Automated Colorimetric Method for Nitrate Analysis

A NON-HAZARDOUS ALTERNATIVE TO TRADITIONAL METHODS FOR AQUEOUS SOLUTIONS *by Craig R. Chinchilla*

"The nitrate-to-nitrite reduction is consistently between 95 percent and 105 percent, which is a dramatic improvement over traditional nitrate methods."

Several methods exist for determining nitrate in aqueous solutions. However, the most commonly performed USEPA-approved methods are problematic and can be unreliable. USEPA methods 353.1 nitrate Hydrazine Reduction[1,2] and 353.2 nitrate Cadmium Reduction[3] utilize chemicals that are carcinogenic and highly toxic. Hazardous waste is generated when performing these methods and disposal is costly. Other methods performed by ion chromatography (IC)[4,5] and ion selective electrode (ISE)[6] are slow and can have issues when performing analysis on samples with high ionic strength, such as wastewater, ground water and soil extracts. The new Systea Easy (1 – Reagent) nitrate method was developed to eliminate the problems associated with these traditional methods and improve performance.

Method summary

The method was primarily designed to be performed by automated discrete analysis. The method can also be performed by traditional flow analysis instrumentation. However, discrete analysis is rapidly becoming the preferred technique for environmental ion analysis in laboratories throughout the United States. The advantages include ease of use, minimization of waste and reagent consumption, and true unattended operation.

The procedure for determining nitrate utilizes a reaction in which nitrate is reduced to Nitrite by a proprietary reagent "R1." The reaction is slow and requires greater than 12 minutes for 100 percent reduction of nitrate to Nitrite at 50°C. The reduced nitrate is then treated under acidic conditions to form a highly colored soluble dye, which is measured colorimetrically between 520 and 550 nm. The final product measured represents the Nitrite ion originally present, plus that formed from the reduction of nitrate (nitrate+Nitrite). In order to determine the true nitrate concentration, the sample must also be analyzed separately for Nitrite to determine the amount originally present in the sample. The value obtained for Nitrite is then subtracted from the nitrate + Nitrite value to determine the true value for nitrate.

The method has several advantages over USEPA methods 353.1 nitrate Hydrazine Reduction, and 353.2 nitrate Cadmium Reduction, including elimination of hazardous waste and hydrazine and cadmium exposure. The method utilizes a non-hazardous, non-enzymatic reducing agent, protecting personnel and the environment. Also, potential liability associated with waste handling and disposal is significantly reduced or eliminated. Discrete analysis usually reduces waste generation one-third to one-sixth compared to traditional flow analysis techniques, with total reaction volumes of 300 to 700 µl per test.

Analytical performance is greatly enhanced by the use of the new method. The nitrate to Nitrite reduction is consistently between 95 percent and 105 percent, which is a dramatic improvement over traditional nitrate methods. In the cadmium reduction method, the reduction efficiency changes over time. Depending on the matrix, efficiency of the cadmium reduction can change quickly, causing the analysis to be outside of quality control limits. Examples include samples with high ionic strength, surfactants, and oils and grease, all commonly found in environmental matrices. Charging and recharging of the cadmium coil or column can also be uncertain from procedure to procedure. The introduction of air into a cadmium coil or column also reduces the efficiency of the reduction. When performing the hydrazine method, adjustments to the reagent quantity must be made for proper reduction. High chlorides are known to interfere with the reduction in the hydrazine method. IC and ISE methods experience similar matrix interference problems with samples of high ionic

strength. After extensive testing on various matrices, no matrix interference problems have been observed when using the Easy (1 – Reagent) nitrate method.

Depending on how the method is performed, the associated reagent cost is approximately four cents or less per test. Furthermore, the reagent cost is dramatically less than that of other non-hazardous methods for nitrate, such as enzymatic tests. There are also savings from a labor standpoint, when considering the amount of time that is required to perform the traditional tests. Since the overall performance of the new method is better—and matrix interference problems are not present—analytical runs need not be re-run, saving laboratories time. Finally, since the method has been developed for the discrete analyzer, it can truly run unattended.

"Discrete analysis is rapidly becoming the preferred technique for environmental ion analysis in laboratories throughout the United States."

