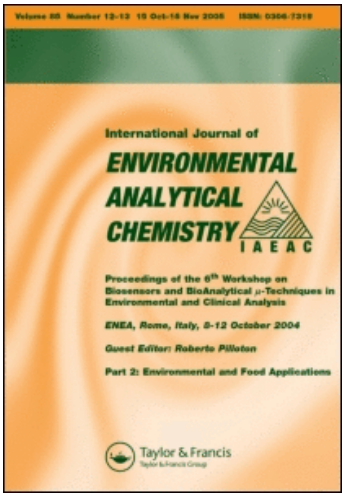

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Purification procedure for $\delta^{15}N$, $\delta^{18}O$, $\Delta^{17}O$ analysis of nitrate

Greg Michalski[a]
[a] Department of Earth and Atmospheric Sciences, Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA

Online publication date: 24 March 2010

To cite this Article Michalski, Greg(2010) 'Purification procedure for $\delta^{15}N$, $\delta^{18}O$, $\Delta^{17}O$ analysis of nitrate', International Journal of Environmental Analytical Chemistry, 90: 7, 586 — 590
To link to this Article: DOI: 10.1080/03067310902783593
URL: http://dx.doi.org/10.1080/03067310902783593

Intern. J. Environ. Anal. Chem.
Vol. 90, No. 7, 15 June 2010, 586–590

Taylor & Francis
Taylor & Francis Group

Purification procedure for $\delta^{15}N$, $\delta^{18}O$, $\Delta^{17}O$ analysis of nitrate

Greg Michalski*

Department of Earth and Atmospheric Sciences, Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA

(*Received 14 November 2008; final version received 28 January 2009*)

A solid phase extraction technique has been developed for purification of nitrate in environmental solutions for isotopic analysis. Three materials, C18, silica gel and cross linked poly-vinylpyrrolidone, were used to remove non-polar, slightly polar and humic compounds from solutions with high dissolved organic loads. The clean up procedure does not impact the isotopic composition of the nitrate analyte for samples in the 2–6 mg (NO_3^-) range and improves accuracy and precision of the oxygen and nitrogen isotope analysis using the $AgNO_3$ decomposition and thermal conversion elemental analysis techniques.

Keywords: nitrate; isotope; oxygen; purification

1. Introduction

Nitrate is an important compound in atmospheric chemistry [1] and in the biogeochemical cycling of nitrogen [2], and isotopic studies are becoming increasingly important for understanding its chemical and biological transformation in the environment [3–9]. Various methods have been developed to analyse the $^{15}N/^{14}N$, $^{18}O/^{16}O$ [10–12], and $^{17}O/^{16}O$ [13,14] ratios in nitrate samples and to assess their $\delta^{15}N$, $\delta^{18}O$, $\Delta^{17}O$ values with respect to atmospheric N_2 and VSMOW, the accepted isotopic standards for nitrogen and oxygen. For $\Delta^{17}O$ analysis ($\Delta^{17}O \sim \delta^{17}O - 0.52\delta^{18}O$) O_2 is the required analyte because of isobaric interferences with more conventional gases such as CO_2 and N_2O. Nitrate sample preparation for the $AgNO_3$ pyrolysis analysis method [14] is sensitive to the presence of extraneous dissolved compounds, such as dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) because these low volatility compounds will co-precipitate with $AgNO_3$ at the final stage of freeze-drying/evaporation. These compounds can react with O_2 produced during $AgNO_3$ thermal decomposition or N elemental analysis leading to isotopic exchange, gas contamination and loss of product O_2 ($C_{org} + O_2 \rightarrow CO/CO_2$). Purification by anion exchange resin works well in samples with low DOC and high nitrate concentrations, but it has proven challenging for samples with high DOC loads such as organic rich soils and eutrophic lakes/stream waters. Therefore we have developed a new technique of sample purification using a suite of solid phase extraction (SPE) resins to eliminate DOC from environmental samples for more accurate isotopic analysis of NO_3^-.

