

A Method for the Extraction of Ammonium from Freshwaters for Nitrogen Isotope Analysis

Moritz F. Lehmann,* Stefano M. Bernasconi, and Judith A. McKenzie

Geological Institute, Swiss Federal Institute of Technology (ETH), Sonneggstrasse 5, 8092 Zurich, Switzerland

The measurement of $\delta^{15}\text{N}$ values of inorganic nitrogen species is an important analytical tool to trace nitrogen species in order to understand nitrogen cycling in aquatic systems. Nitrogen isotope analysis of freshwater ammonium has, however, been hindered by the lack of a simple and reliable technique to measure $\delta^{15}\text{N}$ values at natural abundance levels. We present a simple and rapid method to concentrate ammonium from freshwater samples for on-line N-isotope ratio determination. Ammonium is collected by adsorption on N-free cation exchange resins. The dried N-loaded exchange resin is then directly combusted to produce N_2 gas for subsequent $\delta^{15}\text{N}$ analysis. The method was evaluated with simulated freshwater solutions containing varying amounts of standard $\text{NH}_4^+\text{-N}$ ($\delta^{15}\text{N} = 2.1\text{‰}$) and potentially interfering inorganic and organic compounds. In general, the cation exchange resin method gives accurate and reproducible $\delta^{15}\text{N}$ values ($\sigma_1 < 0.3\text{‰}$; $n = 10$). Because of adsorption interference, high concentrations of cations in solution may cause ammonium loss but do not result in measurable isotope fractionation. Replicate extractions of the ammonium standard added to water collected from four Swiss lakes demonstrate the good performance of this method when applied to low ionic strength natural water samples with modest concentrations of dissolved organic nitrogen.

Although nitrogen isotope tracers at the natural abundance level have often been used for studying biogeochemical processes in aquatic systems,^{1–7} the exact controls on N-isotope cycling are still poorly understood. The interpretation of N-isotope data in

autochthonous organic matter is particularly hampered by the paucity of $\delta^{15}\text{N}$ values for ambient inorganic N species. Despite the high potential to allow for a better understanding of the nitrogen isotopic dynamics during algal assimilation, nitrification, and microbial decomposition, N-isotopic data for dissolved ammonium are scarce.

In previous studies, ammonium has been removed from the water sample by steam distillation and oxidized with a hypobromite solution, followed by purification of N_2 for mass spectrometric analysis by combustion in evacuated sealed tubes filled with Cu/CuO .^{8,9} Other methods include steam distillation with subsequent collection of ammonium on zeolite¹⁰ or sample concentration and slow diffusion of ammonium onto acidified glass fiber disks to produce ammonium sulfate^{11,12} prior to quartz-tube combustion¹³ or continuous-flow isotope analysis.

These methods in use for determining $\delta^{15}\text{N}_{\text{NH}_4^+}$ values are either labor- or time-intensive or both and, therefore, are not suitable for routine analyses of large quantities of samples. In addition, the distillation component has been reported to be subject to contamination and isotope fractionation,^{12,14,15} especially when it is applied to large sample volumes with low ammonium concentrations. For these reasons, there is a strong demand for a rapid and simple technique to collect freshwater ammonium from natural water samples for automated continuous-flow nitrogen isotope analysis.

Using ion exchange followed by resin elution and further sample processing to concentrate groundwater nitrate for isotope analyses has become very popular.^{12,16–18} Silva et al.¹⁸ investigated adsorption interference caused by high concentrations of anions during nitrate collection on an ion exchange resin, leading to the depletion of ^{15}N in the sample. They reported on admissible

* Corresponding author. Phone: +41-1-6323691. Fax: +41-1-6321080. E-mail: lehmann@erdw.ethz.ch.

