analytical chemistry

Article

pubs.acs.org/ac

Chemical Method for Nitrogen Isotopic Analysis of Ammonium at Natural Abundance

Dongwei Liu,[†,§] Yunting Fang,*[,†] Ying Tu,[†] and Yuepeng Pan[‡]

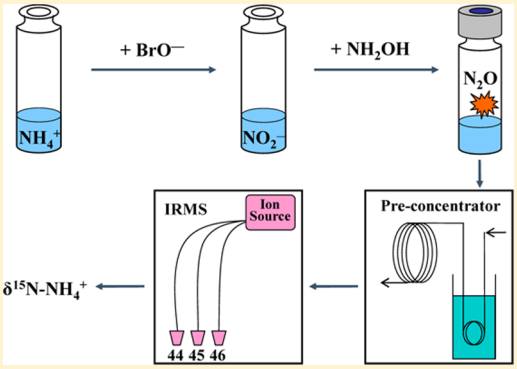
[†]State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, Liaoning 110164, China

[‡]State Key Laboratory of Atmospheric Boundary Layer Physics and Atmospheric Chemistry (LAPC), Institute of Atmospheric Physics, Chinese Academy of Sciences, Beijing, 100029, China

[§]College of Sources and Environment, University of Chinese Academy of Sciences, Beijing, 100049, China

Supporting Information

ABSTRACT: We report a new chemical method to determine the ^{15}N natural abundance ($\delta^{15}N$) for ammonium (NH_4^+) in freshwater (e.g., precipitation) and soil KCl extract. This method is based on the isotopic analysis of nitrous oxide (N_2O). Ammonium is initially oxidized to nitrite (NO_2^-) by hypobromite (BrO^-) using previously established procedures. NO_2^- is then quantitatively converted into N_2O by hydroxylamine (NH_2OH) under strongly acid conditions. The produced N_2O is analyzed by a commercially available purge and cryogenic trap system coupled to an isotope ratio mass spectrometer (PT-IRMS). On the basis of a typical analysis size of 4 mL, the standard deviation of $\delta^{15}N$ measurements is less than 0.3‰ and often better than 0.1‰ (3 to 5 replicates). Compared to previous methods, the technique here has several advantages and the potential to be used as a routine method for $^{15}N/^{14}N$ analysis of NH_4^+: (1) substantially simplified preparation procedures and reduced preparation time particularly compared to the methods in which diffusion or distillation is involved since all reactions occur in the same vial and separation of NH_4^+ from solution is not required; (2) more suitability for low volume samples including those with low N concentration, having a blank size of 0.6 to 2 nmol; (3) elimination of the use of extremely toxic reagents (e.g., HN_3) and/or the use of specialized denitrifying bacterial cultures which may be impractical for many laboratories.



Ammonium (NH_4^+) is one of the most important nitrogen (N) sources for plants and microbes and one of the primary forms of reactive nitrogen (N_r) input into the environment.[1] Depending on the environment, the ^{15}N natural abundance ($\delta^{15}N$) of NH_4^+ can provide insights into N sources and mechanisms of N transformations in terrestrial ecosystems.[2] A variety of methods have been developed for $\delta^{15}N$ determination for NH_4^+ during the past several decades.[3,4] However, these methods still exhibit some drawbacks.

The traditional methods of preparations for $\delta^{15}N$ analysis of NH_4^+ include quantitative separation of NH_4^+ from the solution and conversion to a gaseous species suitable for analysis by an isotope ratio mass spectrometer (IRMS). The main separation methods are distillation,[5] diffusion,[6] mercury precipitation,[7] and cation exchange.[8] However, the former two methods are both time-consuming and labor intensive and not very reliable at low NH_4^+ concentration, while the latter two methods are unsuitable for seawater samples and soil extracts. Furthermore, most currently available methods produce nitrogen (N_2) as an end-product analyte for IRMS using automated Rittenberg oxidation[9] or elemental analyzer (EA) combustion.[6,10] Thus, these methods require a relatively large amount of N (>1 μmol N) to avoid incidental atmospheric N_2 contamination.

Nitrous oxide (N_2O) is a much better alternative to N_2 as an IRMS analyte due to its trace concentration in the atmosphere.[4,11] The isotopic composition of N_2O can now be easily determined using a commercially available purge and trap system coupled to an IRMS (PT-IRMS).[4,12] Laughlin et al.,[11] for the first time, produced N_2O as a byproduct during the oxidation of NH_4^+ by alkaline hypobromite (BrO^-) following NH_4^+ diffusion to determine the ^{15}N of NH_4^+ in soil extract. However, large levels of background N (0.20 μmol test^{-1}) hampered its further use in samples with natural abundance.[11] Koba et al.[12] and Lachouani et al.[13] also developed methods for $\delta^{15}N$ analysis of NH_4^+ by using N_2O as the analyte. In these methods, NH_4^+ is trapped using microdiffusion, then oxidized to NO_3^- by alkaline persulfate, and finally converted to N_2O by the denitrifier method[12] or by chemical reactions[13] (with vanadium chloride (VCl_3) to reduce NO_3^- into NO_2^- and sodium azide (NaN_3) to further reduce NO_2^- into N_2O in a

