

Specific detection of gaseous NO and ^{15}NO in the headspace from liquid-phase reactions involving NO-generating organic, inorganic, and biochemical samples using a mid-infrared laser

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Abstract

Nitric oxide (NO) is an important biological signaling agent. The specific detection of NO represents a continuing challenge in the field of NO research. Many methods are currently employed for the detection of NO. Here, we report a qualitative but specific detection method for gaseous NO liberated in and from solution taking advantage of its low solubility. Importantly, our mid-infrared laser absorption method does not depend on any chemical derivatization of NO, and is applicable over a wide range of concentrations for both protein work and in organic–inorganic modeling work. We also apply this method to the specific detection of ^{15}NO .

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Nitric oxide (NO) is a small paramagnetic molecule that is biosynthesized by the class of heme-containing enzymes called the nitric oxide synthases (NOSs) [1,2]. NO is also produced as a viable intermediate during bacterial denitrification [3]. In eukaryotic organisms, NO serves as a signaling agent to elicit a number of physiological responses such as vasodilation [4,5]. NO signaling is achieved when NO binds to the heme-containing enzyme soluble guanylate cyclase (sGC). Binding of NO to sGC is associated with the cleavage of the proximal iron–histidine bond and activation of the enzyme [4,5].

Nitric oxide interacts with other heme proteins [6]. For example, NO binding to hemoglobin and myoglobin has been studied in detail for many decades. The pigment in cured meat has been described as a heme–NO species on

the basis of electron paramagnetic resonance spectroscopy [7,8]. Other heme proteins that have been shown to bind NO include cytochrome *c* oxidase, peroxidases, nitrite reductase, and nitrophorins [6]. Recently, several prokaryotic heme proteins that are homologous to eukaryotic sGCs have been demonstrated to bind NO, perhaps for signaling purposes in bacteria [4,5,9].

Organic nitroso compounds such as alkyl thionitrites (RS–N=O, nitrosothiols) are also biologically relevant [10–13]. *S*-Nitrosoglutathione (GSNO) has bronchodilatory activity [14], and the effect is not correlated with prior NO release [15]. In recent papers, Stamler and coworkers [16,17] report that GSNO levels are depleted in asthmatic airways and suggest an important protective role for GSNO in asthma. Several RSNO compounds have been used as physiological and chemical NO donors [18]. The possible role of protein-bound copper in RSNO decomposition has been discussed [19]. English and coworkers [20] have shown that CuZn–SOD catalyzes NO transfer from

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RSNO to the Cys β 93 residues of oxyHb. Williams and coworkers [21] have also shown that peptide- and protein-bound Cu(II) can be reduced to Cu(I) which then participates in RSNO decomposition.

We recently reported the single-crystal X-ray crystal structure of the *S*-nitrosocysteine ethyl ester hydrochloride [22]. Montfort and coworkers [23] have demonstrated RSNO formation from the reaction of NO with the iron–thiolate bond in the heme active site of *Cimex* nitrophorin; thus, RSNO formation during NO signaling is proposed to occur in vivo for this system.

There is a lot of interest in determining the factors that control the release of NO from heme proteins and from RSNO compounds. It is generally accepted that the binding of NO to ferrous heme is greater than that for ferric heme [24]. The insect nitrophorins from *Rhodnius prolixus*, however, utilize ferric-NO compounds in their saliva for feeding purposes. The decomposition of RSNO compounds is now known to be catalyzed by some transition metal ions such as Cu(I) [19,25,26].

Accurate and specific identification of NO as a product of decomposition of heme-NO and RSNO compounds has been a subject of significant interest over the years. Several excellent methods are currently in use for the assessment of the in vitro and in vivo production of NO [27,18], and an excellent review describes recent advances in the bioimaging of NO [28]. The *Griess method* [29] detects nitrite which is an oxidative metabolite of NO. The method is based on diazotization of sulfanilic acid followed by azo coupling with 1-naphthylamine; the product is then monitored by UV–vis spectroscopy at 545 nm. A recent advance due to combination of flow injection analysis with the Griess reaction has improved the sensitivity from the μ M to nM levels of nitrite [30]. Contamination from other nitrogen oxides in the samples may complicate the results, however.

