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Research Paper

Nitrite removal improves hydroxylamine analysis in aqueous solution by conversion with iron(III)

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Environmental context. Nitrogen is an essential nutrient for marine organisms, and thus an understanding of the marine nitrogen cycle is a crucial factor in predicting the sensitivity of marine life to environmental change. Hydroxylamine is a short-lived intermediate in nitrogen transformation processes, and reliable detection of this compound in seawater can help to identify these processes within the marine nitrogen cycle.

Abstract. Dissolved hydroxylamine (NH_2OH) is a short-lived compound produced in the oceanic environment during nitrification and dissimilatory reduction of nitrate to ammonium (DNRA). The ferric ammonium sulfate (FAS) conversion method is the only method available so far to determine dissolved NH_2OH in nanomolar concentrations in seawater. We show that side reactions of dissolved nitrite (NO_2^-) can result in a significant bias in the NH_2OH concentration measurements when applying the FAS conversion method. We propose to scavenge dissolved NO_2^- by addition of sulfanilamide to suppress effectively the undesired side reactions by NO_2^-. This modification of the FAS conversion method will allow a NH_2OH determination even in oceanic regions with high NO_2^- concentrations. A reliable detection of NH_2OH in seawater samples can give us a clue about the occurrence of active nitrification or DNRA in the ocean and, therefore, will provide further insights about the oceanic nitrogen cycle.

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Introduction

Hydroxylamine (NH_2OH) is a short-lived compound of the marine nitrogen cycle.[1] Two microbial pathways that involve NH_2OH have been identified so far: Ammonium (NH_4^+) oxidation to nitrate (NO_3^-) (i.e. nitrification) and dissimilatory reduction of nitrate to ammonium (DNRA). The idea that NH_2OH also occurs during the anaerobic ammonium oxidation (anammox) pathway could not be verified.[2,3]

NH_2OH is formed as an intermediate during the first step of nitrification by ammonia-oxidising bacteria (AOB)[4,5]:

$$NH_4^+ \rightarrow NH_2OH \rightarrow NO_2^-$$

There is increasing evidence that ammonium oxidation in the ocean is dominated by ammonia-oxidising archaea (AOA) and not AOB.[6,7] Little is known about the pathway of ammonium oxidation by AOA, however, and unlike AOB, a set of genes encoding the NH_2OH oxidoreductase has not been identified in AOA yet.[8] Thus, potential formation of NH_2OH during archaeal nitrification remains to be proven.

In contrast to nitrification, which occurs at oxic conditions, bacterial DNRA is an anaerobic process that requires anoxic conditions.[9–11] During DNRA, NH_2OH evolves as an enzyme-bound intermediate in the reduction of nitrite to ammonium. It may be released from the binding site of the enzyme under acidic conditions[12]:

$$NO_3^- \rightarrow NO_2^- \rightarrow (NH_2OH) \rightarrow NH_4^+$$

Dissolved NH_2OH is not stable in seawater and its turnover times range from 4 h (in artificial seawater) to 8 h (in natural seawater).[13,14] The decomposition of NH_2OH in aqueous solution is strongly enhanced under alkaline conditions, where NH_2OH rapidly reacts with ambient oxygen.[15] In addition, several reactions with transition metal ions and complexes[16–18] and the catalytic effect of copper and other heavy metal ions in the decomposition of NH_2OH[17,19] have been reported. Protonation of NH_2OH to NH_3OH^+ under acidic conditions results in the stabilisation of the molecule.[15]

First attempts to determine NH_2OH in seawater with a spectro-photochemical method were hampered by a high detection limit.[14,20] On the basis of the work by von Breymann et al.,[21] Butler and Gordon[13] developed a method for the determination of NH_2OH in seawater at nanomolar concentrations. This method is based on the oxidation of NH_2OH to nitrous oxide (N_2O) by iron(III) using ferric ammonium sulfate ($NH_4Fe(SO_4)_2$, FAS) as oxidation agent[18,22]:

$$Fe(NH_2OH)^{3+} \rightarrow Fe^{2+} + H_2NO^{\cdot} + H^+ \quad (1)$$

$$Fe^{3+} + H_2NO^{\cdot} \rightarrow Fe^{2+} + HNO + H^+ \quad (2)$$

$$2HNO \rightarrow N_2O + H_2O \quad (3)$$

The resulting N_2O is subsequently analysed quantitatively using a gas chromatograph equipped with an electron capture detector (GC-ECD). The chemical conversion of NH_2OH into