- (1) Cifuentes, L. A.; Fogel, M. L.; Pennock, J. R.; Sharp, J. H. *Geochim. Cosmochim. Acta* **1989**, *53*, 2713–2721.
- (2) Altabet, M. A.; Francois, R. In *Carbon Cycling in the Glacial Ocean: Constraints on the Ocean's Role in Global Change; Quantitative Approaches in Paleocceanography*; Zahn, R., Pedersen, T. F., Kaminski, M. A., Labeyrie, L., Eds.; Springer-Verlag: Berlin, Federal Republic of Germany, 1993; Vol. 17, pp 281–306.
- (3) Ostrom, N. E.; Macko, S. A.; Deibel, D.; Thompson, R. J. *Geochim. Cosmochim. Acta* **1997**, *61*, 2929–2942.
- (4) Bernasconi, S. M.; Barbieri, A.; Simona, M. *Limnol. Oceanogr.* **1997**, *42*, 1755–1765.
- (5) Ostrom, N. E.; Long, D. T.; Bell, E. M.; Beals, T. *Chem. Geol.* **1998**, *152*, 13–28.
- (6) McCusker, E. M.; Ostrom, P. E.; Ostrom, N. E.; Jeremiason, J. D.; Baker, J. E. *Org. Geochem.* **1999**, *30*, 1543–1557.
- (7) Teranes, J. L.; Bernasconi, S. M. *Limnol. Oceanogr.* **2000**, *45*, 801–813.
- (8) Bremner, J. M. *Isotope Ratio Analysis of Nitrogen in Nitrogen-15 Tracer Investigations*; American Society of Agronomy: Madison, Wisconsin, 1965.
- (9) Bremner, J. M.; Edwards, A. P. *Soil Sci. Soc. Proc.* **1965**, *29*, 504–507.
- (10) Velinsky, D. J.; Pennock, J. R.; Sharp, J. H.; Cifuentes, L. A.; Fogel, M. L. *Mar. Chem.* **1989**, *26*, 351–361.
- (11) Sigman, D. M.; Altabet, M. A.; Michener, R.; McCorkle, D. D.; Fry, B.; Holmes, R. M. *Mar. Chem.* **1997**, *57*, 227–242.
- (12) Downs, M. R.; Michener, R. H.; Fry, B.; Nadelhoffer, K. J. *Environ. Monit. Assess.* **1999**, *55*, 211–220.
- (13) Kendall, C.; Grim, E. *Anal. Chem.* **1990**, *62*, 526–529.
- (14) Mulvaney, R. L. *Soil. Sci. Am. J.* **1986**, *50*, 92–96.
- (15) Liu, K. K.; Su, M. J.; Hsueh, C. R.; Gong, G. C. *Mar. Chem.* **1996**, *54*, 273–292.
- (16) Aravena, R.; Robertson, W. D. *Groundwater* **1998**, *36*, 975–982.
- (17) Mengis, M.; Schiff, S. L.; Harris, M.; English, M. C.; Aravena, R.; Elgood, R. J.; MacLean, A. *Groundwater* **1999**, *37*, 448–457.
- (18) Silva, S. R.; Kendall, C.; Wilkison, D. H.; Ziegler, A. C.; Chang, C. C. Y.; Avanzino, R. J. *J. Hydrol.* **2000**, *228*, 22–36.

threshold anion concentrations in simulated solutions, which characterize the suitability of the ion exchange method for natural aquatic systems.

Here, we describe an analytical procedure for the collection of sample ammonium on a cation exchange resin and the subsequent combustion of the N-loaded resin to produce N₂ for isotope analysis. We tested the accuracy and the reproducibility of the method, as well as its applicability to natural water samples. We also investigated expected adsorption interference caused by Ca²⁺ and Mg²⁺ ions and the impact of dissolved N-containing compounds on N_{NH₄⁺} isotope measurements.

EXPERIMENTAL SECTION

Ammonium Extraction. Natural water samples were filtered through Whatmann GF/F and subsequently through 0.2-μm membrane filters. Adapting the technique of using ion exchange resin to extract inorganic N species from water samples for isotope analysis,^{12,18,19} the filtrate and simulated ammonium solutions were passed through prefilled, prerinsed (with deionized water) Poly-Prep cation exchange resin columns (Biorad, AG 50W-X8, 100–200 mesh, H⁺ form) by gravity flow. The recommended flow rate, which can be regulated by changing the elevation of the reservoir, is 6 mL/minute. The columns hold a bed volume of 2 mL of nitrogen-free chromatographic media composed of sulfonic acid functional groups attached to styrene divinylbenzene copolymer with an ion exchange capacity of 1.7 meq/mL lattice.²⁰ To prevent potential incomplete sorption of ammonium-N, the ion exchange capacity should not be saturated. After concentration of ammonium-N on the resin, the resin is dried at 50 °C.