Received: November 6, 2013
Accepted: March 24, 2014
Published: March 24, 2014

   dx.doi.org/10.1021/ac403756u | Anal. Chem. 2014, 86, 3787−3792

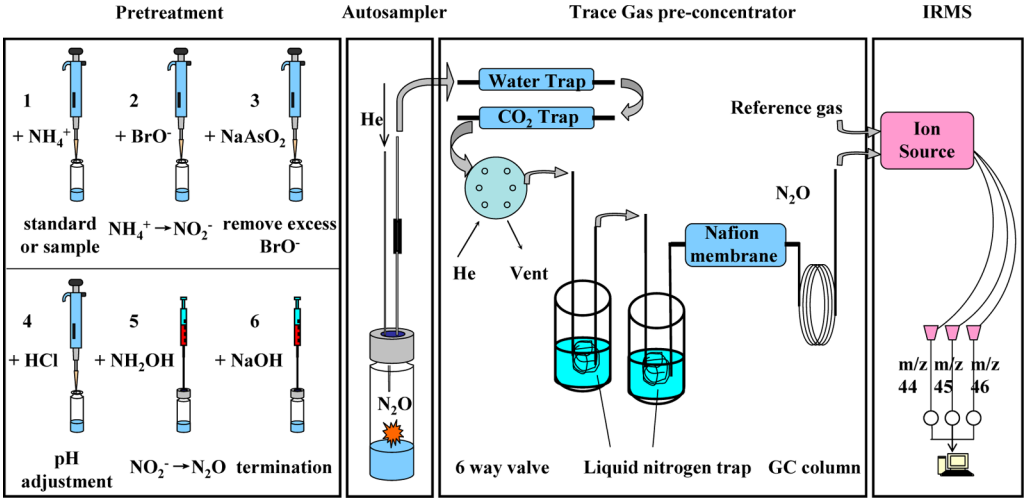


Figure 1. Method schematic for the isotopic analysis of ammonium that combined hypobromite oxidation and hydroxylamine reduction.

coupled reaction). While reducing the N requirement of sample NH_4^+ to 10 nmol and extending the range of samples to those having lower concentrations of NH_4^+ (e.g., μM range[14]), these two methods are still time-consuming and labor intensive.

Zhang et al.[4] introduced a novel method without the need of physical separation of NH_4^+ from solution. In this method, NH_4^+ is first quantitatively oxidized to NO_2^- by BrO^- and subsequently NO_2^- is reduced to N_2O using NaN_3 in an acetic acid buffer. This approach is readily applicable at low concentration, as the end-product N_2O is an excellent analyte for IRMS analysis. In addition, this method requires substantially less processing time, since there is no need for separation of NH_4^+ from solution. However, the main disadvantage of this method involves the safety precautions required for the handling of the azide buffer reagent and its subsequent disposal.[15] Hydrazoic acid (HN_3) which is the dominant species below pH 4.6 is extremely toxic and volatile, and it must be handled carefully in a fume hood at all time. Recently, Felix et al.[16] used the denitrifier method to convert NO_2^- produced by BrO^- oxidation into N_2O (in lieu of reduction by azide) in order to measure $\delta^{15}N$ values for passively collected NH_3. Nevertheless, this method requires the maintenance of specialized denitrifier cultures, which may be impractical for some isotope laboratories.

Here, we report, for the first time, a robust and quantitative chemical method for the conversion of NH_4^+ to N_2O suitable for $\delta^{15}N$ analysis. We adopted the BrO^- oxidation method to oxidize NH_4^+ to NO_2^-, as described in Zhang et al.,[4] in order to obviate the need for extraction of NH_4^+ from solution. Then, we used a hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) solution for the conversion of NO_2^- to N_2O, replacing reduction of NO_2^- by either HN_3[4] or the denitrifier method.[16] The solution of $NH_2OH{\cdot}HCl$ is nonvolatile and much less toxic. The reaction mechanism[17] between NO_2^- and NH_2OH is:

$$NH_2OH + HNO_2 \rightarrow N_2O + 2H_2O \quad (1)$$

Steven and Laughlin[18] had used this reaction to determine the ^{15}N content for ^{15}N-enriched NO_2^- in 2 M KCl soil extract. However, the precision was poor (CV > 10%) for all samples at 0.01 atom % excess and only acceptable (CV < 3%) when at ≥0.5 μmol N per test.

One objective of our study was to optimize the reaction conditions between NO_2^- and NH_2OH, with varied reagent concentration, pH, reaction time, and temperature, so that this reaction can be used to determine $\delta^{15}N$ for samples with low NH_4^+ concentration and at natural abundance. Another objective was to accomplish all reactions in the same small volume reaction vials (e.g., 20 mL) that can directly be used on the automated PT-IRMS for N_2O analysis, greatly simplifying preparation procedures and reducing the time required for analysis.

MATERIALS AND METHODS

Conversion of NH_4^+ to NO_2^-. The sample preparation before $^{15}N/^{14}N$ analysis as N_2O involves two primary reactions, oxidation of NH_4^+ to NO_2^- and reduction of NO_2^- to N_2O, requiring little time and effort (Figure 1). For NH_4^+ conversion, we adopted the BrO^- oxidation method as described in Zhang et al.[4] The BrO^- solution was prepared using the same method described by Zhang et al.[4] Briefly, a bromate/bromide stock solution was made by mixing 0.6 g of sodium bromate and 5 g of sodium bromide in 250 mL of deionized water (DIW). This stock solution has a shelf life of more than 6 months. BrO^- working solution was prepared daily by adding 1 mL of the stock solution to 50 mL of DIW followed by adding 3 mL of 6 M HCl to produce Br_2. After reacting in the dark for 5 min, 50 mL of 10 M NaOH was added quickly to produce BrO^-.