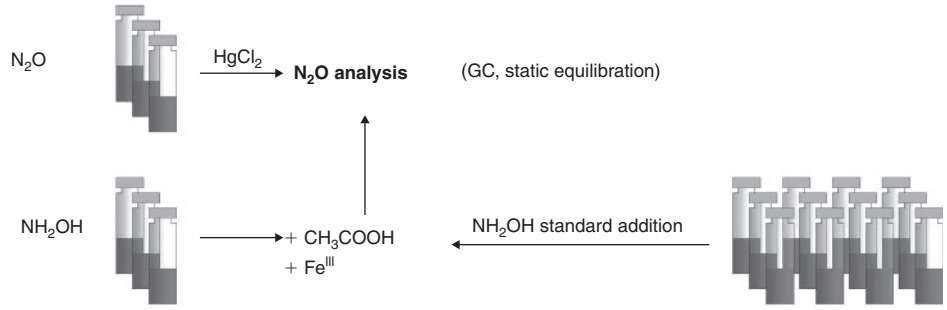

Fig. 1. Sampling procedure for hydroxylamine (NH_2OH) analysis: triplicate samples are taken for the conversion of NH_2OH into N_2O and for background N_2O analysis. The efficiency of the NH_2OH conversion is determined by standard addition.

N_2O with FAS involves numerous side reactions,[22] which results in a conversion efficiency significantly lower than 100 %.[21] The conversion mechanism involves a reaction sequence with short-lived nitrogen compounds as intermediates that easily undergo undesired side reactions.[22] In addition, alternative reaction pathways giving different reaction products or a different stoichiometry have been proposed for other iron species.[18,23]

Up to now the FAS conversion method is the only known method to determine NH_2OH in seawater. Only a few measurements of NH_2OH concentrations in oceanic waters are known so far and range from 0 to 360 nmol L^{-1}.[21,24–27] NH_2OH concentrations in coastal waters show a strong seasonal variability[28] and the observed maximum concentrations in coastal areas are generally higher than the NH_2OH concentrations found off the coast.[21]

Butler and Gordon[13] did not discuss a potential NO_2^- interference with the FAS conversion method. However, NO_2^- has to be considered as a potential source of uncertainty as it may interfere through some of its side reactions: At a pH of 3 (which is the recommended pH at which the FAS conversion should be performed according to Butler and Gordon[22]), NO_2^- is prevalent mainly in its protonated form, nitrous acid (HNO_2, $pK_a = 3.398$). This can directly bias the NH_2OH determination by FAS conversion into N_2O in two ways:

(1) HNO_2 is not stable in aqueous solution and one of the decomposition pathways leads to N_2O[29,30]:

$$4HNO_2 \rightarrow 2HNO_3 + N_2O + H_2O \quad (4)$$

Although the formation of N_2O is not the main decomposition pathway of HNO_2, the amount of N_2O produced from NO_2^- concentrations encountered in oceanic waters has to be taken into account. NO_2^- concentrations in the oceanic water column can reach up to 13 µmol L^{-1} [31] and only nanomolar concentrations of additional N_2O produced from NO_2^- decomposition would be sufficient for a significant bias. Moreover, HNO_2 is also known to react with organic matter and several metal cations to form N_2O,[32,33] which may lead to an additional source of N_2O in natural waters.

(2) NH_2OH reacts with HNO_2 to form hyponitrous acid ($H_2N_2O_2$), which, in turn, can rapidly decompose to N_2O and water[34,35]:

$$NH_2OH + HNO_2 \rightarrow H_2N_2O_2 + H_2O \quad (5)$$

$$H_2N_2O_2 \rightarrow N_2O + H_2O \quad (6)$$

The overall net reaction of Reactions 5 and 6 has a different stoichiometry than the conversion of NH_2OH by iron(III) (Reactions 1–3), which requires two molecules of NH_2OH to form one molecule of N_2O. Different conversion efficiencies of these concurring reactions may therefore lead to a further bias.

The aims of this study were (i) to evaluate the potential bias of the side reactions of NO_2^- in the FAS conversion method and (ii) to find an appropriate treatment to determine NH_2OH in seawater samples by avoiding interferences caused by NO_2^-.