On-Line Isotope Analysis. For N-isotope analysis, up to 25 mg of well-homogenized, dried resin is placed into tin capsules and combusted at 1040 °C in an elemental analyzer (Carlo Erba NCS 2500) coupled in continuous flow with a mass spectrometer (Fisons Optima) with standard setup for N₂ gas. For low-concentration samples, large amounts of N-loaded resin are needed to warrant a minimum of 20 μg of N sample for δ¹⁵N analysis. With the resin, very large amounts of carbon are introduced into the EA/IRMS system, potentially contaminating the system and, therefore, biasing N-isotope analyses as CO⁺ derived from dissociation of CO₂ in the ion source that produces an isobaric interference on mass 28. CO₂ and H₂O are stripped off the combustion gas before isotope ratio measurements by means of a large volume (20-cm length, 0.8-mm internal diameter), two-component chemical trap containing two-thirds Carbosorb (12–20 mesh) followed by magnesium perchlorate (18 mesh). The complete oxidation of the resin requires a sufficient amount of oxygen being injected into the reaction column. The oxygen injection duration and the volume of the oxygen injection loop can be used to regulate it (we used a 10-mL loop and an oxygen injection duration of 80 s). Insufficient combustion, that is, oxidation, of the N-loaded resin is likely to cause N-isotope fractionation. No blank correction needs to be applied to the measurements, because the amount of N₂ produced by the combustion of blank resin in a tin capsule was negligible. Nevertheless, standards were measured as a standard-resin mixture with adequate N/resin ratio in order to account for the

Table 1. Accuracy and Reproducibility

vol test soln, mL	concn, mg NH ₄ ⁺ -N/L	δ ¹⁵ N of std NH ₄ ⁺ , ‰	ave δ ¹⁵ N of recovered NH ₄ ⁺ , ‰	std dev, σ ₁ , ‰ ^a
50	33	2.10	2.04	0.26 (<i>n</i> = 10) ^a
1800	0.6	2.10	2.00	0.29 (<i>n</i> = 10) ^a
50	33	20.34	20.28	0.17 (<i>n</i> = 3)

^a Calculated from duplicate isotope analyses of five replicate samples.

potential influence of the large amounts of organic matter on combustion and chromatography characteristics.

Isotope ratios are defined as δ¹⁵N_{sample} = [(¹⁵N/¹⁴N_{sample})/(¹⁵N/¹⁴N_{standard}) - 1] × 1000 and expressed in permil deviations vs atmospheric N₂ (0‰). Instrumental precision based on repeated analyses of (NH₄)₂SO₄ standards (IAEA-N1, IAEA-N2) is generally better than ±0.2‰. On the basis of the amount of cation exchange resin used, nitrogen yields were calculated by the elemental analysis of the N-loaded resin. The analytical precision of the N analysis and, therefore, the reproducibility of calculated N yields are better than ±4%.

RESULTS AND DISCUSSION

Analytical Precision. To test the adequacy of the procedures for the ammonium adsorption and isotopic analysis, test solutions made from (NH₄)₂SO₄ standard salts (2.1‰, 20.34‰) and distilled water were passed through the cation exchange resin columns and processed. Determined by ion chromatography, the ammonium concentrations in standard solutions after passage through the exchange resin columns revealed that NH₄⁺ was completely removed. Nitrogen yields were generally better than 90%. The average δ¹⁵N values for standard NH₄⁺ removed from multiple aliquots of standard solutions with a typical range of ammonium concentrations was 2.02‰ ± 0.27‰ and 20.28‰ ± 0.17‰, respectively (Table 1). No significant concentration or volume effect has been observed. We processed 1800-mL test solutions with concentrations as low as 0.3 mg NH₄⁺-N/L (21 μM) with satisfying results.