Zhang et al.[4] conducted several experiments to optimize experimental parameters for maximizing NH_4^+ oxidation yield, including reaction time, BrO^- amount, and NH_4^+ concentration. They found that 30 min was the optimal reaction time and that reducing or increasing the amount of BrO^- affected the NH_4^+ oxidation yield. Under optimal reaction time and BrO^- amount, the oxidation efficiencies of NH_4^+ to NO_2^- were more than 90% over a NH_4^+ concentration range of 0.5−20 μM in DIW and 0.5−10 μM in fresh seawater.

In the study of Zhang et al.,[4] 20 mL samples or standards were placed in the 60 mL reaction vessels. In our study, to reduce the sample volume and to have all chemical reactions occurring in the same vials, 20 mL headspace glass vials (Chromacol, 125 × 20-CV-P210) were used as reaction vials, which had been acid rinsed and combusted in an oven at 450 °C for 4 h before use. To maximize the yield of NO_2^- during BrO^- oxidation of NH_4^+, we checked the effect of NH_4^+

dx.doi.org/10.1021/ac403756u | Anal. Chem. 2014, 86, 3787−3792

concentration (Figure 2A), using the optimal reaction time and BrO^- amount suggested by Zhang et al.[4] To this end, we

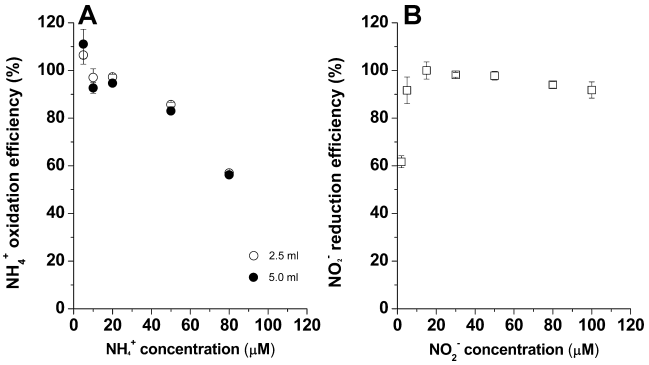


Figure 2. Effects of initial NH_4^+ concentration on NH_4^+ oxidation efficiency in 2.5 and 5.0 mL of solution (A), and effects of initial NO_2^- concentration on NO_2^- reduction efficiency (B). DIW was used as the matrix. Shown are means ± one SD, n = 3 for A and 5 for B.

pipetted 2.5 or 5.0 mL of laboratory $(NH_4)_2SO_4$ solution with varied concentrations from 5 to 80 μM to the 20 mL vials, and then, 0.25 or 0.5 mL of BrO^- working solution (volume ratio of BrO^- working solution to $(NH_4)_2SO_4$ solution of 1:10, as used by Zhang et al.[4]) was added to each vial and shaken vigorously. After 30 min of oxidation, 0.05 mL of sodium arsenite ($NaAsO_2$) solution was added to remove the excess BrO^- and terminate the reaction. The arsenite solution was made by dissolving 5.1 g of $NaAsO_2$ in 100 mL of DIW.[4] Afterward, the concentration of NO_2^- produced from oxidation of NH_4^+ was determined colorimetrically.

Conversion of NO_2^- to N_2O. The second step converts NO_2^- to N_2O (Figure 1). The azide and acetic acid buffer[4,13,19] and denitrifying bacteria[20] are popularly used in this step for N and oxygen (O) isotope analysis of NO_3^- and NO_2^-. The use of the azide method, however, requires purging of the buffer solution with helium (He) to remove any N_2O produced from NO_2^- contained in the azide and acetic acid buffer reagents. Furthermore, all reactions are required to be performed in a fume hood due to the high volatility and toxicity of HN_3. As for the denitrifier method, the maintenance of bacterial cultures is not always practical. Here, we used $NH_2OH{\cdot}HCl$, which is much less toxic and nonvolatile, as an alternative reductant for conversion of NO_2^- to N_2O. Hydroxylamine hydrochloride reagent stock solution was made by dissolving 0.2778 g of $NH_2OH{\cdot}HCl$ in 100 mL of DIW (shelf life ≤1 week). The working solution of $NH_2OH{\cdot}HCl$ is prepared daily by adding 3 mL of stock solution to 500 mL of DIW.

Previous studies have showed that N_2O yield was increased by higher acidity, chloride ion (Cl^-) concentration, NH_2OH concentration, and incubation time, but large reaction volumes (50 mL) were used.[18] To further optimize the conditions of NO_2^- reduction by NH_2OH with small volumes (e.g., several mL), we performed several additional experiments in this study to investigate the effects of temperature, time, pH, NH_2OH concentration, and NO_2^- concentration of the reaction. In the first experiment, 4 mL of 15 μM (10−20 μM was found to be the optimal concentration of NH_4^+ oxidation in the first step as shown in Figure 2A; see more in Results and Discussion) laboratory nitrite ($NaNO_2$) solution was pipetted into 20 mL vials. Then, 0 to 0.5 mL of 6 M HCl solution was pipetted to produce different solution acidity (Figure 3A). Afterward, 0.5