Methods

For the analysis of dissolved NH_2OH we followed the measurement procedure of Schweiger et al.,[28] which was slightly modified from the procedure by Butler and Gordon[13] (Fig. 1). The NH_2OH concentration in the samples was calculated as follows:

$$[NH_2OH] = \left([N_2O]_{FAS} - [N_2O]_{BG}\right)/RC \quad (7)$$

$$RC = 2 \times m_{stadd} \quad (8)$$

where m_{stadd} is the regression slope of the standard addition and $[N_2O]_{FAS}$ and $[N_2O]_{BG}$ are the N_2O concentrations of samples with and without FAS conversion. The factor of two in the calculation of the recovery factor (Eqn 8) results from the stoichiometry of the reaction between NH_2OH and FAS.

In this manuscript, we investigated the effect of the side reactions of HNO_2 on the different stages of the NH_2OH analysis according to Schweiger et al.[28] in several laboratory experiments. Therefore, instead of calculating the final NH_2OH concentrations, we present the N_2O concentrations in the results and discussion section, as the N_2O produced from the side reactions may bias the actual NH_2OH calculations. Similarly, the side reaction between NH_2OH and NO_2^- involves a different stoichiometry than the reaction between NH_2OH and FAS. In experiments that involve the conversion of NH_2OH into N_2O we calculated a conversion factor instead of the recovery factor defined above. The conversion factor was calculated in two ways: (a) in experiments with only one standard concentration added it was calculated as the ratio between the difference of N_2O concentrations with and without NH_2OH addition and the concentration of the NH_2OH standard and (b) in experiments with different standard concentrations added it was calculated as the slope of the linear regression between measured N_2O concentrations and NH_2OH standard additions. Error bars

shown in the figures reflect the standard deviation of triplicate measurements, calculated according to David.[36]

The laboratory experiments were prepared as follows: 10 mL of MilliQ or seawater was placed into opaque vials (20 or 24 mL) that were subsequently sealed with butyl rubber stoppers and crimped before addition of the reactants. In order to remove the background N_2O, the vials were purged for 20 min with N_2O-free nitrogen gas (99.999 %, AirLiquide, Düsseldorf, Germany) at a flow rate of ~80–100 mL min^{-1} or the measurements were corrected for background N_2O concentrations that were obtained from triplicate control samples. Sodium nitrite solutions (100 µL) were added to the samples, leading to final concentrations between 0.1 and 10 µmol L^{-1} and thereby covering the range of ambient NO_2^- concentrations.[31]

Experiments were carried out with three different water types in order to simulate typical matrix effects: We used (i) MilliQ water, (ii) aged filtered surface seawater from the tropical North Atlantic Ocean (~10°N, 30°W, from December 2009) or (iii) unfiltered seawater from the Boknis Eck Time Series Station, located in the Eckernförde Bay in the south-western Baltic Sea (hereafter referred to as BE water; sampling depth 15 m, samples were taken between June 2010 and February 2011).[37,38] Experiments with BE water were carried out within 7 days after sampling. BE water has a lower salinity (typically between 12.5 and 24.5) than the surface seawater from the tropical North Atlantic Ocean (~35).

Sample preparation and general treatments

Stock solutions of $NaNO_2$ (p.a., Merck KGaA, Darmstadt, Germany, ~20 mg per 100 mL, the exact concentration was calculated from the mass weight) were prepared in MilliQ water a maximum of three days before analysis and stored at 4 °C before analysis. If necessary, the stock solution was diluted further to obtain different NO_2^- concentrations.

FAS solutions (p.a., Merck KGaA, 1.206 g per 100 mL) were prepared in MilliQ water at least three days before the experiments to ensure the complete dissolution of the FAS. The FAS solutions were used for multiple experiments but were renewed at least on a monthly basis to prevent contamination.

Stock solutions of hydroxylammonium chloride (p.a., Merck KGaA, ~20 mg per 100 mL, the exact concentration was calculated from the mass weight) were prepared in an aqueous solution of acetic acid (p.a., Merck KGaA) (3 mL of acetic acid (glacial) per 1 L of MilliQ water, pH ~3) to stabilise the NH_2OH solutions. The stock solutions were diluted further to obtain four different standard concentrations leading to final concentrations in the vials between 0 and 100 nmol L^{-1} at an addition of 100 µmol per vial. All standard solutions were prepared a maximum of 7 days before analysis and stored in the dark at 4 °C.

