



An improved method for the determination of dissolved nitric oxide (NO) in seawater samples

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Abstract. Nitric oxide (NO) is a short-lived intermediate of the oceanic nitrogen cycle; however, due to its high reactivity, measurements of dissolved NO in seawater are rare. Here we present an improved method to determine NO concentrations in discrete seawater samples. The set-up of our system consisted of a chemiluminescence NO analyser connected to a stripping unit. The limit of detection for our method is 5 pmol NO in aqueous solution, which translates into 0.25 nmol L⁻¹ when using a 20 mL seawater sample volume. Our method was applied to measure high-resolution depth profiles of dissolved NO during a cruise to the eastern tropical South Pacific Ocean. It is fast and comparably easy to handle; thus it opens the door for investigating the distribution of NO in the ocean, and it facilitates laboratory studies on NO pathways.

laboratory-scale waste water sludge reactor showed that denitrification indeed could be a dominating process of NO release (Kampschreur et al., 2007). The denitrification pathway has been investigated in great detail, and therefore its enzymatic NO production and the subsequent reduction of NO via the intermediate nitrous oxide (N₂O) to nitrogen (N₂) are well understood (Zumft, 1997). Another process where NO is involved as an intermediate is anammox (Strous et al., 2006; Kartal et al., 2011). The latest discovery was the enzymatic dismutation of NO resulting in the microbial production of oxygen (O₂) used to oxidize methane under anaerobic conditions (Ettwig et al., 2010, 2012). Additionally, NO can be produced in the ocean surface layer by the photochemical reduction of dissolved nitrite (NO₂⁻) (Zafriou and True, 1979; Olasehinde et al., 2010).

1 Introduction

Nitric oxide (NO) is a short-lived intermediate of various microbial processes of the nitrogen cycle (see e.g. Thamdrup, 2012). Molecular analysis and lab culture experiments have shown that various kinds of bacteria are able to metabolize NO, e.g. ammonium-oxidizing bacteria (Lipschultz et al., 1981), nitrite-oxidizing bacteria (Freitag and Bock, 1990), methanotrophic bacteria (Yoshinari, 1985) and denitrifying bacteria (Firestone et al., 1979). However, it is still unclear which processes are responsible for the occurrence of NO in natural environments. Although ammonium- and nitrite-oxidizing bacteria can produce NO, there is no evidence for NO as an intermediate during nitrification. A study which compared mathematical models with the results from a

In summary, there are various potential microbial NO production/consumption pathways in the ocean. Unfortunately, our knowledge about the oceanic NO distribution and the major pathways of NO is very poor. There are only a few published NO concentration measurements available (Bange, 2008) because a reliable and easy-to-use method to determine dissolved NO at in situ concentrations in seawater samples is lacking.

Gaseous and dissolved NO is a very reactive and, thus, short-lived molecule because it is a free radical. Its occurrence is predominantly dependent on the presence of O₂ (Lewis and Deen, 1994). Therefore, the determination of dissolved NO under in situ conditions is challenging. A summary of the existing methods for the determination of NO is given by Hetrick and Schoenfish (2009). The published methods for measurement of dissolved NO in seawater are listed in Table 1. The detection limits range from 0.0015

Table 1. Overview of published methods for NO detection in seawater with the respective limit of detection (LOD) for each method.

Method	LOD [nmol L ⁻¹]	Reference
Microelectrode	140	Zhang et al., 2003
Microelectrode	42	Xing et al., 2005
Microelectrode	30	Schreiber et al., 2008
Fluorometric	0.0124*	Olasehinde et al., 2009
Chemiluminescence	0.0015	Ward and Zafiriou, 1988
Chemiluminescence	0.25	This study

* LOD for the conversion product from the reaction of NO with the trapping compound

to 140 nmol L⁻¹. (Please note that the fluorometric detection of NO as described by Olasehinde et al., 2009, is suitable only for formation rates of NO from NO₂⁻). The sensor of Schreiber et al. (2008) was developed for sediments but works in seawater samples as well. The chemiluminescence system of Zafiriou and McFarland (1980) consisted of an NO analyser connected to a stripping unit and is, thus, similar to the set-up described here. The method by Zafiriou and McFarland (1980) is the only one yet to be applied on board to measure NO depth profiles during a cruise (Ward and Zafiriou, 1988). However, the required intensive cleaning of the Niskin bottles prior to the CTD/rosette casts together with the fact that each depth was sampled with a separate cast resulted in a time-consuming and unhandy sampling procedure.