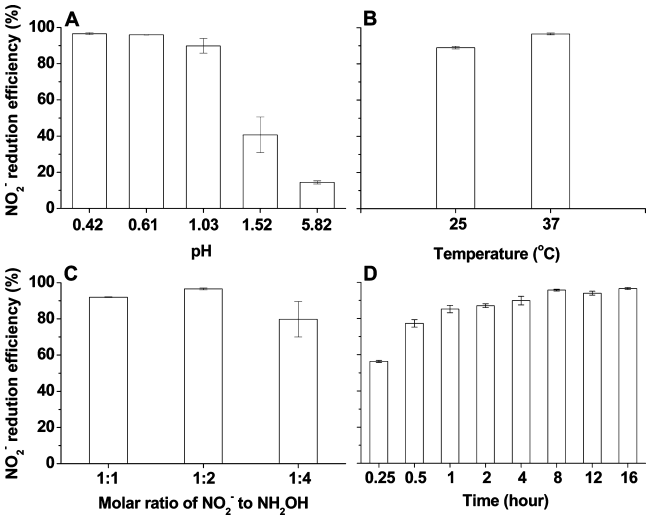


Figure 3. Effects of incubation pH (A), temperature (B), molar ratio of NO_2^- to $NH_2OH{\cdot}HCl$ (C), and incubation time (D) on the reaction of NO_2^- to N_2O by $NH_2OH{\cdot}HCl$. DIW was used as the matrix. Shown are means ± one SD, n = 5.

mL of $NH_2OH{\cdot}HCl$ working solution (molar ratio of NO_2^- to NH_2OH = 1:2) was added, and the vials were crimp sealed. The reaction took place on the shaker at 37 °C (120 cycles/min) for 16 h.

In the second experiment, we examined if room temperature (around 25 °C) was suitable for reaction (Figure 3B). In this experiment, 4 mL of 15 μM $NaNO_2$ solution was pipetted into 20 mL vials. Then, 0.5 mL of 6 M HCl solution and 0.5 mL of $NH_2OH{\cdot}HCl$ working solution was added to each vial to reach a pH of 0.42. The reaction took place on the shaker at room temperature for 16 h. In the third experiment, the effects of NH_2OH concentration were investigated while holding the reaction time for 16 h, temperature at 37 °C, and pH of 0.42. In the fourth experiment, we examined if reduced reaction time affected reduction efficiency at the conditions with temperature of 37 °C, pH of 0.42, and NO_2^-/NH_2OH ratio of 1:2. In the fifth experiment, we investigated the effect of NO_2^- concentration while holding the same reaction conditions as in the fourth experiment. After incubations of these experiments, 0.5 mL of 5 M sodium hydrate (NaOH) was injected to make the solution basic and stop the reaction and to avoid flushing volatile hydrochloric acid into the PT-IRMS equipment during isotopic analysis of N_2O. The NO_2^- concentration remaining in the reaction vials was determined colorimetrically.

Overall Reaction Performance. We found that 10 and 20 μM was the optimal NH_4^+ concentration for NH_4^+ oxidation (Figure 2A) and that a reaction pH of 0.42, temperature at 37 °C, NO_2^-/NH_2OH ratio of 1:2, and time of 16 h were the optimal conditions for NO_2^- reduction by NH_2OH (Figures 2B and 3). Under these optimal conditions, we investigated the overall reaction performance using three international standards (IAEA N1, +0.4‰; USGS 25, −30.4‰; USGS 26, +53.7‰) both in DIW and 2 M KCl solution (Figure 4). First, 4 mL of 15 μM international standard solution prepared in DIW or 2 M KCl solution (KCl reagent was combusted for 48 h at 450 °C prior to use) was added into 20 mL vials (Figure 1). Then, 0.4 mL of BrO^- working solution was added to each vial and shaken vigorously. After 30 min of oxidation, 0.05 mL of $NaAsO_2$ solution was added to remove excess BrO^- and terminate the reaction. Afterward, 0.5 mL of 6 M HCl solution

dx.doi.org/10.1021/ac403756u | Anal. Chem. 2014, 86, 3787−3792

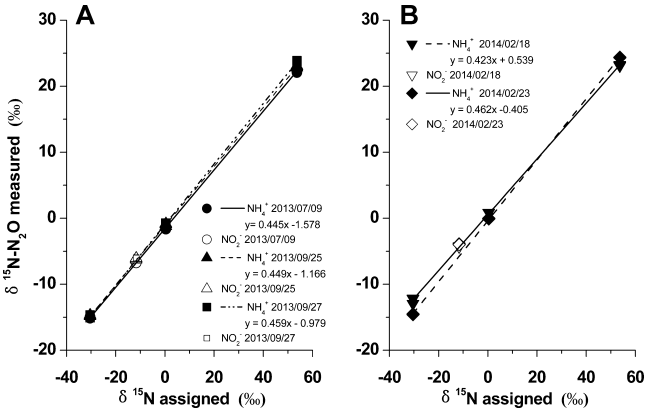


Figure 4. Correlations between the $\delta^{15}N-N_2O$ and the $\delta^{15}N$ assigned for three international standards (IAEA N1, USGS25, and USGS26) in DIW (A) and 2 M KCl solution (B). The regression curves were across three NH_4^+ standards. R^2 = 0.9997–0.9999, n = 3 except for that on 2014/02/23 in which n = 5. $\delta^{15}N-N_2O$ measured was corrected for blank. The results of laboratory nitrite (NO_2^-) were also shown.

was pipetted to acidify the solution and the vials were capped tightly with gray butyl septa (Chromacol, 20-B3P, No. 1132012634) and aluminum crimp seals (ANPEL Scientific Instrument (Shanghai) Co. ltd., 6G390150). After that, 0.5 mL of $NH_2OH{\cdot}HCl$ working solution was added to each vial by a gastight syringe, and the vials were kept in a shaker for 16 h at a temperature of 37 °C (Figure 1). Finally, 0.5 mL of 5 M NaOH solution was injected to make the solution basic and stop the reaction. The produced N_2O within the vials was analyzed for N_2O isotopes as described below. We also checked if excess BrO^- removal by $NaAsO_2$ addition was necessary by comparing it to no $NaAsO_2$ addition (Figure S-1, Supporting Information).