All samples were analysed for their N_2O concentrations using a static headspace equilibration method. A 9 to 9.5 mL volume of the headspace was extracted from the equilibrated samples using a gas-tight syringe (VICI Precision Sampling, Baton Rouge, LA, USA). The headspace subsamples were analysed with a GC-ECD system (HP 5890 II, Agilent Technologies, Santa Clara, CA, USA, or Carlo Erba HRGC 5160 Mega Series, ThermoFisher Scientific, Waltham, MA, USA) that was calibrated using at least four different standard gas mixtures (N_2O in synthetic air, Deuste Steininger, Mülheim; calibrated against NOAA standard scale at the Max Planck Institute for Biogeochemistry, Jena, Germany) or dilution of the highest standard gas mixture. For details of the analytical method for N_2O see Kock et al.[39] and Walter et al.[40]

Table 1. Results from the acidification experiment at the CVOO Time Series Station

Treatment	N_2O (mean of triplicate measurements) (nmol L^{-1})	N_2O standard deviation (nmol L^{-1})[35]
Untreated	174.02	134.46
$HgCl_2$	32.46	0.94
HCl	32.01	2.39
$HgCl_2$ + HCl	32.13	1.47

In contrast to Butler and Gordon,[13] samples were not treated with mercuric chloride ($HgCl_2$) during the experiments, as all samples were analysed within a few days after sampling and acidification of the samples was tested to be efficient to prevent further N_2O production in an earlier experiment.

We tested acidification for conservation with seawater samples from the Cape Verde Ocean Observatory (CVOO) Time Series Station in a comparison experiment with different treatments of the samples. Four triplicates of N_2O samples were taken in 24-mL vials from a depth of 250 m and treated with (a) 50 µL of saturated mercuric chloride solution (p.a., Merck KGaA), (b) 100 µL of hydrochloric acid (2 mol L^{-1}, p.a., Merck KGaA), (c) 50 µL of mercuric chloride solution and 100 µL of hydrochloric acid and (d) were left untreated. The samples were stored over a period of three weeks and analysed using a static equilibration method.[39] Samples treated with mercuric chloride or hydrochloric acid did not show significant differences in N_2O concentrations within the standard deviation of the measurements. The measurements of the untreated samples showed a large variability and strongly differing values (Table 1). Although nitrite measurements were not available for this experiment, no nitrite is usually found at this depth in the North Atlantic Ocean.[41] Therefore, any interference by N_2O production from nitrite can be excluded for this experiment.

Experiments

N_2O production from HNO_2

Background production of N_2O from acidification of NO_2^--containing samples was tested in MilliQ and BE waters. In the experiments with BE water the vials were purged with nitrogen gas to remove background N_2O from the vials whereas MilliQ water samples were corrected for background N_2O concentrations measured from a control sample at t_0. Samples were treated with 100 µL of two different stock solutions of NO_2^- with concentrations of 0.504 and 9.54 µmol L^{-1} and acidified with 100 µL of acetic acid (glacial). A potential influence of N_2O production from background NO_2^- in the BE water was taken into account by measurements of a control run without addition of NO_2^-. The nitrite concentration of the sampled water at the Boknis Eck Time Series Station was 0.02 µmol L^{-1}. Samples were incubated in the dark at 30 °C with the N_2O concentration being measured between 0 and 350 h.

Reaction of NH_2OH with NO_2^- to form N_2O

The conversion of NH_2OH by different concentrations of NO_2^- was tested in MilliQ water, as background N_2O production from HNO_2 became significant in BE water within the time of the conversion reaction (Fig. 2). Five different NO_2^- stock