Therefore, it was the aim of this study to develop an improved (easy to handle) method for the detection of dissolved NO in discrete seawater samples at in situ concentrations in order to decipher its role in the oceanic nitrogen cycle.

2 Material and methods

2.1 Instrumental set-up

The set-up of our system consisted of a NO analyser connected via a four-way gas stream selecting valve to a stripping unit and to the gas cylinders for reference gas and carrier gas (Fig. 1).

The carrier gas (N₂) and the reference gas (1000 ppb NO in N₂) were connected to a two-channel mass flow controller with mixing chamber (HTK Hamburg GmbH, Hamburg, Germany) to ensure a constant gas flow rate of 1 L min⁻¹ and to enable the calibration of the detector signal (see Sect. 2.4.1). The mass flow controller, in turn, was connected to the gas stream selecting valve. This four-way valve enabled us to switch between two modes of gas flow: mode A enabled the direct measurement of the reference gas and carrier gas, and mode B allowed detection of the gas stream after going through the stripping unit.

The sample and stripping vials were connected to the gas line by needles (diameter 1.2 mm) pushed through the respective septa. Two inline filters (Whatman Solvent IFD, 0.2 µm, GE Healthcare UK Limited, Buckinghamshire, Eng-

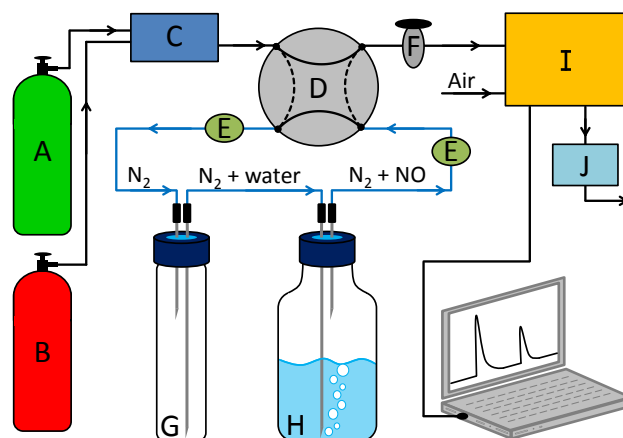


Figure 1. Schematic set-up of the developed measurement system consisting of an NO analyser connected to a stripping unit (blue lines). A: N₂ gas cylinder; B: reference gas cylinder; C: mass flow controller; D: four-way valve (solid lines: mode A; dashed lines: mode B); E: inline filter; F: needle valve; G: sample vial; H: stripping vial filled with water; I: NO analyser with air supply for ozone generator; J: vacuum pump with vent.

land) were installed to remove aerosols from the gas stream. Between the four-way valve and the NO analyser a needle valve was installed to reduce pressure variations. For a detailed description of the measurement procedure see Sect. 2.2 (samples) and 2.4 (standards) below.

For detection, we used a chemiluminescence NO analyser (model 42i-TL, Thermo Fisher Scientific Inc, Waltham, MA, USA) with a detection range from 0 to 1000 ppb. In the reaction chamber of the analyser, NO reacts with ozone (O₃) generated by an O₃ generator and produces NO₂ in an excited state (NO₂^{*}). By relaxation to the ground state the NO₂^{*} emits a photon. The emitted light passes an optical filter to remove interferences from other compounds and is detected by a photomultiplier. The signal recording was done with the open-source software PuTTY 0.62 (http://filehippo.com/de/download_putty/11216/).

2.2 Sample handling

Sampling took place with a commonly used conductivity temperature depth sensor, equipped with a Niskin bottle rosette (CTD/rosette) as well as with a pump CTD (pCTD) system (Strady et al., 2008) during the *Meteor* cruise M93 from 6 February to 11 March 2013 to the eastern tropical South Pacific off Peru (Callao, Peru, to Panama City, Panama). Seawater samples were taken bubble free in 20 mL brown glass vials, closed with rubber plugs and crimped with aluminium caps. Immediately after sampling all samples were stored in a cooling box (~6 °C) until they were measured. From each water depth three to six replicates were taken. From the CTD/rosette all samples were taken as soon as possible, after the CTD was back on the ship's working

deck, and they were analysed within 1 h. The samples from the pCTD were taken as soon as the target depth was reached and were measured immediately within a few minutes after sampling.