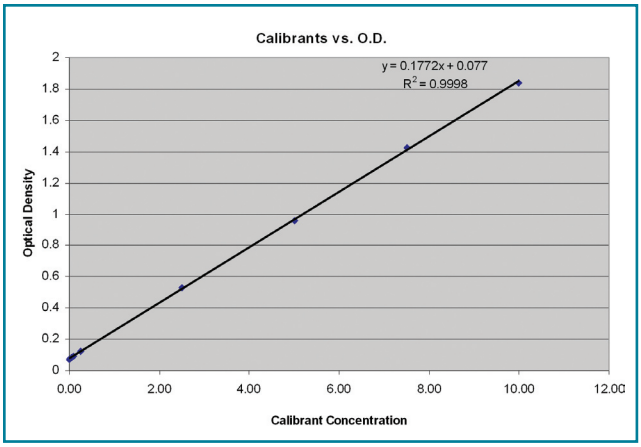
Inter-laboratory study

In order to substantiate the new method's reliability and performance, a comparative inter-laboratory study utilizing various USEPA-approved methods and sample matrices was conducted. The approach taken by the study was different from that of most studies, because the study not only analyzed a variety of sample matrices, but also compared the results of several different EPA-approved methods. Ten laboratories were selected to participate in the study. Each laboratory selected had a unique matrix type, which enabled the Systea method to be compared more rigorously to current USEPA methodology. The laboratories were also selected based on their expertise with particular matrices and the variety of instrumentation used.

The 10 laboratories chosen and various matrices were as follows:

(4) Wastewater treatment plants
(2) Commercial laboratories testing drinking water and wastewater
(1) University testing seawater
(1) Private laboratory testing seawater
(1) Drinking water treatment plant
(1) Laboratory testing soils

Selected samples from laboratories analyzing treated and untreated wastewater included various matrices with the following characteristics: Total Suspended Solids (TSS) greater than 40 mg/L, Total Dissolved Solids (TDS) greater than 100 mg/L, oil and grease greater than 20 mg/L, pre-treatment, and sludge samples. At laboratories analyzing seawater, real seawater samples were analyzed with NaCl concentrations greater than 120 mg/L (near coastal). At the chosen soils laboratory, samples that had been extracted with 2 N KCl were analyzed. Drinking water samples were analyzed from a drinking water plant performing chlorination, and from two commercial testing laboratories. Finally, samples from the commercial test laboratories included steel mill effluent and groundwater.



▲ *Regression Analysis of Calibration Curve 0.050-10.0 mg/L*

Each of the laboratories analyzed its matrix-specific, round robin, initial precision recovery (IPR) and method detection limit (MDL) samples. Standards, quality control (QC) samples and round robin samples were purchased from a third-party manufacturer (Analytical Products Group or APG) and split into 10 duplicate sets labeled 1 through 10 A and B. Each laboratory performed the analytical runs using its current methodology and range. Concurrently, Systea Scientific performed the same analytical tests using the new nitrate method with a range of 0.050–10.0 mg/L. Each of the 10 participating laboratories performed four analytical runs, for a total of 40 runs. Each of the runs was duplicated by Systea Scientific utilizing the new method. To confirm the operation of the method at other calibration ranges, a smaller test group of samples was also run at the following ranges: 0.003 – 0.150 mg/L, 0.020 – 2 mg/L and 0.5 – 50 mg/L.

Sample	Standards	Optical Density	Calc. Conc.
<BLANK>		0.0727	-0.001
<CAL1>	0.00	0.0716	-0.007
<CAL2>	0.05	0.0816	0.050
<CAL3>	0.10	0.0918	0.107
<CAL4>	0.25	0.1219	0.276
<CAL5>	2.50	0.5306	2.576
<CAL6>	5.00	0.9599	4.992
<CAL7>	7.50	1.422	7.592
<CAL8>	10.00	1.835	9.916

▲ *Calibration Data 0.050-10.0 mg/L*

Study results

A statistical analysis of the participating laboratories and the new method data was performed. An assumption was made that the accuracy of the APG samples would ultimately determine the accuracy of the analysis. If the APG round robin samples were found accurate and cross-instrument sample analysis results were erroneous, the APG results would be used as the standard of accuracy. No significant variation between the new method data and the participating laboratories' method data was observed. The results from the Systea method data were equal or superior to the results from the various data from the participating laboratories. Participating laboratories had variable amounts of recoveries and matrix interference issues, depending on the type of instrumentation and methodology employed. In general, labs performing flow analysis methods experienced fewer problems with matrix interferences than labs using IC and ISE techniques.

As part of the study, 10 mg/L nitrate and a Nitrite sample were tested at the end ofeach nitrate run to determine the percentage of recovery of nitrate to Nitrite. The average nitrate-to-nitrite recovery for all the analytical runs performed with the new method was 95.9 percent. This is quite remarkable, considering that perhaps the biggest problem with using the traditional USEPA colorimetric methods for nitrate is poor recovery. The data also demonstrated that the percentage of recovery was very consistent from run to run, with little or no variation.

