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Determination by ion chromatography and spectrophotometry of the effects of preservation on nitrite and nitrate

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ABSTRACT

EPA method states that unpreserved samples analyzed for nitrite and nitrate must be analyzed within 48 h. If samples are preserved with sulfuric acid at pH < 2, then nitrite and nitrate are allowed a 28-day holding time. Early indications suggested that nitrite was not stable under acidic solutions and was in fact converted to nitrate within the holding time set by the methodology. Owing to this discrepency, an investigation was made of the effects of preservation on the stability and equilibrium of nitrite and nitrate in reagent-grade water, groundwater, leachate and surface-type waters. The results showed that when reagent-grade water is spiked with nitrite and nitrate, both anions are stable for > 30 days if simply kept refrigerated at 4°C, but when acidified to pH < 2 with sulfuric acid nitrite is rapidly converted to nitrate. When nitrite and nitrate were added to the matrices found in the environmental samples and held at 4°C, conversion of nitrite to nitrate only occurred in the surface water sample. This conversion is believed to have been caused by the presence of nitrogen-fixing bacteria. As it was believed that acid preservation would oxidize nitrite to nitrate, and bacteria could also cause conversion of nitrite and nitrate, we spiked these samples under highly basic conditions (preservation with sodium hydroxide at pH 12). It was hoped that these basic conditions would destroy the nitrogen-fixing bacteria and stabilize nitrite. The results indicated no conversion of nitrite during the 37-day test period in any of the test matrices when samples were preserved in base.

INTRODUCTION

Nitrite and nitrate are important parameters in ground water analysis [1]. Excessive amounts of nitrites have been shown to increase the methemoglobin in the blood of infants. A 35–50% increase causes headaches and a 70% increase is lethal. Nitrite in acidic solutions forms nitrous acid, which can react with secondary amines to form nitrosamines, many of which are carcinogenic [2].

U.S. Environmental Protection Agency (EPA) methodology states that unpreserved reagent-grade water and environmetal-type waters when analyzed for nitrite and nitrate are to be kept at 4°C and analyzed within 48 h. However, if samples are tested after 48 h, the procedure requires that samples need to be preserved with sulfuric acid at pH < 2 and stored at 4°C. Under these conditions, the samples are allowed a holding time of 28 days [3]. Early indications suggested that nitrite was not

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stable under acidic solutions and was in fact converted to nitrate within the holding time set by the methodology. Owing to this discrepency, an investigation was made of the effects of preservation on the stability and equilibrium of nitrite and nitrate in reagent-grade water, groundwater, leachate and surface-type waters.

The stabilities of nitrite and nitrate in acidic, basic and neutral waters were determined using a spectrophotometric method as stated in EPA Method 353.2 and by ion-exchange chromatography (IC) which is described in EPA Proposed Method B1011 [4,5]. The spectrophotometric method first measures nitrite, after reaction to form a colored complex, at a wavelength of 520 nm. Nitrate is reduced to nitrite and the combined nitrate plus nitrite is measured. The concentration of nitrate is determined by difference. In the chromatographic method, nitrite and nitrate are separated on an ion-exchange column with borate–gluconate as the eluent. The separated ions are measured by UV detection at 214 nm.

EXPERIMENTAL

This study was carried out in two parts. The first part focused on establishing the stability of nitrite and nitrate in neutral and acidic reagent-grade water. Four aliquots of reagent-grade water (obtained from a Milli-Q water purification system) were spiked with nitrite and nitrate at 0.5, 1.0, 2.0 and 5.0 mg/l. Four more aliquots were acidified to pH < 2 with sulfuric acid and spiked at the same levels with nitrite and nitrate.

The second part of the study focused on the stability of nitrite and nitrate in ground water, surface water and leachate samples, both unpreserved and preserved (basic). These field samples were collected from a local landfill in 5-gallon polyethylene containers and stored at 4°C. Each 5-gallon sample was split in order to obtain enough sample to test both preserved and unpreserved (samples were preserved with reagent-grade sodium hydroxide to pH 12). The three types of environmental samples, both unpreserved and preserved, were spiked with nitrite at levels of 0.5 and 1.0 mg/l for analysis on a TRAACS 800 AutoAnalyzer and 10.0 and 50.0 mg/l nitrite for analysis using a Waters ion chromatograph. Prior to analysis, samples tested by IC were diluted ten-fold and filtered through a 0.45-μm filter. Samples tested by the spectrophotometric method were filtered through a 0.45-μm filter prior to analysis. Once the initial samples had been prepared they remained in storage at 4°C except when sampled and analyzed at various intervals during the 37-day period.