For the measurement, the four-way valve was switched to mode A to enable the connection of the sample vial by the needles. In the next step the four-way valve was switched to mode B to reroute the gas flow through the stripping unit. The water of the sample was pushed with the carrier gas into the stripping vial. The stripping vial had a larger volume (50 mL) than the sample vial to allow purging of the sample. The dissolved NO was stripped from the sample by N₂ and transported with the carrier gas stream into the analyser. The sample stayed connected with the stripping unit (mode B) until the detector signal came back to the baseline. Then the four-way valve was switched to mode A and the next sample was connected.

2.3 Experiments

For sample storage experiments we took 18 samples from the pCTD at two stations from the oxygen minimum zone (OMZ) at depths between 60 and 90 m and stored nine of them at room temperature (~24 °C) and nine at ~6 °C in the dark. For the time series, triplicates per temperature were measured in various time steps.

For NO₂⁻ addition tests we added 20 µL of a 20 mmol L⁻¹ sodium nitrite (NaNO₂) aqueous solution to about 100 samples taken at different stations and depths; this corresponds to a concentration of 20 µmol L⁻¹, in addition to the natural concentration already present in the sample. Samples were stored for different time periods, between some minutes and some hours in warm (~24 °C) and cold (~6 °C) environments and then measured like normal samples. Additionally we stored control samples without NO₂⁻ addition under the same conditions.

2.4 Calibration

2.4.1 Detector calibration

To calibrate the detector signal, the carrier gas (N₂) was blended with the reference gas (1000 ppb NO in N₂) by the mass flow controller (see above). The resulting NO mixing ratios covered the whole detection range of the NO analyser (0 to 1000 ppb).

2.4.2 Gas standard injection

Discrete volumes of reference gas ranging from 0.5 to 10 mL were injected with a gas-tight syringe (series A-2, Valco Instruments Company Inc., Houston, TX, USA) into the empty stripper. Two different reference gases with concentrations of 1000 ppb NO and 10 ppm NO were used.

2.4.3 Aqueous NO standard solutions

For preparation of aqueous NO standard solutions a 20 mL brown glass vial filled with 10 mL Milli-Q water was purged with N₂ for 1 h at a flow rate of 100 mL min⁻¹ and then with pure NO or a reference gas (1 % NO in N₂), at a flow rate of 5 mL min⁻¹ for 2 h. Assuming a solubility of 1.94 ± 0.03 mmol L⁻¹ atm⁻¹ at 25 °C for NO (Zacharia and Deen, 2005, and references therein) the final concentrations of the solutions were 1.94 mmol L⁻¹ and 19.4 µmol L⁻¹ respectively. The standards were stored in the dark at room temperature.

For the actual measurements 20 mL Milli-Q water was deoxygenated with N₂ for 1 h at a flow rate of 150 mL min⁻¹ in a 50 mL vial. Then the vial was connected to the stripping unit, followed by an injection of varying volumes (in the range from 1 to 100 µL) of standard through the septum of the vial.

2.4.4 In situ NO formation from NO₂⁻ reduction

This calibration method is based on the in situ formation of NO by chemical reduction of NO₂⁻ with iodide in an acidic aqueous medium (Cox, 1980). The preparation of the NO₂⁻ solution started with a stock solution of 1 mol L⁻¹ NaNO₂ in Milli-Q water and was followed by a two-step dilution series (100 µL in 100 mL Milli-Q water) to get two NO₂⁻ standards with concentrations of 1 mmol L⁻¹ and 1 µmol L⁻¹. They were stored in the dark at room temperature.

The reaction solution is made of two solutions: 11 mL glacial acetic acid was added to 100 mL Milli-Q water, yielding a 10 % acetic acid (with a concentration of 1.68 mol L⁻¹; Kester et al., 1994), and 3 g KI was dissolved in 100 mL Milli-Q water to get a 3 % *w/v* KI solution (Garside, 1982).