Another well-used assay for NO is based on chemiluminescence [31]. In one approach, gas-phase NO is reacted with ozone to generate activated NO₂* which relaxes to ground-state NO₂ with concomitant evolution of light. Advantages of this method include the low solubility of NO in aqueous solutions, the high sensitivity down to the pmol range [32], and the insensitivity of the method to ground-state NO₂ [31]. Specificity, however, may be compromised depending on sample preparation (e.g., acidification) or by the presence of other compounds that could also chemiluminesce under the reaction conditions [32]. In another approach, gas phase NO is reacted with luminol-H₂O₂, and the resulting peroxyxynitrite (from the reaction of NO with H₂O₂) is reported to be an excellent reagent for the chemiluminescence reaction at both neutral and alkaline pH [33,34], and detection limits down to 1 nM have been reported when the system is used in combination with microdialysis [35].

Electron paramagnetic resonance (EPR) has been used extensively for the detection of NO in physiological media [36]. The EPR spectrum of gas-phase NO is known [37], but for biological detection the NO is usually complexed

with a transition metal compound in solution. Nitrosyl complexes of iron diethyldithiocarbamates [38–40] and hemoglobin [41] are popular targets for the EPR detection of NO in biological systems, and the application of EPR spectroscopy to the bioimaging of NO is an emerging field [28]. A related NO detection (optical) method using horseradish peroxidase has been reported with detection limits down to 10 nM [42].

Electrochemical sensors for NO are gaining popularity [43–46], and some of the more recent NO-specific electrodes are reported to have detection limits down to the nM–pM ranges (reviewed in [45]). In general, the NO sensing is achieved through oxidation of NO at the electrode surface and measuring the resulting current. Reliability can be compromised from interference of other species in solution that are oxidized at the potential needed for NO oxidation (typically +0.86 V vs. Ag/AgCl); examples include dopamine and norepinephrine and their metabolites. A convenient way to get around this complication is the use of gas-permeable membranes to select against these interfering chemicals; the resulting selectivity for NO is thus greatly improved [45].

An increasingly popular method for the detection of NO in physiological media is the use of NO-reactive fluorescent probes [28]. An excellent commentary on the advantages and limitations of the use of diaminofluorophores such as DAF-2 DA (4,5-diaminofluorescein diacetate) in NO detection has been published recently [47]. Of some concern is the possibility of additional NO generation from UV–photolysis of nitrate in the samples. Lacza et al. [48] have recently reported that the chromophore diaminorhodamine-4M is a suitable red-fluorescent qualitative probe for reactive nitrogen species but is not specific for NO alone. The coordination of NO to metal ions that results in the release of metal-bound fluorophores and luminophores has been explored as a potential for NO sensing [49–52].

We have been interested in the direct and accurate detection of NO from chemical and biochemical reactions, and in a method that does not rely on chemical derivatization of NO (e.g., to nitrite), and in one that is free from contamination of other species that could interfere with the signal intensity ascribed to NO. Mid-infrared laser absorption spectroscopy has been recognized for many years as a powerful and precise technique for the detection of trace gases [53,54]. The technique is unique and capable of resolving the transition lines of molecules due to the narrow linewidth of the laser. The method is based on the measurement of the wavelength and intensity of the absorption of infrared light by a molecular system.

In the manuscript, we report (i) the successful adaptation of a mid-infrared tunable diode laser absorption spectrometer for the detection of NO released from chemical reactions, (ii) the use of this system for reactions in both organic and aqueous media, and (iii) the use of this system to detect isotopically labeled ¹⁵NO. We show that this real-time measurement is *sensitive and specific* to NO and to

^{15}NO without the need for chemical derivatization of these gases. We also show the inherent flexibility of the system and the ease of use of this prototype.