*Email: gmichals@purdue.edu

ISSN 0306–7319 print/ISSN 1029–0397 online

DOI: 10.1080/03067310902783593
http://www.informaworld.com

2. Experimental

2.1 *Reagents*

Three solid phase extraction materials were selected. The first is water insoluble, cross linked poly-vinylpyrrolidone (PVPP, Sigma, St. Louis, MO, USA), a more highly cross linked version of water soluble PVP. PVPP is used to bind impurities because the amide bonds of PVPP form hydrogen bonds with the hydroxyl groups of polyphenols, polysaccharides and humic substances, thus removing them from solution [15]. The second SPE material is high purity silica gel (Alltech, Deerfield, IL, USA), which is a well known absorbent of slightly polar compounds, such as weak organic acids. The third SPE phase was Prevail^{TM} C-18 (Alltech), a styrene material with an 18 atom (C) hydrocarbon chain attached that makes it very selective for non-polar hydrocarbon type compounds [16]. The absorption properties of this suite of SPE materials thus reflects the type of intra molecular forces typical of DOC while being unselective for strong anion/cations (see below).

Each resin type was rinsed in Millipore water in 300 mL beakers, allowed to settle, and the supernatant decanted off. The resins were loaded into 10 mL disposable polypropylene chromatography columns after which the resins were again rinsed using ~250 mL of Millipore water. A series of control experiments were set up to test whether any of the SPE materials either absorbed NO_3^- or contributed to background blanks that might interfere with the isotopic analysis. For these tests we used an internal isotopic standard which is a finely ground $NaNO_3$ fertiliser mined from the Atacama Desert in northern Chile (A.H. Hoffman Inc., Lancaster, NY, USA). Natural Chilean nitrates originate from long-term deposition of atmospheric nitrates [8,17] and have high $\delta^{18}O$ and $\Delta^{17}O$ values making them ideal tracers for isotopic analysis development. We use this as an internal standard as it has an oxygen isotopic composition similar to the international nitrate standard USGS35 (another Chilean fertiliser) [14,18]. This internal lab nitrate standard has a $\delta^{18}O$ value of 50.2‰ (±1) and $\Delta^{17}O$ value of 19.9‰ (±0.1) relative to SMOW and a $\delta^{15}N$ value of 4.3‰ (±0.3) *versus* air N_2 (Table 1) based on internal calibrations using nitrate reference materials USGS35, USGS32 and USGS34 [18].

2.2 *Experimental set up*

The lab standard sodium nitrate was passed through columns of the three SPE materials then converted to $AgNO_3$. Nitrate absorption tests were conducted on 3 different column bed volumes (1, 2 and 5 mL) of each SPE material and equal bed volumes in a single column (i.e. 3, 6 and 15 mL of combined PVP-SIilca-C18). A stock 1 L solution of our

Table 1. Treatment impact on isotope analysis.

Treatment	*n*	$\delta^{18}O$‰	$\Delta^{17}O$‰	$\delta^{15}N$‰
Hoffman (cation only)	10	50.2 +/− 1.0	19.9 +/− 0.1	4.3 +/− 0.3
PVPP only	4	50.1 +/− 1.1	19.9 +/− 0.2	4.1 +/− 0.5
Silica gel only	2	49.9 +/− 1.0	19.6 +/− 0.1	4.0 +/− 0.3
Prevail only	2		19.9 +/− 0.1	4.1 +/− 0.3
PVPP + Silica + Prevail (2 mL each)	3	50.2 +/− 1.2	19.7 +/− 0.2	4.2 +/− 0.3
Soil extract (P + S + P)	2	51.1 +/− 0.5	19.3 +/− 0.2	4.4 +/− 0.4