Fractionation within the Column. To test if isotope fractionation due to preferential adsorption within the exchange resin column occurs during the loading process, a standard solution was passed through the column, and samples of N-loaded resin were collected at discrete depths in the column. We observed a distinct concentration gradient (Figure 1). At the ion exchange front, where the N concentration is lowest, δ¹⁵N values for adsorbed NH₄⁺ significantly deviated by -1.5‰ from the standard δ¹⁵N. ¹⁴NH₄⁺ seems to have a higher affinity for the cation exchange resin than ¹⁵NH₄⁺, and little loss of ¹⁵N may result in isotope fractionation at the ion exchange front and in isotope zoning within the resin column. Therefore, incomplete homogenization of the dried sample resin prior to nitrogen isotope analyses may lead to inaccurate results.

Adsorption Interference. During an ion exchange procedure, counterions (H⁺) are replaced by sample ions, which have a greater affinity for the resin. The ability of the cation exchange resin to retain ammonium depends on the capacity of the resin and the amount of other cations in solution competing for

(19) Chang, C. C. Y.; Langston, J.; Riggs, M.; Campbell, D. H.; Silva, S. R.; Kendall, C. *Can. J. Fish. Aquat. Sci.* **1999**, *56*, 1856–1864.

(20) *Bio-Rad Manual*; Bio-Rad Laboratories: Hercules, CA.

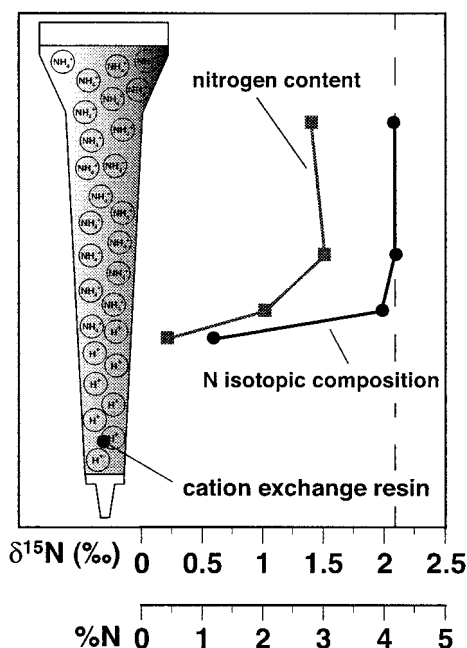


Figure 1. Schematic drawing of a NH_4^+ -loaded (2000 mL of 285 μM standard solution) but not -saturated cation exchange resin column. The nitrogen content of the resin and the $\delta^{15}\text{N}$ for adsorbed ammonium are plotted for four locations along the flow path in the ion exchange column. The calculated bulk $\delta^{15}\text{N} = 1.98\text{‰}$ integrating all four sub-samples is reasonably close to the expected value of the standard reagent (2.1‰) indicated by the dashed line.

exchange sites. If insufficient resin capacity or displacement by cations of higher affinity causes incomplete adsorption of ammonium during sample loading, isotope fractionation could result. Silva et al.¹⁸ reported on N-isotope fractionation caused by high concentration of Cl^- during nitrate adsorption on an anion exchange resin. In natural freshwater, Ca^{2+} , Mg^{2+} , and Na^+ are abundant cations that potentially bias the ammonium extraction from water samples via ion exchange. We tested only the effect of Ca^{2+} and Mg^{2+} ions, because their affinity for the AG 50W-X8 resin, in contrast to Na^+ , is higher than the affinity of NH_4^+ .²⁰ Na^+ ions are less likely to cause adsorption interference, but anomalous behavior during ion exchange cannot be excluded.

To assess the impact of other cations on the nitrogen recovery and to evaluate the effect of incomplete adsorption of NH_4^+ on the measured N isotope ratios, 500-mL test solutions were prepared using varying amounts of Ca^{2+} from $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, Mg^{2+} from $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, and standard NH_4^+ ($\delta^{15}\text{N} = 2.1\text{‰}$) and subsequently were analyzed as described above. Figure 2a–d illustrates the results from experiments with 2.5–12 mg NH_4^+ -N/L and 10–250 mg Ca^{2+} /L, respectively. In general, the nitrogen recovery ranges between 80 and 95% for Ca^{2+} concentrations up to 150 mg/L but decreases to less than 10% if Ca^{2+} concentrations are 200 mg/L or higher. The theoretical saturation concentration to exhaust 2 mL of AG 50W-X8 resin (3.4 meq) should already be reached at Ca^{2+} concentrations higher than 136 mg/L in a 500-mL ammonium-free solution. For the solution with 12 mg of NH_4^+ -N (Figure 2d), the combined concentrations of NH_4^+ and Ca^{2+} seem to exceed the column capacity at Ca^{2+} concentrations of 150 mg/L or higher. If sample volume increases, the admissible cation concentration causing little ammonium loss during the ion exchange procedure is respectively lower (Figure 2b). Mg^{2+}