Method detection limit (MDL) and method limit (ML) data were equally as impressive, or more impressive than, any data obtained during the study. Using a 0.050

Sample	Optical Density	Calc. Conc.		
MDL 0.050 mg/L 1	0.0810	0.046		
MDL 0.050 mg/L 2	0.0814	0.048		
MDL 0.050 mg/L 3	0.0809	0.046		
MDL 0.050 mg/L 4	0.0811	0.047		
MDL 0.050 mg/L 5	0.0809	0.046		
MDL 0.050 mg/L 6	0.0807	0.045		
MDL 0.050 mg/L 7	0.0808	0.045		
MDL 0.050 mg/L 8	0.0807	0.045		
MDL 0.050 mg/L 9	0.0806	0.044	St. Dev.	MDL
MDL 0.050 mg/L 10	0.0804	0.043	0.001433721	0.00404453

▲ *Method Detection Limit Example*

Avg MDL 7 (mg/L)	0.01119
Avg ML 7 (mg/L)	0.03558
MDL Pooled 7 (mg/L)	0.00416
ML (mg/L)	0.01323
Avg MDL 10 (mg/L)	0.011483
Avg ML 10 (mg/L)	0.036515
MDL Pooled 10 (mg/L)	0.004256
ML (mg/L)	0.013535

▲ *Statistical Results of Method Detection Limits*

mg/L sample, detection limits obtained were consistent and ranged between 0.010-0.015 mg/L. Continuous flow analyzers typically report MDL values of about 0.5 to 1 percent of the full-scale concentration of the range. The full-scale concentration of the Systea method study was 10 mg/L. Using the Avg MDL 7 and the MDL Pooled 7, the study obtained a MDL of 0.01119/10 * 100 = 0.11 percent and 0.00416/10 * 100 = 0.04 percent, respectively.

Conclusion

The new Systea Easy (1 – Reagent) nitrate method offers a suitable alternative to traditional nitrate methods for aqueous solutions. Problems associated with performing nitrate analysis, such as poor recovery and matrix interferences, are minimal or nonexistent. Greater method sensitivity and linear range enable both high- and low-range samples to be performed together without compromising performance.

The new method utilizes a non-hazardous reducing agent, eliminating hazardous waste and associated disposal costs. Potential liability associated with disposal of and exposure to carcinogenic chemicals is eliminated. Since the method has been designed for discrete analysis, labor costs associated with performing the analysis are minimized and true unattended operation is possible.

References

1. Methods for Chemical Analysis of Waters and Wastes - Nitrite 353.1, Nitrogen, nitrate-Nitrite (Colorimetric, Automated Hydrazine Reduction, U.S. EPA National Exposure Research Laboratory EPA-600/4-79/020 (NTIS PB 84-128677), Issued 1971; Reissued with Revision 1978.

2. APHA, AWWA,WEF, Standard Methods for the Examination of Water and Wastewater, 20th Edition - 4500-NO3-H Automated Hydrazine, American Public Health Association: Washington, DC, 1998.

3. Methods for Chemical Analysis of Waters and Wastes - nitrate 353.2, Rev 2.0 Determination of nitrate-Nitrite Nitrogen by Automated Colorimetry, U.S. EPA National Exposure Research Laboratory EPA-600/R93/100 (NTIS PB 84-120821), 1993.

4. Methods for Chemical Analysis of Waters and Wastes - nitrate 300.0, Rev 2.1 Determination of Inorganic Anions by Ion Chromatography, U.S. EPA National Exposure Research Laboratory EPA-600/R93/100 (NTIS PB 94-120810), 1993.

5. Methods for the Determination of Organic and Inorganic Compounds in Drinking Water, Volume 1 – 300.1, Rev 1.0 Determination of Inorganic Anions in Drinking Water by Ion Chromatography, U.S. EPA Office of Ground Water and Drinking Water/Technical Support Center (NSCEP PB 815-R-00-014), 1997.

6. APHA, AWWA, WEF, Standard Methods for the Examination of Water and Wastewater 20th Edition - 4500-NO3-D nitrate Electrode Method, American Public Health Association: Washington, DC, 1998.

Craig R. Chinchilla Sr. BS, *is general manager for Systea Scientific, LLC. He has many years of experience developing chemistry methods and working with discrete and flow analysis instrumentation. craig@easychem.com, 630-645-0600.*