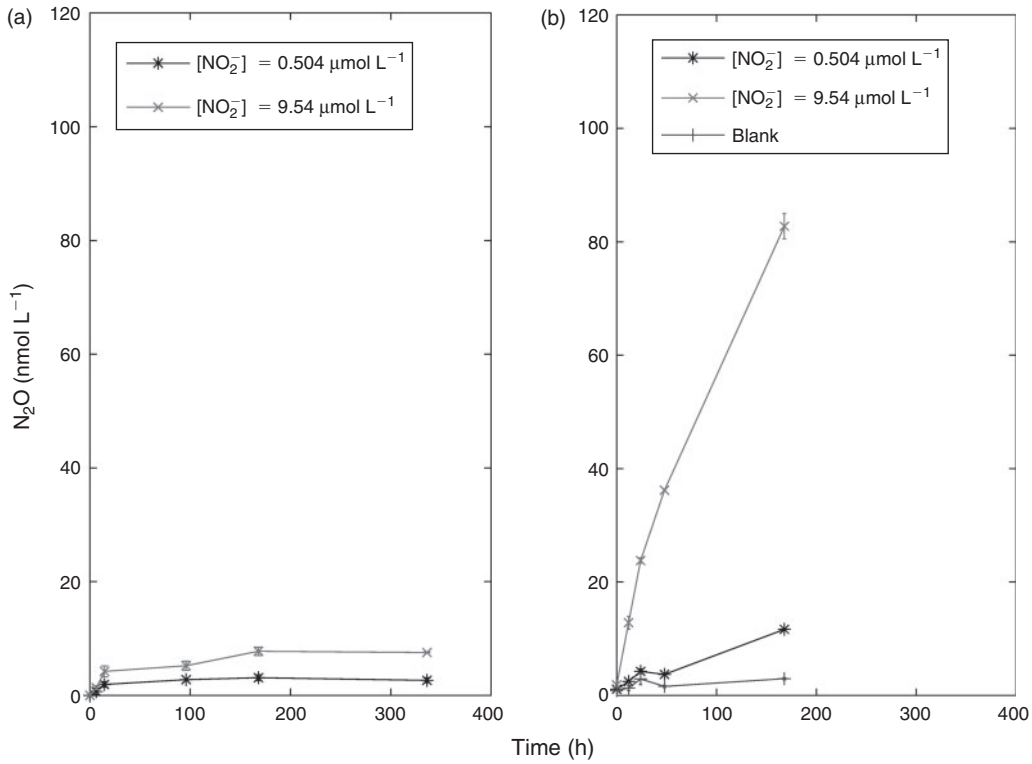

Fig. 2. N_2O production over time from acidified samples with different nitrite (NO_2^-) additions in (a) MilliQ water and (b) unfiltered seawater from the Boknis Eck Time Series Station, located in the Eckernförde Bay in the south-western Baltic Sea (BE water).

solutions (100 µL) were added to the sample vials, resulting in concentrations between 0.23 and 3.45 µmol L^{-1}. Subsequently, the samples were treated with 100 µL of acetic acid and 100 µL of hydroxylammonium chloride solution (5.0 µmol L^{-1}). A set of control samples without addition of NH_2OH was measured for each NO_2^- concentration, thereby accounting for the background production of NO_2^-, and the final N_2O concentrations were background corrected from a sample without nitrite addition. Vials were analysed for N_2O concentrations after 24 h.

However, the influence of this reaction on the NH_2OH analysis depends on the kinetics of the concurring reactions of NH_2OH with NO_2^- or FAS. Therefore, the conversion reaction of NH_2OH with FAS in the presence of different NO_2^- concentrations was also tested: three sets of samples with NO_2^- concentrations of 0.162, 0.539 and 4.27 µmol L^{-1} were treated with 100 µL of glacial acetic acid and four different NH_2OH standards. All samples were subsequently treated with 100 µL of FAS solution and were left at room temperature for 23 h. After 23h, 100 µL of sodium hydroxide solution (8 M) were added, adjusting the pH to 10 to stop the background NO_2^- production. The pH was measured in five random samples for control using pH control strips (pH 0–14, Macherey–Nagel, Düren, Germany), all of them showing a pH close to 10. All samples were analysed for N_2O within three days after conversion.

Removal of NO_2^-

The removal of NO_2^- before acidification of the samples is the simplest way to eliminate negative side reactions during NH_2OH conversion. Appropriate scavengers for NO_2^- need to selectively react with NO_2^- without affecting the conversion reaction between NH_2OH and FAS.

An acidic solution of sulfanilamide (p.a. VWR International, Darmstadt, Germany, 100 µmol L^{-1}) was therefore tested as a scavenger for NO_2^-, as sulfanilamide is widely used as a reagent in the detection of NO_2^- and NO_3^-. It reacts selectively with NO_2^- with formation of a diazonium salt that is coupled to 1-naphthylamine and forms a spectrometrically detectable dye.[42] Without addition of 1-naphthylamine, the diazonium salt is decomposed with formation of nitrogen gas.[43]

To test the efficiency of sulfanilamide as a NO_2^- scavenger, the NH_2OH conversion by FAS was tested during two experiments in filtered seawater from the tropical North Atlantic Ocean with and without addition of sulfanilamide in the presence of NO_2^- at two different concentrations (final concentration: 0.873 and 4.37 µmol L^{-1}). All samples were purged with nitrogen before the experiments. 100 µL of an acidic solution of sulfanilamide (10 mmol L^{-1}) were added to half of the vials directly after acidification and before the addition of NO_2^-. The final sulfanilamide concentration was chosen as 100 µmol L^{-1} to ensure a large excess over ambient nitrite concentrations[31] and to enable a rapid and complete decomposition of nitrite. The vials were subsequently treated with 100 µL of four different NH_2OH standards and 100 µL of FAS solution and were analysed for N_2O after 20 to 24 h.