Isotope Analysis of N_2O. Stable nitrogen isotope of the produced N_2O is analyzed by an automated PT-IRMS (Figure 1), which included a continuous flow IRMS (IsoPrime100, IsoPrime limited, UK) and a 112-slot autosampler (Gilson GX-271, IsoPrime limited, UK) with a cryo-focusing unit (Trace Gas Preconcentrator, IsoPrime limited, UK). The autosampler and PT-IRMS setup can be found in the Supporting Information text.

Calculation of the True $\delta^{15}N$ for NH_4^+. The N_2O isotopic ratios for all standards and samples were normalized to the N_2O reference gas, which had not yet been calibrated for $\delta^{15}N$ and $\delta^{18}O$ and was set to be zero for both $\delta^{15}N$ and $\delta^{18}O$, in order to eliminate analytical variation associated with any drift in IRMS response over the course of long analytical runs. The three international standards (IAEA N1, USGS 25, and USGS 26) were treated with the same protocol for the samples and were used to calibrate $\delta^{15}N-NH_4^+$ of samples. In theory, the slope of the relationship between the true $\delta^{15}N$ of international NH_4^+ and the measured $\delta^{15}N$ of the final produced N_2O should be 0.5, as described by Stevens and Laughlin,[17] since one N atom in the product N_2O originates from NH_4^+ and one from NH_2OH. The intercept reflects the combination of initial $\delta^{15}N$ of NH_2OH and isotopic fractionation associated with the reactions from NH_4^+ to N_2O.

The calibration equation is:

$$\delta^{15}N_{NH_4^+sample} = (\delta^{15}N_{N_2Osample} - \text{intercept})/\text{slope} \quad (2)$$

where the intercept and slope were obtained from the linear regression of the $\delta^{15}N$ measured for the product N_2O produced by the standards and the $\delta^{15}N$ assigned for the standards.

In addition, a laboratory $NaNO_2$ (−11.7‰, determined by EA-IRMS) was run to monitor the performance of the reduction of NO_2^- to N_2O and long-term consistency. On the basis of the N_2O peak produced from the laboratory NO_2^- and from standard NH_4^+, we can also check the efficiency of NH_4^+ oxidation to NO_2^-.

RESULTS AND DISCUSSION

NH_4^+ Oxidation Efficiency. The quantitative oxidation of NH_4^+ to NO_2^- is the first step in the $\delta^{15}N-NH_4^+$ measurement. Nearly complete oxidation from NH_4^+ to NO_2^- is required to avoid inconsistent isotope fractionation.[4] Oxidation efficiency was expressed as percentage of NO_2^- yield oxidized from NH_4^+ with respect to the initial NH_4^+ amount. We found that 10 and 20 μM was the optimal NH_4^+ concentration and oxidation efficiency ranged from 93% to 97% (Figure 2A), consistent with the result of Zhang et al.[4] In the later analysis, 15 μM was used as the reaction concentration and 0.4 mL of BrO^- working solution as oxidant amount when preparing for standards or samples to produce 60 nmol N_2O.

NO_2^- Reduction Efficiency. The remaining NO_2^- concentration was measured after terminating the reaction. The reduction efficiency was expressed as a percentage of NO_2^- consumption to initial NO_2^-. The reaction rate of NO_2^- reduction by NH_2OH was shown to be highly dependent on pH, and its reaction rate increased rapidly at pH below 1 (Figure 3A), because the reaction species was HNO_2 and not NO_2^-.[17] There was no significant difference at pH below 1, and the reduction efficiency was 97%. At pH 1.52 and 5.82, the reaction was slow, and reduction efficiency decreased to 41% and 14%, respectively (Figure 3A).

Elevated temperature (37 °C) above the room temperature (around 25 °C) significantly increased NO_2^- reduction efficiency to 97% (Figure 3B), but NO_2^- reduction efficiency was also high even at room temperature (89%, Figure 3B). Nitrite reduction yield was highest with a molar ratio of NO_2^- to NH_2OH at 1:2 (Figure 3C). Nitrite reduction efficiency was over 90% when the reaction lasted for 4 h or longer and reached a maximum of 97% when incubated for 16 h (Figure 3D). A longer incubation time than 16 h did not further increase reduction efficiency (data not shown). Nitrite reduction efficiency was 92% to 100% when initial NO_2 concentration ranged from 5 to 100 μM, with optimal concentration range from 15 to 50 μM (Figure 2B).

