral slit-width of 0.7 nm. The optimized graphite furnace program is shown in Table 1. Peak-height absorbance was used for determinations. The software of the instrument permits the pipetting speed of the pump of the autosampler to be varied from 40 to 100%, thus a pipetting speed for the autosampler of 40% was programmed to increase the contact time of the sample with the metallic cadmium, which produces a greater amount of oxidized cadmium and so increases the sensitivity. No chemical modifiers were used because nitrate is the most commonly used chemical modifier in cadmium determinations. An injection volume of 6 µl was used for the analysis of samples containing nitrate in the range 0.5–14.0 µg ml⁻¹; for samples containing lower nitrate concentrations (0.09–0.5 µg ml⁻¹) an injection volume of 10 µl was recommended.

The microcolumn containing the reductant was a polytetrafluoroethylene tube (4 cm × 1.5 mm id) packed to 2.0 cm from the top with granulated metallic cadmium (0.5–1 mm grain size). On the end of this column was placed a piece of sample capillary of the sampler arm, in imitation of the sample tip of the sampler arm. Details on the design of this microcolumn can be seen in Fig. 1. Thus the sample tip of the sampler arm was replaced with this microcolumn, permitting normal working of the sampler.

The sequence of operation of the instrument is as follows: the sampler arm lowers the sample capillary into the sample or standard vessel and a fixed volume of solution is loaded and passes through the metallic cadmium grains, oxidising the metallic cadmium, then the sampler arm swings over to the graphite furnace and injects the solution into the graphite tube, with the sample passing a further time through the cadmium grains. At the end of this sequence the sampler arm returns to the wash position and the cycle of furnace operation commences (Table 1).

This system of loading and unloading of the sample avoids the continuous increase in column compactness caused when the flow through the column is always in the same direction. Hence this integrated system represents a powerful system which permits fully automated operation.

Table 1 Graphite furnace temperature programme

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature/°C</th>
<th>Ramp time/s</th>
<th>Hold time/s</th>
<th>Argon flow rate/ml min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>110</td>
<td>1</td>
<td>15</td>
<td>250</td>
</tr>
<tr>
<td>2</td>
<td>130</td>
<td>5</td>
<td>35</td>
<td>250</td>
</tr>
<tr>
<td>3</td>
<td>750</td>
<td>10</td>
<td>15</td>
<td>250</td>
</tr>
<tr>
<td>4</td>
<td>1700</td>
<td>0</td>
<td>5</td>
<td>0 (read 5 s)</td>
</tr>
<tr>
<td>5</td>
<td>2400</td>
<td>1</td>
<td>2</td>
<td>250</td>
</tr>
</tbody>
</table>

**Results and discussion**

**Effect of pH on efficiency of redox reaction.**

The dependence of the redox reaction on the pH of the solution is one of the important parameters that can have a significant influence on the over-all performance of the indirect method. The influence of pH was studied over the range 3.0–10.0; nitrate reference solutions were prepared in HCl of different concentrations and the absorbance (for Cd) was recorded. The results are shown in Fig. 2. The optimum range of pH for the indirect determination of nitrate is 4.2–4.7, adjusted using 0.6 ml of 0.001 mol l⁻¹ HCl for each 25 ml of standard solution.

**Design of the reductant microcolumn.**

The design of the reductant microcolumn was based on the form of the sample tip of the sampler arm, permitting normal working of the sampler when the sample tip is replaced by the microcolumn (Fig. 1).

The column packing was performed according to Bermudez et al., who studied the influence of the grain size and nature of the reductant (cadmium or coppered cadmium). Different heights of packing in the column were tested but always using cadmium grains with a particle size between 0.5 and 1 mm because the time needed to attain reproducible signals with this type of packing is the shortest and the column lifetime is the longest. The optimum height of cadmium packing in the column was established as 2 cm from the top.

**Calibration graph, sensitivity and precision.**

Under the optimum experimental conditions, different linear calibration graphs were obtained with different injection volumes. When high sensitivity is required an injection volume of 10 µl was chosen as the optimum; with this injection volume a linear calibration graph was obtained in the range 0.09–0.5 µg ml⁻¹. With an injection volume of 6 µl, the calibration graph exhibited two linear portions, 0.5–4.0 and 4.0–14.0 µg ml⁻¹. The signal appeared 90 s after sample injection. Within 15 s after injection the analytical system was ready for the next injection, thus giving a sample throughput of about 35 h⁻¹.

The detection and determination limits, defined as the concentrations of analyte giving signals equivalent to three and ten times, respectively, the standard deviation of the blank plus the net blank intensity, were 0.05 and 0.09 µg ml⁻¹, respectively, calculated with an injection volume of 10 µl. The absolute blank value was obtained by using the reduction and determination procedures with de-ionized water adjusted to pH 4.2–4.7.

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**Fig. 1** Schematic diagram of the microcolumn assembly.

**Fig. 2** Effect of pH on efficiency of redox reaction.
The precision of the method for aqueous standards, evaluated as the relative standard deviation (RSD, n = 11), was 2.3% at the 0.1–2.0 µg ml⁻¹ level of nitrate and 2.6% at the 2.0–8.0 µg ml⁻¹ level.