Hoffman isotopic lab standard $NaNO_3$ was diluted to 150 mg kg^{-1} (as NO_3^-) and split into aliquots of 50 mL that were used as control samples. This concentration is what might be expected for dilute nitrate solutions (μg kg^{-1}–mg kg^{-1}) that are pre-concentrated using the method of Silva *et al*. [19] and the total sample size (7.5 mg of NO_3^-) is the minimum needed for triplicate analysis using the thermal decomposition method [14]. The 50 mL stock solutions were analysed for NO_3^- concentration before and after passing through the columns containing the SPE materials using suppressed ion chromatography (Alltech model 626) in order to determine NO_3^- loss on each SPE reagent (Figure 1). After passing through the SPE resins the $NaNO_3$ was converted to weak HNO_3 by passing the solutions through a Bio-Rad AG50W cation exchange resin in H^+ form (BioRad, Hercules, CA, USA) that is pre-cleaned using 50 mL 1 M HCl followed by a 200 mL deionised water rinse. The resulting HNO_3 is neutralised using an excess of pre-washed Ag_2O, which generates aqueous $AgNO_3$, which is then filtered and freeze dried. The resulting $AgNO_3$ salt is re-hydrated with 1–3 mL Millipore water and filtered a second time using a 0.2 micron syringe filter before a final freeze drying step.

We also used our internal standard to simulate a soil extraction procedure that would be used with field collections as described by Silva *et al*. [19]. Approximately 1 kg of soil from a corn field near Purdue University was mixed with 1 L of Millipore water, stirred, and filtered at 50 microns. The soil extract was pale yellow indicating the presence of dissolved organic material (2.5 mg L^{-1}) but IC analysis showed it contained no nitrate. 100 μmol of our Hoffman standard was added to 200 mL of soil extract, which was then passed through a 2 mL of cation resin column (Biorad AG 50W) in order to absorb positively charged DOC compounds [19] and eluant anions were collected on 2 mL of anion resin (Biorad Ag-1 × 8). The anion resins were eluted using 15 mL of 1M HBr and the solutions were neutralised using an excess of clean Ag_2O (neutralisation was tested with pH strips). One mL of a 0.1 M sodium carbonate/bicarbonate was added to the

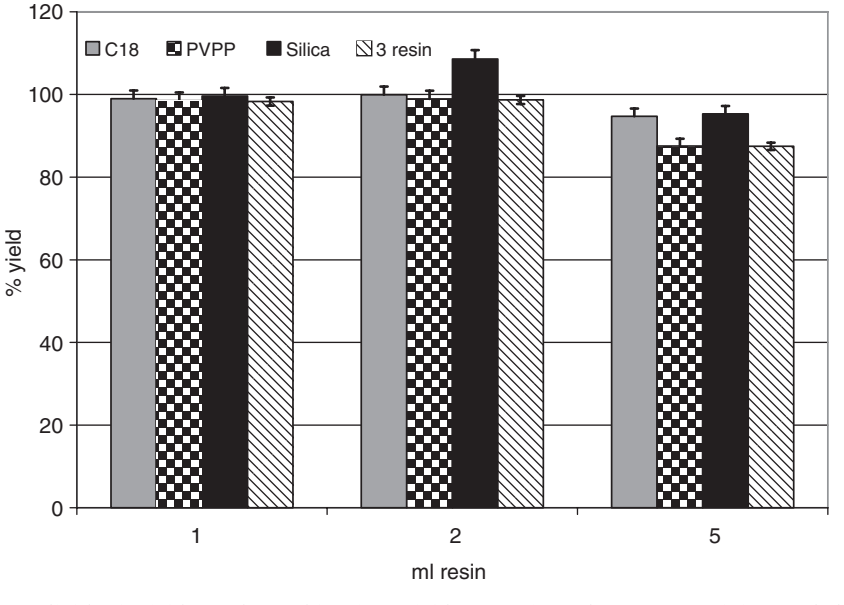

Figure 1. Individual yields and combination yields (3-resin) of SPE material tested for NO_3^- absorption.