Prior to a measurement, 1 mL of the KI solution and 1.5 mL 10 % acetic acid were mixed in a 50 mL vial and Milli-Q water was added to a final volume of 20 mL. The vial was purged for 20 min with N₂ (flow rate 150 mL min⁻¹) to remove the O₂ and was then connected to the stripping unit. Then the NO₂⁻ solution was added via the septum as described above.

2.5 Data analysis

The recorded NO signals (i.e. peaks) were integrated manually with Origin (OriginLab, Northampton, MA, USA). With the obtained peak areas from the standard measurements a linear calibration equation was calculated to convert the peak areas of sample measurements into concentrations.

The signal-to-noise ratio (*S/N*) was calculated by an equation of Stauffer (2008):

$$S/N = 2H/h_N \quad (1)$$

H stands for the height of the signal and *h_N* is the height of the baseline noise. For the limit of detection (LOD) the

Table 2. Overview of the limit of detection (LOD), the limit of quantification (LOQ), the standard deviation (SD) and the estimated stability time of the applied standards types.

Standard	LOD [pmol]	LOQ [pmol]	SD [%]	Stability time
Aqueous NO standard solution	5	20	25	10 months
In situ NO formation from NO ₂ ⁻ reduction	10	40	3	–
Reference gas	15	30	10	1 year

value of S/N was set to 3, and for the limit of quantification (LOQ) a value of 10 was used. NO signals below the detection limit were set to zero in further calculations (e.g. averaging of replicates).

3 Results and discussion

3.1 Method evaluation

3.1.1 Standard measurements

To assess the performance of our instrumental set-up, we calculated the LOD, the LOQ and the standard deviation (SD, average from all measurements over the whole detection range) for the different gaseous and aqueous standards. The results are summarized in Table 2. Please note that the values are given as molar amounts of substance and not as concentrations in order to get volume-independent numbers and thus a better comparability between the different kinds of standards described in Sect. 2.4.2–2.4.4. Concentrations for sample measurements are discussed in Sect. 3.1.2.

For aqueous NO standards (see Sect. 2.4.3) the lowest detectable molar amount of NO was 5 pmol with a SD of 25 %. The SD is relatively high compared to the two other used standards and may be caused by the syringes used in combination with the very small injection volumes. The syringes were open at the needle end and could not be locked. Hence during the transfer from the vial containing the standard to the stripping vial the NO concentrations could be changed by external influences (e.g. contamination by diffusion through the needle), and with a smaller volume the effects get bigger. For nitrite the formation of NO occurs in the closed system, and so the transport from one vial to the other could not affect the measured concentration. The gas standards were injected with a larger volume and gas-tight syringes. We observed that the aqueous NO solutions did not change in 10 months. This indicates that the standard solutions are stable much longer than previously reported (Mesaros et al., 1997; Menon et al., 1991).

For in situ NO formation from NO₂⁻ reduction (see Sect. 2.4.4) the LOD was 10 pmol NO and the SD 3 %. The detection limit is higher than for aqueous NO solutions because NO is formed in situ, which results in broader peaks with lower peak heights. We observed no decrease of the NO₂⁻ concentration in the standards during our measure-

ments and conclude that the NO₂⁻ solutions should be stable when kept in the dark.

Discrete gas standard measurements (see Sect. 2.4.2) had a detection limit of 15 pmol NO. By cleaning the gas-tight syringes after five measurements with 100 % ethanol, the SD could be decreased from 65 to 10 %, whereas cleaning with Milli-Q did not improve the SD. Also a cleaning of the syringe after every measurement resulted in no further decrease of the SD. We observed no influence of the injected volume between 0.5 and 10 mL on the detected NO. The stability of the used reference gases (1 year) was given by the manufacturer.

3.1.2 Sample measurements

With a water volume of 20 mL the LOD and the LOQ for dissolved NO translate into concentrations of 0.25 and 1 nmol L⁻¹ respectively. By enlarging the sample volume the detection limit can be lowered. However, the peaks will get broader with larger volume; thus the detection limit will not decrease in the same amount as the sample volume is increased. We observed, for example, that by increasing the sample volume from 20 to 80 mL the detection limit rose to 10 pmol detectable molar amount of NO but the detectable concentration decreased to 0.125 nmol L⁻¹.