Overall Reaction Performance. The solution produced from the BrO^- oxidization reaction is very alkaline with a pH of about 12. In contrast, the following reduction of NO_2^- by NH_2OH requires highly acidic conditions. So, a volume of 0.5 mL of 6 M HCl was added to reduce pH prior to the injection of $NH_2OH{\cdot}HCl$ solution (Figure 1). Three international NH_4^+ standards were chosen to reflect the natural abundance range (−32.4‰ to +53.7‰, Figure 4). The precision of $\delta^{15}N$ of the product N_2O was better than 0.3‰ and often better than 0.1‰ (one standard deviation, 3 to 5 replicates, Figure 4). This precision is adequate, especially when compared to the large range of natural samples from −56.1‰ for NH_3 emitted from turkey facility[16] to > +20‰ for NH_4^+ in manure and septic waste.[21]

The relationship between the assigned $\delta^{15}N-NH_4^+$ and the measured $\delta^{15}N-N_2O$ was significant and linear. The slopes of

dx.doi.org/10.1021/ac403756u | Anal. Chem. 2014, 86, 3787–3792

linear regressions were 0.42 to 0.46 and did not changed significantly from batch to batch in different days in DIW (Figure 4). The slopes were 0.44 to 0.45 before blank correction for standards in DIW (data not shown). The N_2O peak of the reagent blank was 1% to 3% of the N_2O peak of the standards in DIW, so the slopes should be about 0.49 before blank correction. The slope was slightly lower than the theoretical value (0.5) as predicted by the 1:1 combination of NH_4^+−N and NH_2OH−N. The reasons for the small devivation from the theoretical slope were not clear. However, the excellent correlation of assigned and measured $\delta^{15}N$ demonstrate that the method is a robust and quantitative technique for measurement of $\delta^{15}N$ of NH_4^+.

The intercept values were also relatively constant across different batches of reagents prepared on different days (Figure 4). The intercept represents the combined influence of the $\delta^{15}N$ of NH_2OH and any isotope fractionation during the reactions. The $\delta^{15}N$−N_2O of sample or standard was normalized to the N_2O reference gas, and both $\delta^{15}N$ and $\delta^{18}O$ were assigned as zero in the present study; thus, we were not able to know the isotopic fractionation during the whole reaction. However, we measured $\delta^{15}N$ for the laboratory NO_2^-, which produced almost the same N_2O peak height as that produced from NH_4^+ standards (data not shown). $\delta^{15}N$ of laboratory NO_2^- can be predicted by the regression line generated by international NH_4^+ standards (i.e., the point of NO_2^- was on the regression line, Figure 4), suggesting that the oxidation from NH_4^+ to NO_2^- was almost complete and there was virtually no isotopic fractionation during the NH_4^+ oxidation.

Effects of Excess BrO^-. Zhang et al.[4] reported that the remaining BrO^- should be removed after NH_4^+ oxidation by $NaAsO_2$, because BrO^- would react with the azide reagent in the next step to produce N_2O blank. Because $NaAsO_2$ is a toxic chemical, we examined whether the $NaAsO_2$ addition could be omitted in the NO_2^- reduction by $NH_2OH{\cdot}HCl$ in our study. The results showed that without adding $NaAsO_2$ to remove the excess BrO^- the relationship was still linear across the standards (Figure S-1, Supporting Information). However, the slope was only 0.30, deviating far from 0.5 (Figure S-1, Supporting Information). Omission of the $NaAsO_2$ addition step resulted in a much more negative intercept (Figure S-1, Supporting Information), lower N_2O production (on average 22% of that produced with $NaAsO_2$ addition, Table S-1, Supporting Information), and much larger blanks (on average 41 nmol, Table S-1, Supporting Information) relative to the results that included $NaAsO_2$ addition, indicating that the remaining BrO^- likely reacted with NH_2OH in the reduction step. Thus, $NaAsO_2$ addition was an essential step, and 0.05 mL of $NaAsO_2$ solution was added to each reaction vial in our final protocol.

Blank Sizes. As mentioned above, the blank size was small in this study. The N_2O peak produced from reagents was between 0.6 and 2 nmol of N per test in DIW, or 1% to 3% of the samples or standards peak when producing 60 nmol N_2O for isotopic analysis. The blank size is comparable to that of NO_3^- isotope analysis by the denitrifier method[20] or azide method.[15] Notably, the blank size was relatively larger in the experiments using the 2 M KCl solution, about 4 nmol per test.

Possible sources of this blank include: (1) trace NH_4^+ or NO_2^-, or both, in chemical reagents, and (2) air N_2O inside the 20 mL reaction vial before NO_2^- reduction. In our study, we did not purge the reaction vial to remove air N_2O. We found that the N_2O peak in the reaction vial was about 0.6 nmol N. Thus, the N contaminants in chemical reagents could be 0 to 1.4 nmol per test. We can further reduce blank size by removing air N_2O when handling with the samples with very low NH_4^+ concentration (<5 μM). However, we suggest that this step is not required when the total blank size is less than 3% of the samples or standards so that preparation time can be shortened. Furthermore, we found that purging the NH_2OH solution with He gas before use did not reduce blank size (data not shown), suggesting the effects of N_2O dissolved in solution and other potential N contamination were of minor importance.