**Interferences**

As the described procedure involves the oxidation of cadmium to Cd²⁺, ions that can change the rate of electron transfer or the redox potential can possibly interfere with the analytical method. For this reason, the effect of various ions that commonly occur with nitrate in natural water samples was examined under the optimum working conditions. For this study, different amounts of the ionic species tested were added to the solutions and blanks treated in the same way. Based on the interference study, for the analysis of bottled waters, passage through the Amberlite column is not necessary because the levels of Ca²⁺, Mg²⁺ and Na⁺ were lower than the interference levels (the specifications on the label are given in Table 3). This was confirmed for Aguavida and Lanjarón waters; in these instances the only treatment necessary was adjusting the pH to 4.2–4.7 with 0.001 mol l⁻¹ HCl. However, high results for Evian bottled water were found; this can be attributed to the relatively high Ca²⁺ concentration in the sample (although not exceeding the tolerated ratio). Hence, when these samples were first passed through the Amberlite column, the results obtained showed much better agreement with those obtained by the conventional method. For bottled waters matrix effects were apparent for the ETAAS measurements and the determination was performed with the standard additions method. Blanks were always run in the same way and for each sample examined the value of the blank was subtracted. The results are given in Table 4 as the average of four replicates. As can be seen, the concentrations provided by the conventional and proposed methods agreed much better with those obtained by the ETAAS measurements and the determination was performed with the standard additions method. Blanks were always run in the same way and for each sample examined the value of the blank was subtracted. The results are given in Table 4 as the average of four replicates. As can be seen, the concentrations provided by the conventional and proposed methods.

**Table 2** Interferences in the proposed method

<table>
<thead>
<tr>
<th>Ion or species</th>
<th>Without Amberlite</th>
<th>With Amberlite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ba²⁺</td>
<td>&gt; 2000</td>
<td>98.5</td>
</tr>
<tr>
<td>Chloride</td>
<td>1000</td>
<td>98.5</td>
</tr>
<tr>
<td>Ni²⁺</td>
<td>300</td>
<td>102.0</td>
</tr>
<tr>
<td>Sulfate</td>
<td>200</td>
<td>98.1</td>
</tr>
<tr>
<td>Phosphate</td>
<td>120</td>
<td>95.5</td>
</tr>
<tr>
<td>K⁺</td>
<td>80</td>
<td>95.2</td>
</tr>
<tr>
<td>Zn²⁺</td>
<td>80</td>
<td>101.6</td>
</tr>
<tr>
<td>Fe²⁺</td>
<td>80</td>
<td>99.0</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>50</td>
<td>101.8</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>50</td>
<td>102.0</td>
</tr>
<tr>
<td>Na⁺</td>
<td>24</td>
<td>102.2</td>
</tr>
<tr>
<td>Cu²⁺</td>
<td>—</td>
<td>10</td>
</tr>
</tbody>
</table>

**Results for some water samples**

In order to test the accuracy and applicability of the proposed method to the analysis of real samples, several bottled waters and some locally available spring water samples were analysed for nitrate by the proposed method and by the conventional method based on the modified Griess reaction. The samples were analysed using the proposed procedure. Matrix interference was checked by comparison of the slopes of the calibration graphs with those using the standard addition method. The nitrate concentration in the two spring waters was evaluated from a calibration graph constructed with standard solutions and blanks treated in the same way. Based on the interference study, for the analysis of bottled waters, passage through the Amberlite column is not necessary because the levels of Ca²⁺, Mg²⁺ and Na⁺ were lower than the interference levels (the specifications on the label are given in Table 3). This was confirmed for Aguavida and Lanjarón waters; in these instances the only treatment necessary was adjusting the pH to 4.2–4.7 with 0.001 mol l⁻¹ HCl. However, high results for Evian bottled water were found; this can be attributed to the relatively high Ca²⁺ concentration in the sample (although not exceeding the tolerated ratio). Hence, when these samples were first passed through the Amberlite column, the results obtained showed much better agreement with those obtained by the conventional method. For bottled waters matrix effects were apparent for the ETAAS measurements and the determination was performed with the standard additions method. Blanks were always run in the same way and for each sample examined the value of the blank was subtracted. The results are given in Table 4 as the average of four replicates. As can be seen, the concentrations provided by the conventional and proposed methods agreed much better with those obtained by the ETAAS measurements and the determination was performed with the standard additions method. Blanks were always run in the same way and for each sample examined the value of the blank was subtracted. The results are given in Table 4 as the average of four replicates. As can be seen, the concentrations provided by the conventional and proposed methods.
methods were generally consistent, but the precision in some cases was poor.

Conclusion

This study has shown that the method described allows the rapid determination of nitrate. The analytical scheme of the proposed system is simpler than that of other conventional procedures because it combines reduction and detection in a single analytical set-up. The use of organic reagents is also avoided. The high speed, ease of use and automated handling make this method suitable for nitrate determination in water samples. The selectivity obtained is comparable to those using conventional methods. The detection limit, obtained with an injection volume of 10 µl, was 0.05 µg ml\(^{-1}\) of nitrate, which compares favourably with other values obtained using copperised cadmium or cadmium columns.\(^4\)\(^-\)\(^6\)\(^,\)\(^17\) For example, the detection limits obtained by spectrophotometry using different chelating agents ranged from 0.1 to 1.0 µg ml\(^{-1}\) of nitrate.\(^4\)\(^-\)\(^6\)\(^,\)\(^17\) Further, this work opens a way for the automated indirect determination of other anions by ETAAS, which has been developed very little so far.

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References