Ion chromatographic method

The instrumentation used was a Waters single column ion chromatographic system (Millipore–Waters Chromatography Division, Milford, MA, U.S.A.). The system consisted of a high-performance liquid chromatographic pump delivering borate–gluconate eluent at 1.2 ml/min, an autosampler with a 100-μl injection loop, a high-capacity anion-exchange column with in-line precolumn filter and a UV–VIS detector set at 214 nm. Intergration was performed with a DEC Pro380 PC data station. The eluent consisted of a mixture of lithium gluconate and borate which forms a gluconate–borate anion complex. Sample anions pass into solution by exchange with eluent anions and ultimately are eluted from the column. The increase in absorbance was measured at 214 nm.

Spectrophotometric method

The spectrophotometric instrumentation used was a Bran and Luebbe TRAACS 800 AutoAnalyzer segmented-flow system consisting of a random-access autosampler sampling at 120 samples per hour, a multi-test cartridge for nitrite, a multi-test cartridge for nitrate + nitrite, a reagent sequencer, an IBM PC PS/2 30 data system and a UV–VIS detector set at 520 nm.

In the colorimetric reaction, nitrate is reduced by hydrazine solution to nitrite, which, together with the nitrite originally present, reacts with sulfanilamide to form a diazonium salt. This couples with N-(1-naphthyl)ethylenediamine dihydrochloride to form a highly colored azo dye which is measured at 520 nm. Nitrite and nitrate concentrations are determined by the difference in concentration before and after reduction.

Reagents and chemicals

Ion chromatographic method. Borate–gluconate concentrate contains 7.2 g of reagent-grade lithium hydroxide, 25.5 g of boric acid, 13.2 g of D-gluconic acid and 94 ml of glycerin diluted to 1 with water. The eluent is then prepared by diluting 20 ml of the concentrate and 120 ml of acetonitrile to 1 l with water. Purified (18 MΩ) water was obtained from a Milli-Q water purification system (Millipore-Waters). The eluent was then filtered through a 0.45-μm filter and degassed by suction. Analytical standards for nitrite and nitrate (200 ppm) were purchased from Wescan Instruments.

Spectrophotometric method. Copper(II) sulfate, Brij-35, hydrazine sulfate, hydrochloric acid, N-(1-naphthyl)ethylenediamine dihydrochloride, phosphoric acid, potassium nitrate, sodium hydroxide, sodium nitrite and sulfanilamide were used, with 18 MΩ water as above.

RESULTS AND DISCUSSION

Spiked reagent-grade water preserved at 4°C showed no deterioration of nitrite or nitrate during the 37-day test period. The nitrite levels in the unpreserved ground and leachate waters also remained stable up to 37 days. However, the surface water samples indicated some deterioration of nitrite after 14 days and it was totally converted to nitrate after 37 days (Fig. 1). This conversion of nitrite to nitrate is thought to be caused by nitrogen-fixing bacteria present in the surface water [6]. Reagent-grade water spiked with nitrite and nitrate and acidified with sulfuric acid to pH < 2 showed immediate deterioration of nitrite to nitrate. This would explain the poor nitrite spike recoveries experienced in our laboratory during routine analysis. Total conversion of nitrite to nitrate was observed within 14 days (Fig. 2).

The field water samples referred to above stored at 4°C and preserved with sodium hydroxide to a pH 12 showed excellent stability of nitrite up to 37 days (Fig. 3). The samples for IC were spiked with 50 and 10 mg/l nitrite and diluted 10-fold. The results obtained represent 116% and 111% recoveries, respectively. The slight increase in nitrite concentration is within the analytical error. No conversion of nitrite to nitrate was observed during this test period. It is believed that at this high pH, bacteria, which effect nitrite conversion, were not present. Nitrate was stable under both acidic and alkaline conditions.

In conclusion, base preservation seems to be the method of choice to obtain

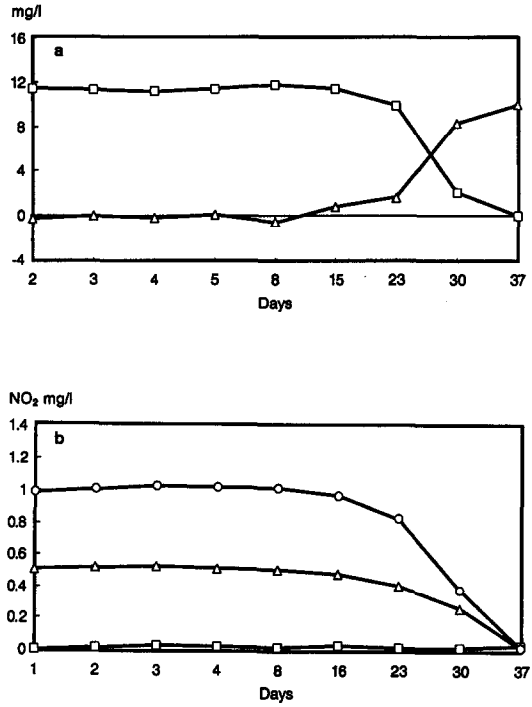

Fig. 1. (a) Results for unpreserved surface water: △ = nitrate; □ = nitrite. (b) Results for unpreserved surface water: ○ = 1.0 mg/l nitrite spike; △ = 0.5 mg/l nitrite spike; □ = unspiked.