Recommendations for the Method. The recommended protocols for the sample preparation before N_2O isotope analysis as outlined in Figure 1 are: (1) pipet samples or standard solution into precombusted reaction vials. Samples should be diluted to 10−20 μM to maximize oxidation yield. Any detectable NO_2^- in samples should be removed prior to NH_4^+ oxidation; (2) add BrO^- working solution (volume ratio of sample to BrO^- solution is 1:10) and shake vigorously; (3) after 30 min, remove excess BrO^- and terminate oxidation by adding 0.05 mL of $NaAsO_2$ solution. The NO_2^- produced from NH_4^+ oxidation can be determined to check the oxidation efficiency. Arsenite solution addition is necessary since the remaining BrO^- will react with the reagents in the next steps; (4) add 6 M HCl solution into vials to lower solution pH below 1 and crimp seal the vials. This pH adjustment should be done before the NH_2OH injection, since NO_2^- reduction requires very low pH conditions; (5) inject $NH_2OH{\cdot}HCl$ working solution with a gastight syringe. The optimal molar ratio of NH_4^+ to NH_2OH is 1:2. Afterward, keep the vials on a shaker (120 cycles/min) with temperature of 37 °C for 8 to 16 h; and finally, (6) inject 0.5 mL of 5 M NaOH solution with a gastight syringe to stop reaction and to absorb carbon dioxide (CO_2) within the vials.

Application to Freshwater and Soil Extract Samples. We applied this newly developed method to precipitation samples collected from an urban site in Beijing,[22] which exhibited a large NH_4^+ concentration range from 0.25 to 8.3 mg N L^{-1} (Figure 5). The results showed that almost all of these precipitation samples had negative $\delta^{15}N$ values for NH_4^+, with a range from −33.0‰ to +14.0‰ and an arithmetic mean of −10.8‰ (Figure 5). These values were comparable to the previous reports for a farm site near Beijing (−13‰ to +13‰),[23] a forest site in southern China (−18‰ to 0‰),[14] and a city in southwestern China (−38‰ to +5‰).[24]

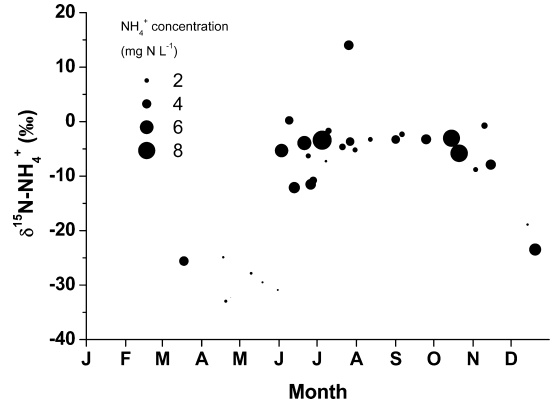


Figure 5. $\delta^{15}N$ of NH_4^+ in precipitation collected from an urban site in Beijing in 2012.

dx.doi.org/10.1021/ac403756u | Anal. Chem. 2014, 86, 3787−3792

We also applied this method to soil KCl extracts. Soil was collected from two grassland sites in northern China in July 2012 and was extracted with 2 M KCl solution (10 g of fresh soil/50 mL of KCl solution) on the sampling day. $\delta^{15}N$ of NH_4^+ at the dry site ranged from +6.5‰ to +13.8‰ and from −0.7‰ to +5.4‰ at the less dry site (Table S-2, Supporting Information).

CONCLUSIONS

To our knowledge, our method allows us to measure, for the first time, ^{15}N natural abundance for freshwater samples and soil KCl extracts by combining NH_4^+ oxidation by BrO^- and NO_2^- reduction by NH_2OH to produce N_2O for isotope compositional analysis in an automated purge and trap system coupled to an IRMS. The improved technique here does not require NH_4^+ separation from solution, and all reactions occur in the same vial, thus substantially reducing preparation time, particularly compared to methods in which diffusion or distillation is involved. The NH_4^+ conversion to N_2O for one batch (95 analyses, including 60 samples and 7 replicates of IAEA N1, USGS 25, USGS 26, laboratory $NaNO_2$, and blank) requires one day, with the NH_2OH reaction running overnight. Analysis of N_2O isotopes by PT-IRMS for one batch takes 1.5 days. The total preparation time is 2.5 days for one batch, therefore increasing the throughput.

Another advantage of our method is the use of a much less dangerous reagent, NH_2OH, to reduce NO_2^- to N_2O, as an alternative to HN_3, which is highly volatile and toxic, and as an alternative to denitrifying bacteria, which are not available in most laboratories. Thus, our method can be set up easily by most stable isotope laboratories. Third, the blank size is small, 0.6 to 2 nmol, corresponding to 1% to 3% of standards when producing 60 nmol N_2O. Therefore, this method is applicable for the samples with low NH_4^+ concentrations and requires smaller sample volumes (e.g., < 4 mL). Finally, our method can work effectively for soil KCl extracts and probably seawater samples. The overall performance of standards in 2 M KCl solution was comparable to that in DIW. In summary, our method has the potential to be used as a routine procedure for $^{15}N/^{14}N$ analysis of NH_4^+.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: fangyt@iae.ac.cn. Phone: +86-24-83970541.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The work was jointly supported by the National Natural Science Foundation of China (Nos. 31370464, 41230642, and 41021004) and State Key Laboratory of Forest and Soil Ecology Institute of Applied Ecology, CAS (No. Y1SRC111J6 and No. LFSE 2013-13). We appreciate Dr. Keisuke Koba at Tokyo University of Agriculture and Technology, Dr. Weixing Zhu at Binghamton University, State University of New York, and Dr. Scott D. Wankel at Woods Hole Oceanographic Institution for their constructive comments and language editing on the earlier version of the manuscript. We thank Mr. Yi Xu, the engineer of Beijing Jade - Element Technology Co., Ltd, for setting up the isotope analysis system in our laboratory. We also thank Chao Wang, Shili Tian, and Yuzhu Xie for their assistance in laboratory analysis.