Materials and methods

S-Nitroso glutathione (GSNO) was prepared from the reaction of glutathione with sodium nitrite according to a literature procedure [55]. The ^{15}N -labeled analogue GS^{15}NO was prepared similarly using $\text{Na}^{15}\text{NO}_2$. $(\text{TTP})\text{Mn}(\text{NO})(1\text{-MeIm})$ [56] and $(\text{TTP})\text{Fe}(\text{NO})$ [57] (TTP, *meso*-tetratolylporphyrinato dianion; 1-MeIm, 1-methylimidazole) were prepared according to literature procedures. The nitrosyl derivative of horse heart myoglobin (MbNO) was prepared by reduction of *met*Mb with sodium dithionite followed by exposure to NO [58]. Silver tetrafluoroborate, sodium dithionite, sodium nitrite, Tris-HCl, ferrous chloride tetrahydrate, ammonium sulfate, ethylenediaminetetraacetic acid (EDTA), and potassium ferricyanide were purchased from Aldrich Chemical Company and used as received. ^{15}N -Labeled sodium nitrite was purchased from Isotec. Cupric sulfate (crystal form) was purchased from Fisher Scientific. Dichloromethane for the heme model chemistry was distilled under nitrogen from calcium hydride just prior to use.

Instrumentation and detection of NO

Nitric oxide detection is based on high-resolution tunable diode laser absorption spectroscopy (TDLAS). The experimental setup is shown in Fig. 1. The system consists of a cold head, optics and control electronics. The cold head, inside the cryostat housing, contains an infrared photovoltaic detector, a foil heater, a temperature sensor, and a mid-infrared tunable diode laser source emitting in the wavelength region around $5.2\ \mu\text{m}$. The optics consist of a gas cell and a series of plane and focusing mirrors for steering and collimating the infrared beam. Associated electronics include a laser current controller, a laser temperature controller, a lock-in amplifier, two waveform generators, and a computer. The present system employs a closed-cycle refrigerator system that cools the diode laser and detector to cryogenic temperatures with no need for liquid nitrogen refills. An auto-tuning temperature controller (LakeShore, OH), working in conjunction with cryo-cooler (IGC Polycold, CA), is used to maintain laser temperatures at set points between 85 and 110 K. The spectrometer takes advantage of a second harmonic detection scheme, and a 100-m long optical multi-pass Herriott cell (Aerodyne, MA).

Molecular absorption experiments are performed by collimating the laser beam with an $f/1$ off-axis-parabolic