Results and discussion

N_2O production from HNO_2

In both media, N_2O concentrations in samples that were treated with NO_2^- significantly increased over time, with a much higher increase in N_2O in BE water than in MilliQ water (Fig. 2). Samples with higher NO_2^- concentrations showed significantly

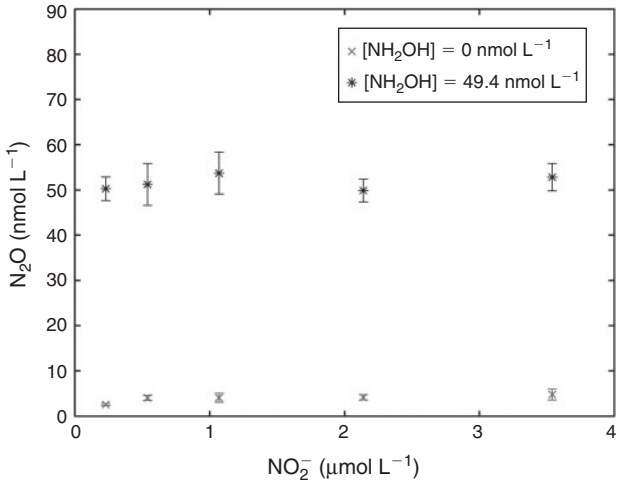

Fig. 3. N_2O production in MilliQ from the reaction between NH_2OH and HNO_2 (black stars). Grey crosses denote the N_2O production from the dissociation of HNO_2. All measured N_2O concentrations were corrected for background N_2O concentrations.

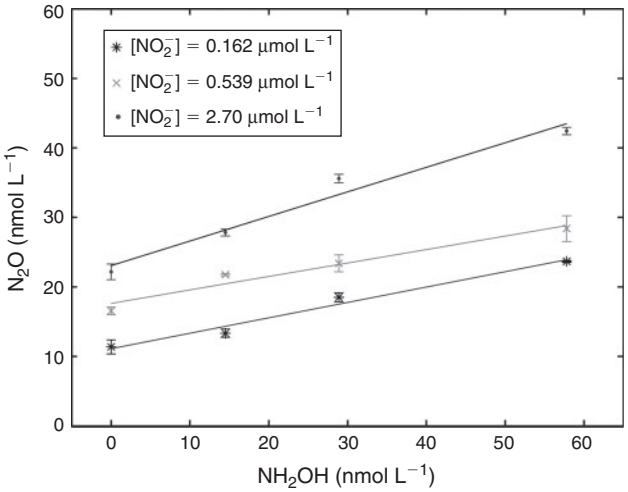

Fig. 4. Reaction of NH_2OH with ferric ammonium sulfate (FAS) in unfiltered seawater from the Boknis Eck Time Series Station, located in the Eckernförde Bay in the south-western Baltic Sea (BE water) in presence of ambient NO_2^- concentrations. Regression parameters are: $[NO_2^-] = 0.162\,\mu mol\,L^{-1}$: $y = 0.22\ (\pm 0.03)x + 11.12\ (\pm 0.79)$, $R^2 = 0.97$; $[NO_2^-] = 0.539\,\mu mol\,L^{-1}$: $y = 0.19\ (\pm 0.03)x + 17.62\ (\pm 0.97)$, $R^2 = 0.93$; $[NO_2^-] = 2.70\,\mu mol\,L^{-1}$: $y = 0.35\ (\pm 0.05)x + 23.06\ (\pm 1.50)$, $R^2 = 0.95$.

higher N_2O production in both experiments. A much larger influence on N_2O production by the medium was found, however. Although N_2O production in MilliQ water stayed moderate and levelled off after 168 h even for high NO_2^- concentrations, concentrations continued to increase in BE water until the end of the incubations. The N_2O production exceeded the statistical uncertainty of the N_2O measurements even at concentrations as low as $0.5\,\mu mol\,L^{-1}$ in less than 24 h. The much higher N_2O production in BE water can be explained by an increased number of side reactions of NO_2^- with organic compounds or trace metal ions that favour N_2O production in the BE water.[30]

Reaction of NH_2OH with NO_2^- to form N_2O

Samples without NH_2OH addition showed little N_2O production with only a slight increase with increasing NO_2^- concentrations, which is in reasonable agreement with the results from the previous experiment (Fig. 3). Elevated N_2O concentrations of $\sim 50\,nmol\,L^{-1}$ were found in all samples with NH_2OH addition. No significant difference between the samples with different NO_2^- concentrations was found and the conversion factors, calculated as the ratio between the difference of N_2O concentrations with and without NH_2OH addition and the concentration of the NH_2OH standard, were close to one, showing that N_2O is produced almost quantitatively from the comproportionation of NO_2^- and NH_2OH even at NO_2^- concentrations as low as $\sim 0.2\,\mu mol\,L^{-1}$. This indicates that under acidic conditions only low concentrations of ambient NO_2^- are necessary to convert NH_2OH into N_2O.