$AgNO_3$(aq) to ensure neutralisation and precipitate Ag^+ as Ag_2CO_3. The solution was diluted up to 50 mL and then passed through a SPE column containing 2 mL of each of the SPE materials followed by nitrate concentration analysis using the IC as above. The silver carbonate precipitation step is performed to eliminate Ag^+ that will precipitate $AgCO_3$ during contact with the IC carbonate eluant, which results in the clogging of the anion column. Excess $BaCl_2$ (1 mL at 1M) was added to precipitate sulfate as $BaSO_4$, after which the solution was filtered at 0.2 micron. These test solutions were then converted to Ag_2NO_3 via the same cation procedure that was used on our control samples.

Oxygen isotopic analysis ($\Delta^{17}O, \delta^{18}O$) was carried out by thermal decomposition of the silver nitrate described by Michalski *et al.* [14] and $\delta^{15}N$ values were determined by Thermal Combustion/Elemental Analyser (TC/EA) techniques [20]. The oxygen isotopes were analysed on a Thermo Delta V isotope ratio mass spectrometer (IRMS) in dual inlet mode and nitrogen isotopes on a Sercon IRMS by continuous flow techniques. All measurements were conducted at the Purdue Stable Isotope (PSI) facility.

3. Results and discussion

None of the SPE resins showed any affinity for NO_3^- with yields, based on IC analysis, being near 100% after accounting for the uncertainties in the IC analysis (±2%). The 5 mL bed volumes showed a slight decrease in NO_3^- concentrations, but this is likely due to a dilution of the stock solution by rinse water retained in the pore space of the resins. For example, 8 mL of PVP contains ~4 mL of water (50% pore space/absorption) that, if corrected for in the concentrations data, would increase the yield to 95%, similar to the other controls. Soil simulations using the 2 mL × 3 resin columns showed a higher degree of loss (92% yield) relative to the control (98%), but again much of this loss can be attributed to retention of NO_3^- in the pore spaces of the AgBr and $BaSO_4$ precipitates and in the syringe filter dead volume that are unavoidable during sample processing. Such loss results in a decrease in concentration after the final dilution to 50 mL.

The isotopic data suggests that there is no isotopic interference occurring due to the clean up procedure (Table 1). All three isotope measures are within the statistical uncertainty of the $AgNO_3$ thermal decomposition and TC/EA techniques. None of the individual SPE resins, or in combination, had a significant impact on the $\Delta^{17}O$ values of our working standard indicating negligible oxygen resin blanks. Precision decreased in the $\delta^{18}O$ values, primarily because small mass dependent fractionations occur during the decomposition phase of the analysis because oxygen is partitioned into O_2 and NO_2, which may slightly vary during decomposition [14]. This effect does not occur in the $\delta^{15}N$ analysis because the N is largely conserved (>90% yields) in the TC/EA method and the high reaction temperatures (~800°C) limits isotopic fractionation. The $\delta^{18}O$ and $\delta^{15}N$ values for the Hoffman nitrate that was added to our soil extract were indistinguishable from our pure standard analysis (within our 2σ uncertainty). The $\Delta^{17}O$ value of the soil extract nitrate was slightly lower (by 0.3‰) relative to our standard deviation on the pure standard and resins treatments. However, this is only 1.5% error, and we are currently exploring additional purification options to reduce this further, including high capacity ion chromatography separations. The isotopic data suggests that the SPE clean up approach yields accurate nitrate isotopic values in environmental samples.

Previous reports of C18 resin impacting $\delta^{15}N$ results were not observed [15]. This may be due to our rehydration/filtration step after the initial freeze dry. It was noticed that

during blank runs, when only Millipore water passed through the P-VPP and 18C resins and was subsequently dried, that there was always a small amount of white material. This material does not dissolve upon rehydration and was removed by filtration. We suspect this material to be residue from a low solubility resin component that precipitates during drying but does not re-dissolve upon rehydration. Since the C18 resins contains N in its structure, this may be the origin of previously reported $\delta^{15}N$ uncertainty.