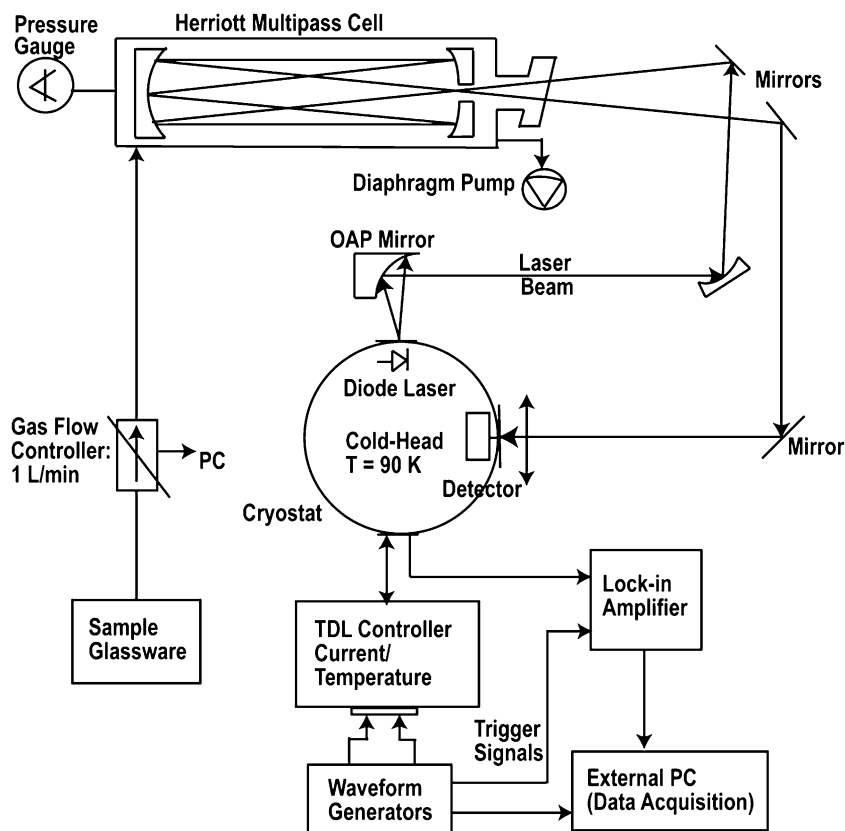


Fig. 1. Schematic diagram of the TDLAS instrument. Major components include a cryostat, Herriott multipass cell, electronics, and an integrated gas sampling interface.

mirror and then focusing the beam with a plano-concave mirror into the pressure controlled absorption cell. The output beam from the cell is then collected and focused onto an HgCdTe infrared detector by an $f/1.5$ aspheric lens. High-resolution absorption measurements are achieved by scanning the laser wavelength over the absorption features of ^{14}NO and ^{15}NO in the 1909.00 to 1909.64 cm^{-1} spectral region with a programmable 100 Hz saw-tooth ramp signal. The computer stores individual ramp scans and can be set to co-add these scans for improving signal-to-noise ratios. An oil-free diaphragm pump and a gas flow controller (Alicat Scientific, AZ) were used to reduce the gas cell pressure down to about 42 Torr and allow controlled gas flow through the glassware at a set value of 1 L per minute. In the case of Fig. 4B, no nitrogen gas flow was used (so as not to dilute the NO gas generated from the reactions), and the pressure dropped to 3 Torr . This system provides sensitivities in the one part-per-billion range for NO measurements. The measured absorption intensities are qualitatively proportional to the concentration of NO in the sample chamber; we have previously shown the direct linear correlation between absorption intensity and NO concentration in the $20\text{--}500\text{ ppb}$ range

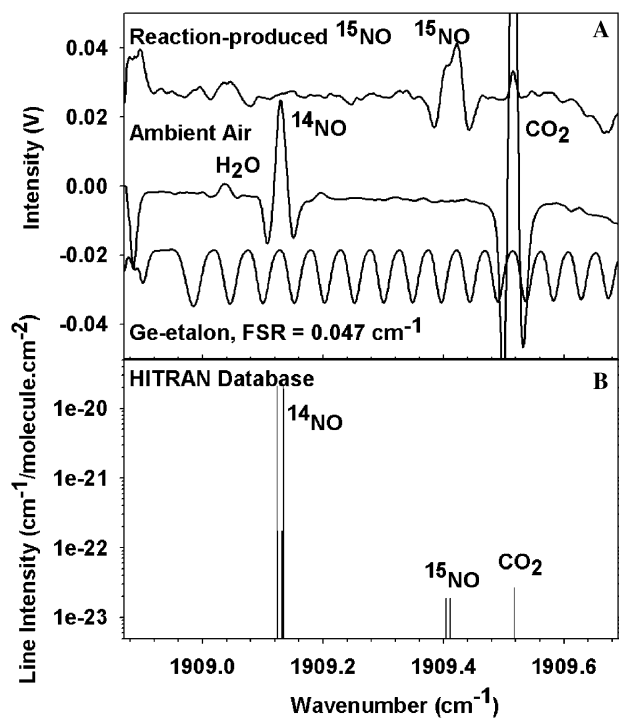


Fig. 2. (A) Second-harmonic spectrum of measured ^{14}NO and ^{15}NO . (Upper): ^{15}NO produced from a chemical reaction in the lab; (Middle): measured absorption spectrum of the ambient air with elevated ground level of ^{14}NO ; (Bottom): Ge etalon calibration spectrum induced by the single mode laser beam with a free spectral range of 0.047 cm^{-1} (Note that the spectra are shifted vertically for clarity). (B) Corresponding line intensities (in $\text{cm}^{-1}/\text{molecule} \times \text{cm}^{-2}$) and frequencies of ^{14}NO , ^{15}NO , and CO_2 from 1909.00 to 1909.64 cm^{-1} found in the HITRAN 1996 database. Etalon spectrum and the HITRAN spectra are used to identify observed absorption peaks.