3.2 Interferences by other components

3.2.1 Hydrogen sulphide (H₂S)

During cruise M93 we faced a sulfidic event close to the coast of Peru. Therefore, some of the samples contained H₂S, which resulted in a strong negative detector signal (Fig. 2a). A visible negative response of the NO analyser (i.e. stronger than the baseline noise of the instrument) was determined down to a concentration of about 80 nmol L⁻¹ H₂S, but even lower H₂S concentrations could have an impact on the NO signal such as neutralization of a positive NO signal.

The reason for the negative peaks is a change of the signal from the continuous background calibration of the NO analyser itself. Parallel to the normal detection in the reaction chamber (described at the end of Sect. 2.1) the sample is mixed in a pre-reaction chamber with a high amount of ozone to remove the NO before the sample is led into the reaction chamber. This leads to a continuous zero calibration of the instrument. H₂S seems to affect this background sig-

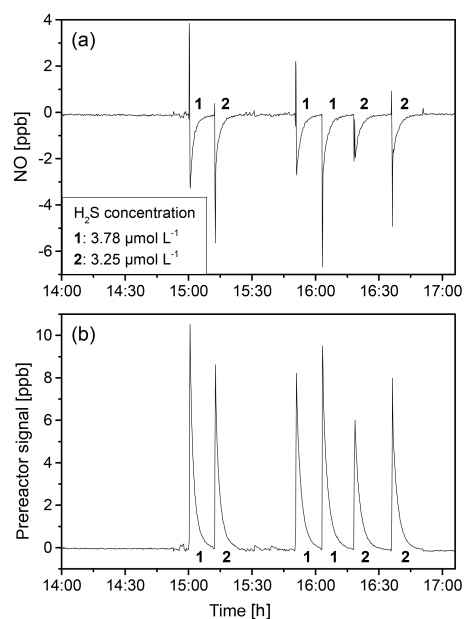


Figure 2. Detector signal for six seawater samples containing H₂S. (a) Signal passing the prereactor. (b) Prereactor signal.

nal (Fig. 2b). The signal from the prereactor channel induced by H₂S is stronger than from the normal channel passing the prereactor, which leads to the negative detector signal.

Tests with addition of ZnCl₂ (in order to precipitate H₂S as ZnS) showed that the negative peaks of H₂S did indeed vanish, but the impact of ZnCl₂ addition on the NO concentration in the sample is unknown. It might be possible that NO is removed from the sample by chemical reduction. Some preliminary lab incubation tests with *Paracoccus denitrificans* DSM 413 showed that ZnCl₂ can also increase the NO concentration (NO concentration without ZnCl₂: 12 nmol L⁻¹; NO concentration after addition of 0.5 mL 50% w/v ZnCl₂ to a 30 mL culture: 4458 nmol L⁻¹). A reason for that may be that NO could be involved in cell death. For example in diatoms a stress surveillance system based on NO and calcium was found which is suggested to be widespread among phytoplankton (Vardi et al., 2006, 2008; Vardi, 2008). Moreover, Hawkins et al. (2013) found a pathway including programmed cell death in a model sea anemone–dinoflagellate symbiosis. Both studies detected an increase of NO coming along with cell death. So it may be possible that the addition of ZnCl₂, a common method to poison plankton, leads to an increase of NO in seawater samples.

3.2.2 Nitrite (NO₂⁻)

NO can photochemically be produced from dissolved NO₂⁻ (Zafiriou and True, 1979; Olasehinde et al., 2010). As NO₂⁻ can be enhanced in the water column (especially in OMZs) we performed NO₂⁻ addition tests to find out if there is any

light-induced production of NO caused by our sample handling.

Our experiments showed no differences in NO concentrations between samples with and without NO₂⁻ addition. The addition of 1 mL of a 1 mmol L⁻¹ NaNO₂ solution to 20 mL Milli-Q water resulted only in a very small NO peak. Thus we conclude that a potential in situ production of NO from NO₂⁻ does not affect the measurement method described here.