In contrast to the experiment without FAS addition, the conversion factors in the experiment with FAS addition in the presence of nitrite were significantly lower, ranging from 0.19 (± 0.03) to 0.35 (± 0.05) (Fig. 4) and were therefore in the range of conversion factors obtained in the reaction between FAS and NH_2OH. Due to the different stoichiometry this reaction yields conversion factors <0.5. A significant change in the conversion factors from ~ 0.2 to 0.35 occurred between samples with low ($<0.54\,\mu mol\,L^{-1}$) and high ($2.695\,\mu mol\,L^{-1}$) NO_2^- additions. This indicates that the conversion of NH_2OH is likely dominated by the reaction with FAS, but concentrations of NO_2^- in the micromolar range may have the potential to bias the conversion factors towards higher values. Therefore, this effect has to be considered as an additional source of uncertainty within the NH_2OH analysis.

Removal of NO_2^-

Samples without sulfanilamide addition showed a significant influence of NO_2^- on the N_2O production, which is in good agreement with the results of the previous experiments. A significant background production of N_2O from nitrous acid was observed for both NO_2^- concentrations, with higher background production from samples with high NO_2^- content (Fig. 5). This agrees well with the results from the N_2O production from HNO_2 experiment (see above). These show a significant increase in N_2O concentrations 24 h after acidification of NO_2^--containing samples due to the background production of N_2O from HNO_2 (Fig. 2). Furthermore, a significant change in the conversion factor could be observed with increasing NO_2^- concentrations, which is the result of an increased influence of the reaction between NO_2^- and NH_2OH with increasing NO_2^- concentrations, as seen in the experiment involving the reaction of NH_2OH with NO_2^- to form N_2O (see above) (Fig. 4).

In contrast, N_2O production from samples with sulfanilamide addition did not change with increasing NO_2^- concentration. No change in the background N_2O concentration or the conversion factor was observed, which leads to the conclusion that NO_2^- was successfully removed from the samples.

Conclusions

All experiments show that the NH_2OH determination by FAS conversion into N_2O is significantly affected by the presence of NO_2^-. On the one hand, the addition of acid to natural waters that