[59]. McCann and coworkers [60] have employed similar methodology for simultaneous detection of NO and CO_2 in exhaled breath. The software, written in LabView 7.0, controls the laser and the heat sink temperature while acquiring data from the detector signal via the lock-in amplifier (SR810, SRS, CA).

The NO released was identified by its specific absorption feature at 1909.12 cm^{-1} using the HITRAN database [61]; ^{15}NO was also identified by its specific absorption at 1909.41 cm^{-1} (Fig. 2). The related absorption features for H_2O and CO_2 at 1909.04 and 1909.51 cm^{-1} , respectively, do not interfere with the measurements of NO and ^{15}NO .

Glassware description

The sample chamber is shown in Fig. 3. It consists of a chamber (A) to hold the reacting species (i.e., the solution chamber, capped with a rubber septum) and a large gas compartment (B) to hold the evolved NO gas. In general, the glassware is initially placed under vacuum and then backfilled with nitrogen gas to achieve anaerobic conditions for the experiments. The solvent is first introduced into compartment (A) via gas-tight syringe (sample volume $0.2\text{--}10\text{ mL}$) while the stopcock V_1 is closed. An anaerobic solution of the reagent is then added via gas-tight syringe to (A) to induce the release of NO, and the stopcock V_1 is opened and the gas captured in compartment (B) and directed to the TDLAS instrument via the stopcock V_2 .

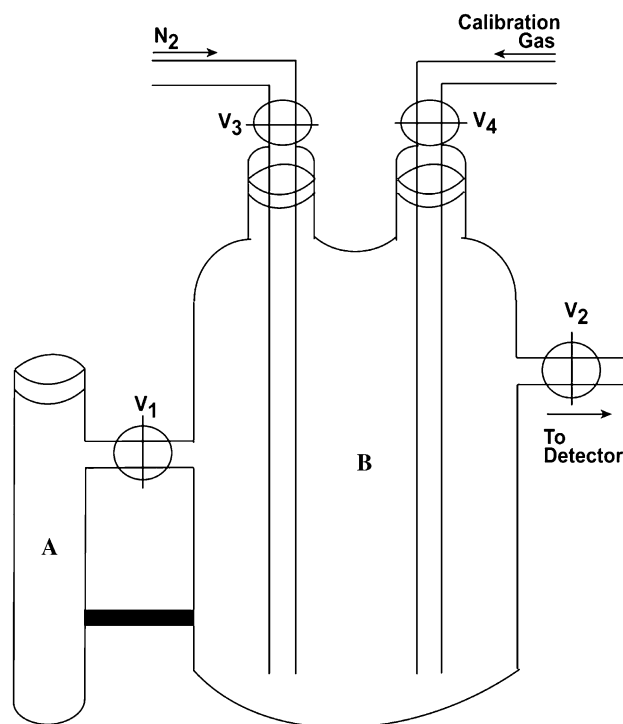


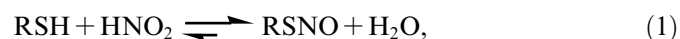
Fig. 3. Drawing of the sample chamber. (A) is the solution chamber. (B) is the large gas compartment. $V_1\text{--}V_4$ are glass stopcocks. V_2 leads to the TDLAS instrument.