REFERENCES

(1) Sutton, S. C. *J. Libr. Scholarly Commun.* **2013**, *1*, 10.
(2) (a) Takebayashi, Y.; Koba, K.; Sasaki, Y.; Fang, Y.; Yoh, M. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 1001−1008. (b) Makarov, M. I. *Eurasian Soil Sci.* **2009**, *42*, 1335−1347.
(3) (a) Feast, N. A.; Dennis, P. F. *Chem. Geol.* **1996**, *129*, 167−171. (b) Risgaard-Petersen, N.; Revsbech, N. P.; Rysgaard, S. *Soil Sci. Soc. Am. J.* **1995**, *59*, 1077−1080. (c) Sebilo, M.; Mayer, B.; Grably, M.; Billiou, D.; Mariotti, A. *Environ. Chem.* **2004**, *1*, 99−103.
(4) Zhang, L.; Altabet, M. A.; Wu, T.; Hadas, O. *Anal. Chem.* **2007**, *79*, 5297−5303.
(5) Preston, T.; Bury, S.; McMeekin, B.; Slater, C. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 965−968.
(6) Stark, J. M.; Hart, S. C. *Soil Sci. Soc. Am. J.* **1996**, *60*, 1846−1855.
(7) Fisher, T.; Morrissey, K. *Mar. Chem.* **1985**, *16*, 11−21.
(8) Lehmann, M. F.; Bernasconi, S. M.; McKenzie, J. A. *Anal. Chem.* **2001**, *73*, 4717−4721.
(9) Brooks, P.; Stark, J. M.; McInteer, B.; Preston, T. *Soil Sci. Soc. Am. J.* **1989**, *53*, 1707−1711.
(10) (a) Burke, I. C.; O'Deen, L. A.; Mosier, A. R.; Porter, L. K. *Soil Sci. Soc. Am. J.* **1990**, *54*, 1190−1192. (b) Holmes, R.; McClelland, J.; Sigman, D.; Fry, B.; Peterson, B. *Mar. Chem.* **1998**, *60*, 235−243.
(11) Laughlin, R.; Stevens, R.; Zhuo, S. *Soil Sci. Soc. Am. J.* **1997**, *61*, 462−465.
(12) Koba, K.; Inagaki, K.; Sasaki, Y.; Takebayashi, Y. Nitrogen isotopic analysis of dissolved inorganic and organic nitrogen in soil extracts. In *Earth, Life and Isotopes: Ecology and Pedosphere*; Ohkouchi, N., Tayasu, I., Koba, K., Eds.; Kyoto Univ. Press: Kyoto, Japan, 2010; pp 17−36.
(13) Lachouani, P.; Frank, A. H.; Wanek, W. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 3615−3623.
(14) Koba, K.; Fang, Y.; Mo, J.; Zhang, W.; Lu, X.; Liu, L.; Zhang, T.; Takebayashi, Y.; Toyoda, S.; Yoshida, N.; Suzuki, K.; Yoh, M.; Senoo, K. *J. Geophys. Res.* **2012**, *117*, G02015 DOI: 10.1029/2010JG001615.
(15) McIlvin, M. R.; Altabet, M. A. *Anal. Chem.* **2005**, *77*, 5589−5595.
(16) David Felix, J.; Elliott, E. M.; Gish, T. J.; McConnell, L. L.; Shaw, S. L. *Rapid Commun. Mass Spectrom.* **2013**, *27*, 2239−2246.
(17) Bothner-By, A.; Friedman, L. *J. Chem. Phys.* **1952**, *20*, 459−462.
(18) Stevens, R. J.; Laughlin, R. J. *Soil Sci. Soc. Am. J.* **1994**, *58*, 1108−1116.
(19) (a) Tsunogai, U.; Kido, T.; Hirota, A.; Ohkubo, S. B.; Komatsu, D. D.; Nakagawa, F. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 345−354. (b) Smirnoff, A.; Savard, M. M.; Vet, R.; Simard, M. C. *Rapid Commun. Mass Spectrom.* **2012**, *26*, 2791−2804.
(20) (a) Sigman, D.; Casciotti, K.; Andreani, M.; Barford, C.; Galanter, M.; Böhlke, J. *Anal. Chem.* **2001**, *73*, 4145−4153. (b) Casciotti, K.; Sigman, D.; Hastings, M.; Böhlke, J.; Hilkert, A. *Anal. Chem.* **2002**, *74*, 4509−4512.
(21) Kendall, C.; Elliott, E. M.; Wankel, S. D. Tracing anthropogenic inputs of nitrogen to ecosystems, Chapter 12. In *Stable Isotopes in Ecology and Environmental Science*, 2nd ed.; Michener, R., Lajtha, K., Eds.; Wiley-Blackwell: Oxford, UK, 2007; pp 375−449.
(22) Pan, Y.; Wang, Y.; Tang, G.; Wu, D. *Atmos. Chem. Phys.* **2012**, *12*, 6515−6535.
(23) Zhang, Y.; Liu, X.; Fangmeier, A.; Goulding, K.; Zhang, F. *Atmos. Environ.* **2008**, *42*, 1436−1448.
(24) Xiao, H.; Xiao, H.; Long, A.-m.; Wang, Y. *Atmos. Environ.* **2010**, *54*, 201−206.