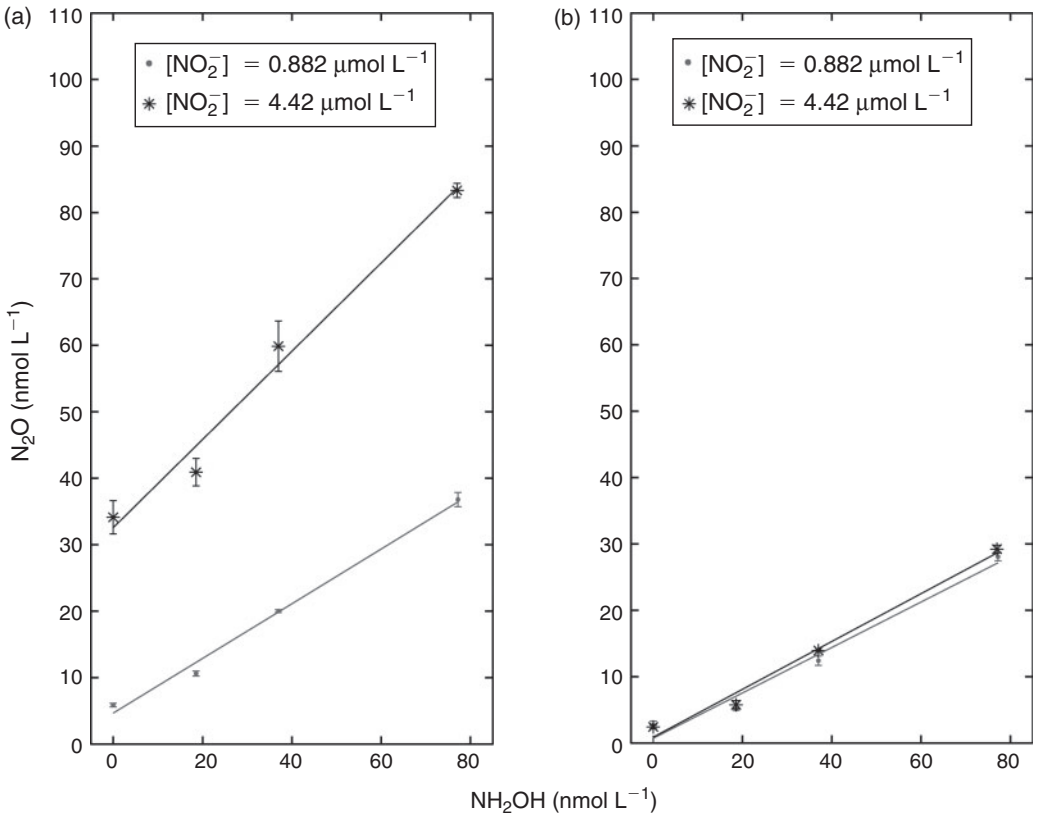

Fig. 5. NH_2OH standard addition with ferric ammonium sulfate (FAS) conversion in presence of NO_2^- without sulfanilamide addition (a) and with sulfanilamide addition (b). Regression parameters are: (a) $[NO_2^-] = 0.882\,\mu mol\,L^{-1}$: $y = 0.41\ (\pm 0.03)x + 4.6\ (\pm 1.2)$; $R^2 = 0.99$; $[NO_2^-] = 4.42\,\mu mol\,L^{-1}$: $y = 0.66\ (\pm 0.07)x + 32.5\ (\pm 2.8)$; $R^2 = 0.97$; (b) $[NO_2^-] = 0.882\,\mu mol\,L^{-1}$: $y = 0.34\ (\pm 0.04)x + 0.70\ (\pm 1.7)$; $R^2 = 0.96$; $[NO_2^-] = 4.42\,\mu mol\,L^{-1}$: $y = 0.36\ (\pm 0.03)x + 0.86\ (\pm 1.4)$; $R^2 = 0.98$.

contain only low amounts of NO_2^- can already lead to a significant production of N_2O through the decomposition of HNO_2. The importance of this background production strongly depends on the composition of the sample matrix and the storage time of the samples under acidic conditions. For seawater samples with NO_2^- concentrations at a micromolar level a significant bias in N_2O within the time of the NH_2OH conversion by FAS cannot be excluded.

On the other hand, it could be shown that N_2O is also produced almost quantitatively by the reaction of NH_2OH with HNO_2 even at very low NO_2^- concentrations, thus showing a much higher conversion factor than in the reaction with FAS. A significant effect of this reaction on the efficiency of the NH_2OH conversion with the FAS method is only observed at NO_2^- concentrations in the micromolar range, however.

Owing to these side reactions, we caution that NH_2OH concentration measurements without NO_2^- scavenging can lead to an overestimation of the true NH_2OH concentrations when NO_2^- is present in large amounts or (acidified) samples are stored over longer periods. Due to the large number of side reactions and their different behaviour in different reaction media it is difficult to determine a threshold NO_2^- concentration that can be tolerated during NH_2OH analysis. This demands the removal of NO_2^- from the reaction medium before NH_2OH analysis.

We could show that sulfanilamide successfully removed NO_2^- from the samples without affecting the FAS conversion, and the reaction of sulfanilamide with NO_2^- is sufficiently fast and quantitative,[44,45] which means that no extra time is required for the removal of NO_2^-. Sulfanilamide thus acts as a suitable NO_2^- scavenger in NH_2OH analysis.

Based on our results, we suggest a modification of the original method[22] by the addition of $100\,\mu mol\,L^{-1}$ acidic sulfanilamide solution to the reaction medium before acidification of the samples to inhibit potential N_2O production from side reactions with NO_2^-.

The proposed modification of the FAS conversion method will allow NH_2OH determination even in oceanic regions with high NO_2^- concentrations such as found in the suboxic zones of the north-western Indian (see e.g. Lam et al.[46]) and eastern tropical North and South Pacific Oceans (see e.g. Codispoti et al.[47]). A detection of NH_2OH in seawater samples could indicate the occurrence of active nitrification or DNRA. Moreover, as nitrification and DNRA take place at different oxygen concentrations, the detection of NH_2OH in seawater samples could be interpreted as a specific indicator for nitrification in oxic and suboxic environments on the one hand and DNRA in anoxic environments on the other hand.

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