3.2.3 Organic compounds

It is imaginable that organic compounds could also influence the detected NO concentration. A well-known fact is the release of NO by photolysis from nitroso compounds which have a NO group bound to an organic moiety (e.g. Ignarro et al., 1981; Adeleke and Wan, 1974). These compounds are ubiquitous in cells because they are used for the transport of NO during cell signalling (Ignarro, 1990).

To our knowledge no measurements of nitroso compounds in the ocean exist so far. Therefore, it is not possible to realistically estimate how much NO could be generated by these compounds during the sample handling. Further tests are needed to evaluate the strength of the interferences. Indeed, several methods for the detection of nitroso compounds by photolytic (e.g. Alpert et al., 1997) or chemical (e.g. Samouilov and Zweier, 1998) release of NO from nitrosylated compounds and the subsequent detection of NO by chemiluminescence exist already. It should be tested if these methods could be applied to seawater samples.

3.3 Sample handling

Two factors influenced the NO concentrations in the samples: the storage time (i.e. the time between sampling and the actual measurement of the sample) and the ambient O₂ concentrations. This is especially important for samples from the OMZ where slight changes in O₂ are expected to have a significant effect on dissolved NO (Lewis and Deen, 1994).

The storage experiments showed a decrease in NO concentrations over time, with a stronger decline at room temperature compared to storage at ~6°C (Fig. 3). The decrease of the NO concentrations may be explained by the well-known common effect of bottle consumption caused by pores in the glass vials and in the rubber stoppers. However, a stronger effect on the NO concentration is probably caused by diffusion of O₂ into the sample. De Brabandere et al. (2012) showed that O₂ contamination can be caused by diffusion of O₂ out of the rubber plugs. At room temperature the diffusion of 1 nmol O₂ into a water sample takes only a few seconds. Compared to this, the diffusion of NO out of the sample, i.e. the bottle consumption, is negligible. Under the assumption that (i) the diffusion coefficients of NO and O₂ are about the same (Zacharia and Deen, 2004; Goldstick and Fatt, 1970), that (ii) both compounds have the same distance

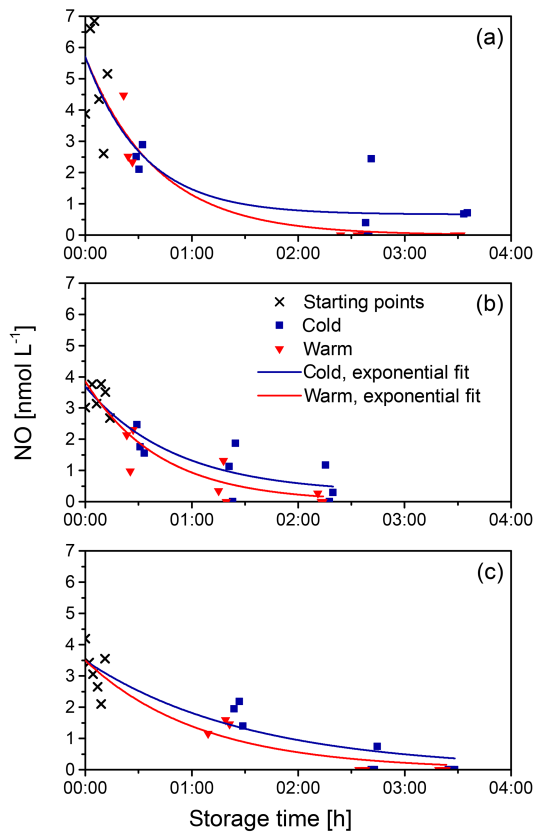


Figure 3. Degradation curves of three sample storage tests. The samples were kept in the dark at room temperature ($\sim 24^\circ\text{C}$, red triangles) and at 6°C (blue squares). The measurements from the regular sampling (black crosses) were used as starting points for the curve fitting of both temperature settings. (a–b) M93 station 399-4 at 12.525°S , 77.597°W ; sampling depth: 77 and 67 m respectively. (c) M93 station 391-4 at 12.668°S , 77.821°W ; sampling depth: 87 m.

to “travel” and that (iii) the target concentration (outside the sample for NO and in the sample for O_2) is zero, only the starting concentrations are important for the diffusion speed. With a starting concentration of 5 nmol L^{-1} NO, the diffusion of NO out of the sample is 208 600 times slower than for O_2 into the sample in relation to the values given by De Brabandere et al. (2012) for O_2 .