accurate determinations of individual levels of nitrite and nitrate in environmental samples while maintaining a holding time of more than 28 days. Acid preservation of samples is not recommended if one wishes to determine individual nitrite and/or nitrate levels in environmental samples.

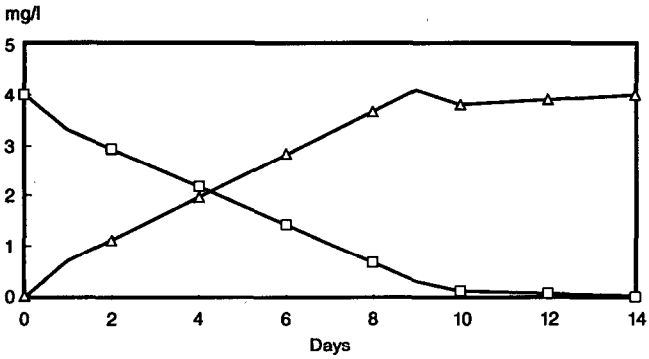

Fig. 2. Results for nitrite-spiked water acidified to pH < 2. △ = Nitrate; □ = nitrite spike.

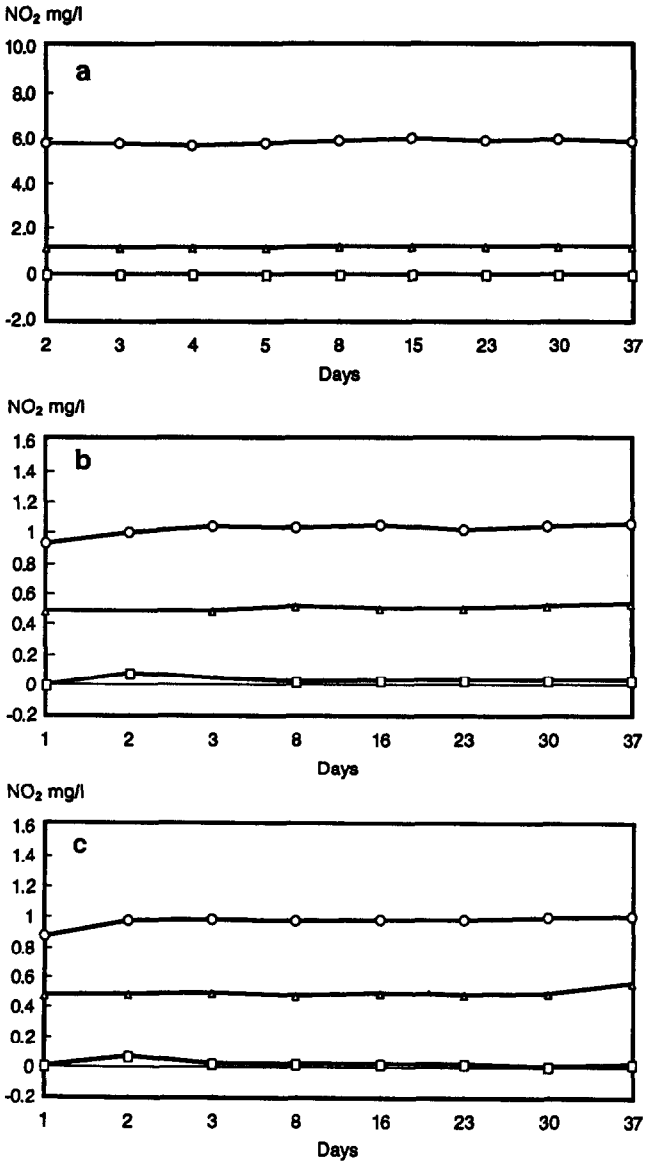

Fig. 3. (a) Results for preserved groundwater (pH 12): ○ = 5.0 mg/l nitrite spike; △ = 1.0 mg/l nitrite spike; □ = unspiked. (b) Results for preserved surface water (pH 12): ○ = 1.0 mg/l nitrite spike; △ = 0.5 mg/l nitrite spike; □ = unspiked. (c) Results for preserved leachate water (pH 12): ○ = 1.0 mg/l nitrite spike; △ = 0.5 mg/l nitrite spike; □ = unspiked.

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