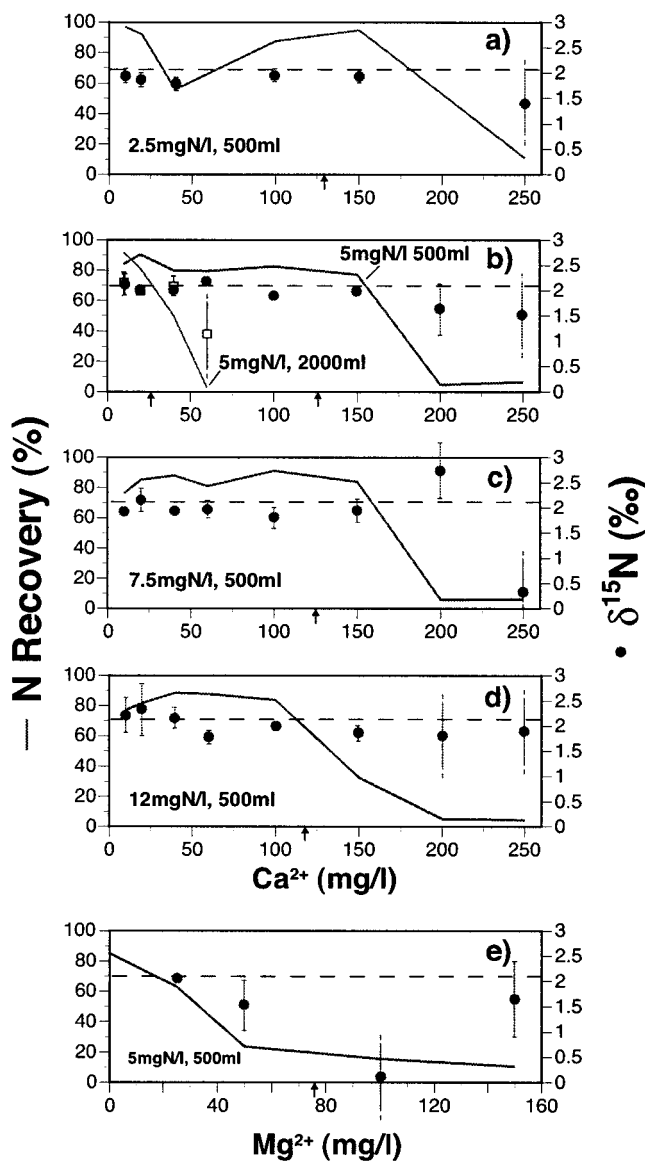


Figure 2. Effect of interfering Ca^{2+} ions (a–d) and Mg^{2+} ions (e) on nitrogen yields (lines) and $\delta^{15}\text{N}$ values (circles) of standard ammonium (2.1‰, dashed lines) recovered from simulated 500-mL solutions with different NH_4^+ concentrations (2.5–12 mg N/L). The open squares in b indicate results from experiments with 2000-mL solutions. The symbols represent average values from replicate isotope measurements with respective standard deviations σ_1 . Unclosed error bars indicate $\sigma_1 > 1\text{‰}$. Arrows point to ion concentrations at which the theoretical exchange capacity of the column is reached.

seems to have a greater potential for inhibiting adsorption of NH_4^+ on cation exchange columns than does Ca^{2+} . Even at Mg^{2+} concentrations that should not saturate the exchange capacity, NH_4^+ -N recovery was poor, and further decreased with increasing $[\text{Mg}^{2+}]$ (Figure 2e). This observation is difficult to explain, because Mg^{2+} is expected to have a lower affinity for the AG 50W-X8 resin than does Ca^{2+} .²⁰