Results and discussion

We have utilized TDLAS technology previously for the simultaneous measurement of biomarkers such as CO, CO₂, ¹³CO₂, N₂O, and NO in human breath [60,62]. We have now extended this technique for the *specific detection of NO* that is released from organic, inorganic, and biochemical compounds through the action of an added reagent. The infrared absorption feature at 1909.12 cm⁻¹ is specific for NO (1909.41 cm⁻¹ for ¹⁵NO; Fig. 2), and is not affected by the presence of other nitrogen oxides such as nitrite, nitrate, peroxy-nitrite, nitrogen dioxide, and nitrous oxide. Furthermore, we show that this method is widely applicable to aqueous and non-aqueous solvents due to the low solubility of NO in these solvents.

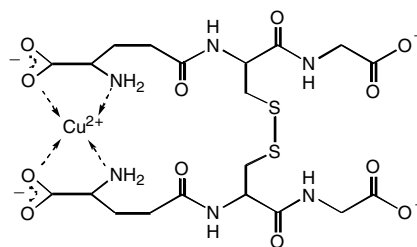
NO dissociation from nitrosoglutathione (GSNO)

McAninly et al. [63] showed, in 1993, that some trace metal ions catalyze the decomposition of organic thionitrites (RS–N=O, nitrosothiols). NO dissociation from nitrosoglutathione is a well-studied system [64], hence we chose this as a model for our gas-phase infrared NO detection method. A rapid release of NO was observed to occur as shown in Fig. 4A (dashed line) when GSNO was added to a solution of Cu(II) sulfate (1.5 equiv.). Similar responses are obtained over a wide range of GSNO concentrations (100 nM–0.9 mM), and the signal intensities correlate qualitatively with the amount of GSNO in the reaction chamber (e.g., Fig. 4B). Eqs. (1)–(3) represent the probable pathway by which the RSNO is decomposed by added copper ions. Williams and others [19,64–67] have shown that the true catalyst in the copper-catalyzed decomposition of RSNO is Cu(I) (Eq. (3)), generated by reduction of Cu(II) by thiolate ions (Eq. (2)) which are present due to the slight reversibility of the RSNO-forming reaction (Eq. (1)).



In comparison, the addition of GSNO to an iron(II) chloride solution did not elicit a strong response for NO release (Fig. 4A, solid line). This efficient GSNO decomposition by added Cu(II) is similar to that reported by Williams and coworkers [64] in which NO release was monitored using an NO electrode system. We further investigated the GSNO concentration dependence on its decomposition catalyzed by the addition of free Cu(II) ions. The results are shown in Fig. 5. When trace Cu(II) (50 μL of a 1.4 mM copper sulfate solution) is added to the GSNO solution, a rapid NO release is observed (peak 1 in Fig. 5). The NO release diminishes rapidly,

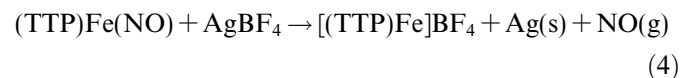
probably due to complexation of the regenerated Cu(II) ions by the GSNO-decomposition product GSSG [64,68].¹



A repeat addition of Cu(II) results in a renewed larger NO release (peak 2 in Fig. 5) due to an increased [Cu(II)]/[GSNO] ratio (i.e., reduced [GSNO] due to its decomposition). This pattern of NO release is repeated upon subsequent additions of Cu(II). Similar observations have been reported by Noble and Williams [69], who used an NO electrode for their measurements. As the GSNO gets depleted from solution, the extent of NO release is also depleted after several sequential additions of Cu(II) (e.g., peak 5 in Fig. 5).

NO release from iron nitrosyl and manganese nitrosyl porphyrins, and from MbNO

Nitric oxide binds to ferrous porphyrins to give relatively stable (porphyrin)Fe(NO) compounds [6]. The relatively high off-rate of NO when bound to ferric porphyrins, however, ensures that many ferric-NO heme compounds will lose their NO ligands. The effect of oxidation of the five-coordinate (TTP)Fe(NO) with silver tetrafluoroborate in an organic solvent (dichloromethane) is shown in Fig. 6A (Eq. (4)), and shows the formation of NO gas upon oxidation of the ferrous porphyrin to the ferric derivative.