4. Conclusions

Analysis of nitrate that has been processed through a suite of three solid phase extraction resins has been shown to maintain its isotopic integrity. The removal of DOC/DON by the three resin clean up procedure on natural samples is visually observable as the resins turn discoloured and black while the solution goes from opaque to clear. The elimination of DOC/DON by the procedure improves yields during the thermal decomposition of $AgNO_3$ and results in accurate and precise isotopic measurements of environmental nitrates.

References

[1] P.J. Crutzen and J. Lelieveld, Ann. Rev. Earth Plan. Sci. **29**, 17 (2001).
[2] J.N. Galloway, F.J. Dentener, D.G. Capone, E.W. Boyer, R.W. Howarth, S.P. Seitzinger, G.P. Asner, C.C. Cleveland, P.A. Green, E.A. Holland, D.M. Karl, A.F. Michaels, J.H. Porter, A.R. Townsend, and C.J. Vorosmarty, Biogeochemistry **70**, 153 (2004).
[3] A. Amberger and H.L. Schmidt, Geochim. Cosmochim. Acta **51**, 2699 (1987).
[4] R. Aravena and W.D. Robertson, Ground Water **36**, 975 (1998).
[5] W. Durka, E.D. Schulze, G. Gebauer, and S. Voerkelius, Nature **372**, 765 (1994).
[6] M.G. Hastings, D.M. Sigman, and F. Lipschultz, J. Geophys. Res. **108**, 4790 (2003).
[7] C. Kendall, D.H. Campbell, D.A. Burns, J.B. Shanley, S.R. Silva, and C.C.Y. Chang, IAHS Publ. **228** (Biogeochemistry of Seasonally Snow-Covered Catchments), 339, (1995).
[8] G. Michalski, Z. Scott, M. Kabiling, and M. Thiemens, Geophys. Res. Lett. **30**, 1870 (2003).
[9] G. Michalski, T. Meixner, M. Fenn, L. Hernandez, A. Sirulnik, E. Allen, and M. Thiemens, Envir. Sci. Tech. **38**, 2175 (2004).
[10] K.L. Casciotti, D.M. Sigman, M.G. Hastings, J.K. Böhlke, and A. Hilkert, Anal. Chem. **74**, 4905 (2002).
[11] K. Revesz, J.K. Böhlke, and T. Yoshinari, Anal. Chem. **69**, 4375 (1997).
[12] D.M. Sigman, K.L. Casciotti, M. Andreani, C. Barford, M. Galanter, and J.K. Böhlke, Anal. Chem. **73**, 4145 (2001).
[13] J. Kaiser, M.G. Hastings, B.Z. Houlton, T. Rockmann, and D.M. Sigman, Anal. Chem. **79**, 599 (2006).
[14] G. Michalski, J. Savarino, J.K. Böhlke, and M. Thiemens, Anal. Chem. **74**, 4989 (2002).
[15] G. Haberhauer and K. Blochberger, Anal. Chem. **71**, 3587 (1999).
[16] B. Tippins, Nature **334**, 273 (1988).
[17] G. Michalski, J.K. Böhlke, and M.H. Thiemens, Geochim. Cosmochim. Acta **68**, 4023 (2004).
[18] J.K. Böhlke, S.J. Mroczkowski, and T.B. Coplen, Rapid Comm. Mass Spec. **17**, 1835 (2003).
[19] S.R. Silva, C. Kendall, D.H. Wilkison, A.C. Ziegler, C.C.Y. Chang, and R.J. Avanzino, J. Hydrol. **228**, 22 (2000).
[20] C.C.Y. Chang, S.R. Silva, C. Kendall, G. Michalski, S. Wankel, and K.L. Casciotti, *Preparation and Analysis of Nitrogen-Bearing Compounds in Water for Stable Isotope Ratio Measurement*. pp. 305–347, P. A. DeGroot, Elsevier, Amsterdam, 2004.