The Ca^{2+} , as well as Mg^{2+} , experiments produced no detectable (with given procedural precision) isotope fractionation, even if adsorption interference produced significant NH_4^+ -N loss (Figure 2). Assuming preferential adsorption of $^{14}\text{NH}_4^+$, we would expect lower $\delta^{15}\text{N}$ values for measured samples when the recovery was incomplete. However, this was not the case, because the relative

affinity of $^{14}\text{NH}_4^+$ for the AG 50W-X8 resin is apparently not enough different from the affinity of $^{15}\text{NH}_4^+$ -N to cause a preferential displacement of $^{15}\text{NH}_4^+$ by Ca^{2+} or Mg^{2+} . Therefore, there is no measurable isotope fractionation. When nitrogen recovery dropped below 25%, $\delta^{15}\text{N}$ values tended to be significantly lower than expected. We cannot exclude isotope fractionation in the ion exchange column, but the low values are rather due to analytical artifacts, making these measurements unreliable. Poor nitrogen recovery causes the amount of nitrogen analyzed to be too small for mass spectrometric analysis. We found that isotopic measurements of sample resin having a nitrogen content of $<0.04\%$ become somewhat erratic with standard deviations $\sigma_1 > 1\%$ (Figure 2). Therefore, even if NH_4^+ -N loss during adsorption on cation exchange resins does not produce isotope fractionation, we strongly recommend not saturating the ion exchange capacity of the resin. The amount of cation exchange resin used to collect ammonium from freshwater samples should be kept at a minimum, depending on the volume of water needed at given NH_4^+ concentrations and the concentration of interfering cations.

Blank Contribution from Dissolved Organic Matter. Dissolved organic matter (DOM) includes a wide spectrum of organic substances that are regularly found in groundwater and surface water samples. In common freshwater pH ranges, the amino groups of common amino acids are largely ionized and, therefore, amino acids are potentially retained on a strong acid ion exchange resin. Test solutions containing varying amounts of different amino acids and water-soluble yeast extract, mimicking natural dissolved organic nitrogen (DON), were passed through the AG 50W-X8 resin column. We found that between 83 and 100% of the nitrogen of the amino acids and the yeast extract was adsorbed. The DOM might interfere with the adsorption of sample ammonium by occupying exchange sites, but its biggest drawback is that it is combusted together with loaded NH_4^+ , and DON contributes to the total $\delta^{15}\text{N}$ of the sample. Figure 3 illustrates the results from experiments with 500 mL of $357\ \mu\text{M}$ NH_4^+ solutions amended with varying amounts of L-proline (12.1% N) and yeast extract ($\sim 11\%$ N). Simulated solutions were processed and analyzed as described before. Resulting $\delta^{15}\text{N}$ values represent intermediate isotopic compositions between two end-members. Processing of solutions containing only L-proline or yeast extract revealed that nitrogen from these substances is adsorbed without isotope fractionation. Simple mixing calculations show that the adsorption efficiency for amended DON is comparable to the one for ammonium; therefore, it is imperative that DOM accumulation in the cation exchange column is prevented.

Methods to remove DOM from water include ultrafiltration, dialysis,²¹ and scavenging by activated charcoal or other adsorbents.^{18,22} We tested the use of activated carbon (Darco G-60) to remove DOM from samples prior to adsorption onto cation exchange resins. Our experiments revealed that activated carbon did not reduce the nitrogen blank from the amino acid L-proline; however, it might be reasonably effective when applied to samples containing other dissolved organic compounds.¹⁸

Passing the sample solution through an anion exchange resin column prior to cation exchange can largely eliminate DON contribution. As with the AG 50W-X8 resin, DOM bonds to