Importantly, this result shows the utility of this gas-phase NO detection method for both aqueous solutions (previous section) and organic mixtures.

Manganese(II)-substituted heme compounds have been employed as models for the kinetically unstable and valence isoelectronic iron(III) analogues. We have reported the synthesis and structural characterization of several manganese nitrosyl porphyrins of the form (porphyrin)Mn(NO)(1-MeIm) [56]. These compounds contain relatively strong Mn–NO bonds. We have, however, shown that (electro)oxidation of these compounds results in prod-

¹ Although X-ray crystal data for this proposed structure are currently unavailable, the structure of a related 2:1 Cu(II)/GSSG complex is known [68].

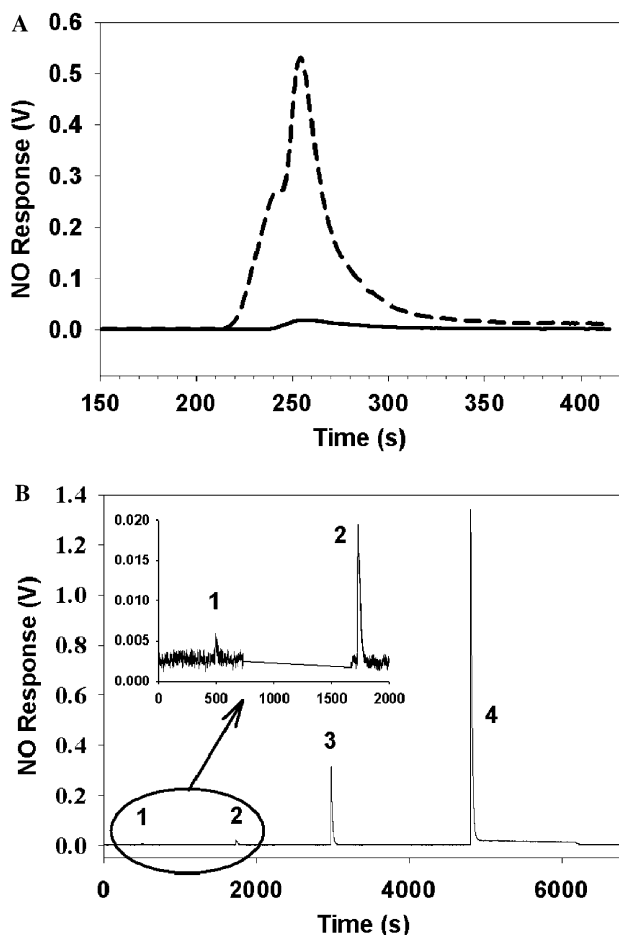
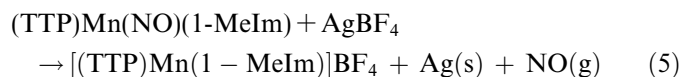


Fig. 4. NO release from metal-ion-catalyzed GSNO decomposition. (A) A comparison of the release of NO from GSNO in aqueous solution catalyzed by copper and iron ions under anaerobic conditions with a nitrogen flow of 1 L/min. Dashed line: NO release after GSNO was added at $t = 212$ s to the Cu(II) solution (1.4 mM) to give an initial concentration of GSNO of 0.89 mM. Solid line: NO release after GSNO was added at $t = 236$ s to an Fe(II) solution (1.6 mM) to give an initial concentration of GSNO of 0.99 mM. (B) NO release from Cu(II)-catalyzed GSNO decomposition as a function of [GSNO] using a constant initial [Cu(II)] of 40 μ M without using a nitrogen gas flow. After each run, the solution in the reagent chamber was replaced with a fresh solution of Cu(II) and then the appropriate amount of GSNO was added to result in a renewed release of NO: (1) 100 nM GSNO, (2) 1 μ M GSNO, (3) 10 μ M GSNO, and (4) 100 μ M GSNO. This Cu(II)-catalyzed NO release is not linearly dependent on [GSNO] (see [69]).