Even though NO is very O_2 sensitive (Lewis and Deen, 1994), oxidation of NO by O_2 alone cannot explain the decrease of NO, because it is too slow. With concentrations of 5 nmol L^{-1} NO and $1\text{ }\mu\text{mol L}^{-1}$ O_2 , the half-life period of the reaction is higher than 6000 h, and for lower concentrations it is even longer. That means that other reactions have to be responsible for the short lifetime of NO in biological systems (Kharionov et al., 1994; Ignarro et al., 1993). One reason could be that in OMZ waters slight changes in O_2 concentrations at nanomolar levels induce strong changes of metabolic rates (Dalsgaard et al., 2014; Tian et al., 2014;

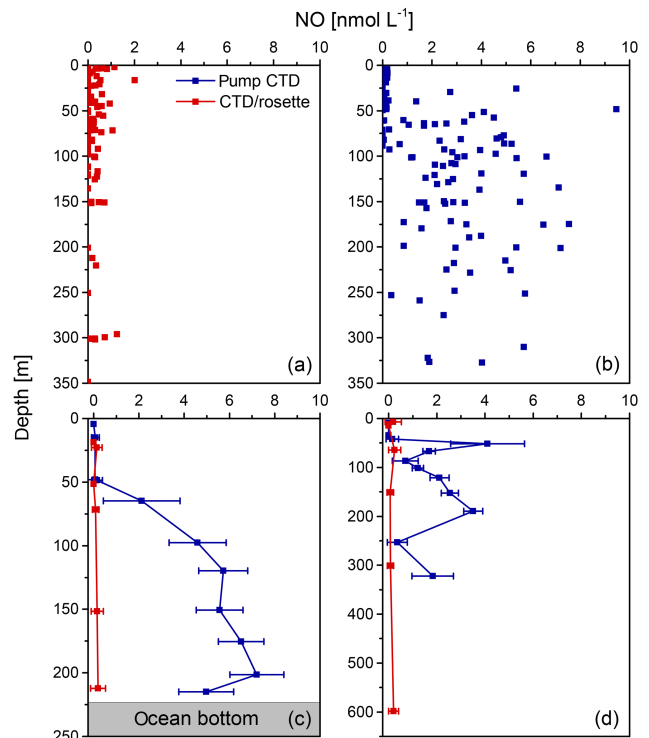


Figure 4. Comparison of the NO measurements from the CTD/rosette (red) and from the pCTD (blue). (a–b) All NO measurements during M93 between 0 and 350 m. (c) M93 station 391-4 at 12.668°S , 77.821°W ; the bottom depth was 1654 m.

Kalvelage et al., 2011). Overall, it can be assumed that biological processes affected by O_2 impurities are the main reason for decrease of NO in the sample vials.

The potential biological consumption of NO, e.g. by denitrification and anammox, in the samples from the OMZ can also explain the enhanced decrease at room temperature compared to storage at $\sim 6^\circ\text{C}$. As the metabolic activity is higher at room temperature compared to $\sim 6^\circ\text{C}$, more NO could be used up. Another reason for the temperature effect is that at higher temperatures the diffusion of O_2 into the sample is faster and thus the effect on the NO metabolism is stronger.

The choice of the water sampling system had a large impact on the NO concentrations in the samples (Niskin bottles or pCTD). The scatter plot with our measurements from the Niskin bottles of the CTD/rosette (Fig. 4a) shows that the NO concentrations were mostly near or below the detection limit. Only a few samples showed NO concentrations of up to 2 nmol L^{-1} . Contrasting to this, samples from the pCTD (Fig. 4b) showed a broad range of concentrations up to 10 nmol L^{-1} . This has been confirmed by direct comparison of both CTD systems on two stations (Fig. 4c–d). No change in NO concentrations with depth was evident from CTD/rosette casts, whereas from the pCTD plausible shapes of the NO depth profiles were obtained.