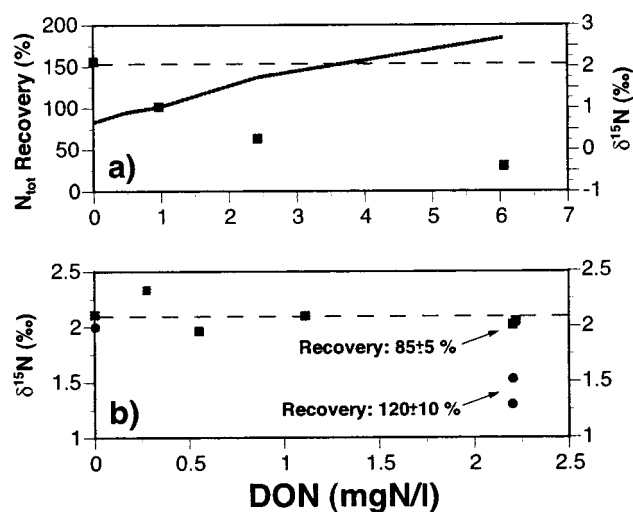


Figure 3. (a) Offset between the true $\delta^{15}\text{N} = 2.1\text{‰}$ of standard ammonium (dashed line) and measured $\delta^{15}\text{N}$ (squares) due to the N blank of DON in experiments with 500-mL solutions containing 5 mg NH_4^+ -N/L ($357\ \mu\text{M}$). The $\delta^{15}\text{N}$ value for L-proline amended to the solutions (1 to 6 mg/L) is $-2.4 \pm 0.15\text{‰}$. N from L-proline was recovered to a similar extent as NH_4^+ -N and adds to the N recovery (line), leading to N_{total} recoveries above 100%. (b) Effects of the use of anion exchange columns on measured $\delta^{15}\text{N}$ values. Squares depict the presence of anion exchange resin, and circles represent sample processing without anion exchange resin. Simulated solutions (500 mL) contained 5 mg NH_4^+ -N/L and DON from yeast extract, a water-soluble mixture of amino acids, peptides and carbohydrates, with a $\delta^{15}\text{N}$ of $-0.46 \pm 0.15\text{‰}$. Anion exchange largely eliminated the DON blank, resulting in measured $\delta^{15}\text{N}$ values close to the expected value of the standard (dashed line).

strongly basic anion exchange resins by both ion exchange and hydrophobic interaction. Chang et al.¹⁹ reported that an average of 50% DON was retained on an anion exchange resin when it was applied to natural samples. In our experiments with an AG 1-X8 anion exchange resin in OH^- form,²⁰ more than 90% of DON could be removed from simulated solutions containing ~ 2.2 mg DON/L from yeast extract. Blank contribution from the anion exchange resin containing ammonium functional groups was negligible. It is crucial to use the AG 1-X8 resin with OH^- counterions (AG 1 resins are supplied in the chloride form and must be converted into the OH^- form). This ionic form may be considered most activated, because the affinity of OH^- for the AG 1 resin is lowest.²⁰ When we used the anion exchange resin in the chloride form, not more than 23% of the initial DON in test solutions, which contained yeast extract or different kinds of α -amino acids, was retained. Most of the DON seems to have a too low affinity for the cation resin to displace Cl^- ions. Significant amounts of positively charged DOM, although being partially removed by hydrophobic interaction with an anion resin, may lead to high nitrogen blanks.

To test the potential of the AG 1-X8(OH^-) resin to reduce the DON blank contribution to $\delta^{15}\text{N}$ - NH_4^+ values, we arranged the two ion exchange columns in series, and introduced simulated solutions containing standard NH_4^+ (2.1‰) and DON from yeast extract (-0.46‰). $\delta^{15}\text{N}$ analyses of the loaded cation resin indicated no measurable influence of nitrogen from yeast extract, even at DON concentrations higher than 2 mg N/L (Figure 3b). The mean $\delta^{15}\text{N}$ value for all 5 samples was $2.02 \pm 0.21\text{‰}$, and the NH_4^+ -N yield was comparable to the yields determined in

(21) Feuerstein, T. P.; Ostrom, P. H.; Ostrom, N. E. *Org. Geochem.* **1997**, *27*, 363–370.

(22) Haberhauer, G.; Blochberger, K. *Anal. Chem.* **1999**, *71*, 3587–3590.

Table 2. Cation Concentrations, $\text{NH}_4^+\text{-N}$ Recovery, and $\delta^{15}\text{N}$ Values for Natural Freshwater Samples

sampling location	no. replicates	$[\text{NH}_4^+\text{-N}]$ mg/L	$[\text{Ca}_2^+]$ mg/L	$[\text{Mg}_2^+]$ mg/L	$[\text{Na}^+]$ mg/L	$[\text{K}^+]$ mg/L	ave N recovery %	ave $\delta^{15}\text{N}$ ‰
Amended with Standard NH_4^+ (2.1‰)								
Lake Lugano ^b	2	2.0	35.09 ^a	8.24 ^a	5.74 ^a	1.38 ^a	81	2.05 ± 0.35
Lake Lucerne ^b	3	0.5 to 1.0	38.52	3.32	2.53	1.09	91.1	1.86 ± 0.2
Lake Zürich ^b	3	1.5	49.88	6.47	4.08	1.49	79	1.98 ± 0.23
Lake Zug ^b	4	1.0 to 1.5	48.61	8.81	6.16	2.05	88.7	1.95 ± 0.3
Natural NH_4^+								
Laveggio River	2	1.18	na ^d	na ^d	na ^d	na ^d	88.3	17.81 ± 0.15
Lake Lugano 7/99 ^c	2	0.88	35.09 ^a	8.24 ^a	5.74 ^a	1.38 ^a	44.0	12.37 ± 0.36
Lake Lugano 10/99 ^c	2	0.76	35.09 ^a	8.24 ^a	5.74 ^a	1.38 ^a	74.2	12.67 ± 0.22

^a Average March concentration for 1983–1999. ^b Natural $\text{NH}_4^+\text{-N}$ concentrations < 0.01 mg/L. ^c From anoxic bottom waters. ^d Not analyzed.

experiments without DON. Although the DOM collected from various natural sources may behave quite differently on the ion exchange columns,¹⁹ this indicates the good performance of our method to measure $\delta^{15}\text{N}$ of ammonium when applied to water samples with modest DON concentration.

Natural Samples. To test the ion exchange method applied to natural water samples, we added the ammonium standard to surface water from four Swiss lakes containing no or negligible amounts of natural ammonium (Table 2). We recovered and analyzed the standard $\text{NH}_4^+\text{-N}$ as outlined before. The nitrogen recoveries determined from elemental analysis ranged between 76 and 94%, and apparently, the natural inventory of other cations (Table 2) did not greatly interfere with ammonium adsorption. The mean measured $\delta^{15}\text{N}$ of recovered standard ammonium ranges between 1.86 and 2.05‰ for the lakes, with standard deviations better than 0.35‰ (Table 2), and they are, thus, reasonably close to the expected value of the standard reagent (2.1‰). As observed in previous experiments, the slight nitrogen loss in some samples did not produce measurable isotope fractionation. Similar standard deviations were obtained for duplicate $\delta^{15}\text{N}$ analyses of natural ammonium extracted from anoxic lake bottom and river water samples (Table 2) from a recent N-cycling study in Lake Lugano.

CONCLUSION

Cation exchange provides an efficient and reliable means of isolating ammonium from freshwater samples for nitrogen isotope analysis. The relative simplicity of the ion exchange procedure coupled with the speed of the automated, on-line isotope analysis

provides minimal sample preparation time without sacrificing analytical precision. Experimental results indicate that the method is applicable to freshwater with moderate ion strength even at low NH_4^+ -concentrations. The method is not subject to measurable isotope fractionation.

In most cases, DON probably contributes only a small fraction to the total N analyzed. When its concentration is higher, DON can be quantitatively removed by adsorption on an anion exchange resin. The fact that organic compounds were almost completely retained on a N-free cation resin if not previously removed may be a benefit; that is, in future studies, the cation exchange resin method could be adapted to extract certain DON species for N-isotope analyses.

Although our method has been applied to only a small number of natural water samples, it has proven to be a promising tool to isolate and isotopically characterize ammonium. This analytical technique may, therefore, offer an important advance for evaluating nitrogen cycling in natural freshwater bodies.

ACKNOWLEDGMENT

We thank N. Andersen and A. Barbieri for their technical assistance and analytical support. Two anonymous reviewers made constructive comments. This study was supported by Swiss National Science Foundation grant NF 2-77631-98.

Received for review February 20, 2001. Accepted July 18, 2001.

AC010212U