One reason for the obvious difference between the two CTD systems is most likely the time effect as found in our sample storage experiments, which might have occurred in the Niskin bottles as well. The seawater stays between 30 min and several hours in the Niskin bottles during the time of a CTD cast until sampling. During this time an O₂ contamination of 1 μmol L⁻¹ can be induced (Alarcón and Ulloa, 2009). This O₂ entry into the bottles together with a comparably long CTD cast time may result in a strong decrease of NO even before the samples could be taken. For the pCTD system a diffusive O₂ input to the water while being pumped up of only 20 nmol L⁻¹ after 1 h of pumping has been estimated (Canfield et al., 2010), resulting in a low O₂ contamination. The short residence time of the seawater in the tubing in combination with a smaller O₂ contamination might have led to comparable lower NO degradation and thus in turn to higher detectable NO concentrations in the samples taken from the pCTD. However, another point should be taken into account: during the in situ pumping, cells are heavily stressed and could be disrupted, which might result in a release of intracellular NO from broken cells and/or an enhanced stress-induced NO production similar to the system found by Vardi et al. (2006).

Overall, NO samples are unstable after sampling so they have to be processed very fast. Thus, it is recommended to use a pCTD as a sampling system, and O₂ contamination should be reduced to a minimum (e.g. by using deoxygenated materials). However, it may be possible that NO concentrations are rather underestimated due to sampling time and delayed until measurement.

4 Summary

Here we present an improved method to determine dissolved NO in discrete seawater samples. The set-up of our system consisted of a chemiluminescence NO analyser connected to a stripping unit. The lower limit of detection for our method was 5 pmol NO in aqueous solution, which corresponds to 0.25 nmol L⁻¹ when using a 20 mL seawater sample volume. Our method was applied to measure high-resolution depth profiles of dissolved NO during a cruise to the eastern tropical South Pacific Ocean. One CTD cast (including sampling) can be processed in less than 2 h. However, for the sampling we recommend using a pCTD rather than a conventional CTD/rosette with Niskin bottles. In general, contamination by O₂ diffusion into the samples should be minimized by using appropriate materials. Dissolved H₂S interferes strongly with the NO detection, whereas the in situ production of NO from dissolved NO₂⁻ seems to be negligible.

The in situ formation of NO from NO₂⁻ reduction (with iodide in acidified aqueous medium) would also allow applying our set-up for the measurements of dissolved NO₂⁻ (and nitrate) in seawater samples (Garside, 1982) down to very low concentrations.

The method for the determination of dissolved NO as described here is fast and comparably easy to handle; thus it opens the door for comprehensive measurements of the distribution of oceanic NO, and it facilitates laboratory studies on NO pathways.

Appendix A

Nitrogen (N_2 , 99.999 %), reference gases (1000 ± 50 ppb NO in N_2 , 10 ± 0.2 ppm NO in N_2 , 1 ± 0.02 % NO in N_2), and nitric oxide (NO, ≥ 99.5 %) were purchased from Air Liquide GmbH (Düsseldorf, Germany). Sodium nitrite ($NaNO_2$, ≥ 99.0 %, p.a.), zinc chloride ($ZnCl_2$, ≥ 98.0 %, p.a.) and acetic acid (100 %) were from Merck KGaA (Darmstadt, Germany). And potassium iodide (KI, ≥ 99.5 %, p.a.) was obtained from Carl Roth GmbH (Karlsruhe, Germany).

All tubings were from stainless steel or PTFE with a diameter of 1/4 and 1/8 in. Valves, fittings and needles were also made of stainless steel.

Processing a measurement with our system is fast, easy and takes only a few minutes. Up to 20 measurements can be done within 1 h.

The instrument showed a stable baseline and no drift over time for 2 years. For measurements in solution and at higher concentration ranges, it is important to include breaks and cleaning of the stripping unit between the measurements to prevent a less sensitive detection limit. The higher the NO concentrations are, the more often breaks and cleaning are needed to keep the baseline stable. The same applies for Milli-Q water and reaction solution for aqueous standard measurements. The two liquids can be reused for several measurements. The number of possible measurements done with the same liquid depends on the concentration. With higher concentrations the liquid should be replaced more often to keep the baseline constant.

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