ucts whose formulations suggest loss of NO [70]. When a dichloromethane solution of one such compound, namely (TTP)Mn(NO)(1-MeIm) is reacted with AgBF_4 , we detect a rapid release of NO as shown in Fig. 6B (Eq. (5)), and a concomitant change in color of the solution from red to green indicative of formation of



a manganese(III) species. This, in essence, is similar to the effect of oxidation of the iron analogue shown in Fig. 6A. To the best of our knowledge, these results represent the first use of vibrational spectroscopy for the *direct and*

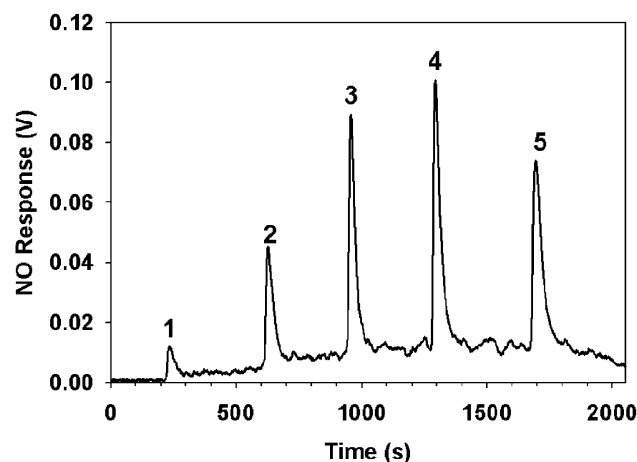


Fig. 5. The release of NO from GSNO in aqueous solution catalyzed by copper ions under anaerobic conditions, as a function of time over which copper ions were introduced. Initial [GSNO] was 0.8 mM, and [EDTA] was 13 μ M. Copper(II) was added at intervals 1–5 to achieve the following total copper concentrations in solution (1) Cu(II) = 27 μ M, (2) Cu(II) = 40 μ M, (3) Cu(II) = 53 μ M, (4) Cu(II) = 65 μ M, and (5) Cu(II) = 78 μ M. The drop in NO response after each peak is attributed to complexation of Cu(II) by the byproduct of GSNO decomposition, namely GSSG (see text). The lower response in (5) is due to a reduced [GSNO] after processes 1–4 (see text).

specific detection of NO resulting from the oxidative decomposition of iron nitrosyl and manganese nitrosyl porphyrins. Feelisch and coworkers [10] have used the chemiluminescence method to detect the NO released during the ferricyanide oxidation of NO-heme adducts. In another study, the released NO from a ferric nitrosyl porphyrin has been trapped with a cobalt porphyrin complex to give a new (porphyrin)Co(NO) compound (i.e., *trans* nitrosylation) [71].

As stated in Introduction, NO binding to myoglobin has been known for quite some time. The ferrous-NO derivative is relatively stable, but the ferric-NO derivative is prone to NO loss. We have detected the release of NO from the ferricyanide oxidation of MbNO in aqueous buffer (Tris-HCl, pH 7.4) as shown in Fig. 6C. This result is similar to that seen for the heme model compound (TTP)Fe(NO) described earlier in Fig. 6A.

Detection of ^{15}N labeled NO

The accurate and specific detection of ^{15}N -isotopically labeled NO is of great interest insofar as being able to identify properly the source of NO (e.g., from ^{15}N -labeling of the reactive guanidine group of L-arginine in the NO biosynthetic pathway). Isotopic ratio mass spectrometry has been utilized for the identification of ^{15}NO and oxidation products such as nitrite/nitrate [72]. We are able to detect gas phase ^{15}NO in the GS ^{15}NO decomposition reaction, and this detection is specific for ^{15}NO . Fig. 7A shows the effect of added Cu(II) to a GS ^{15}NO solution, and demonstrates that ^{15}NO can indeed be

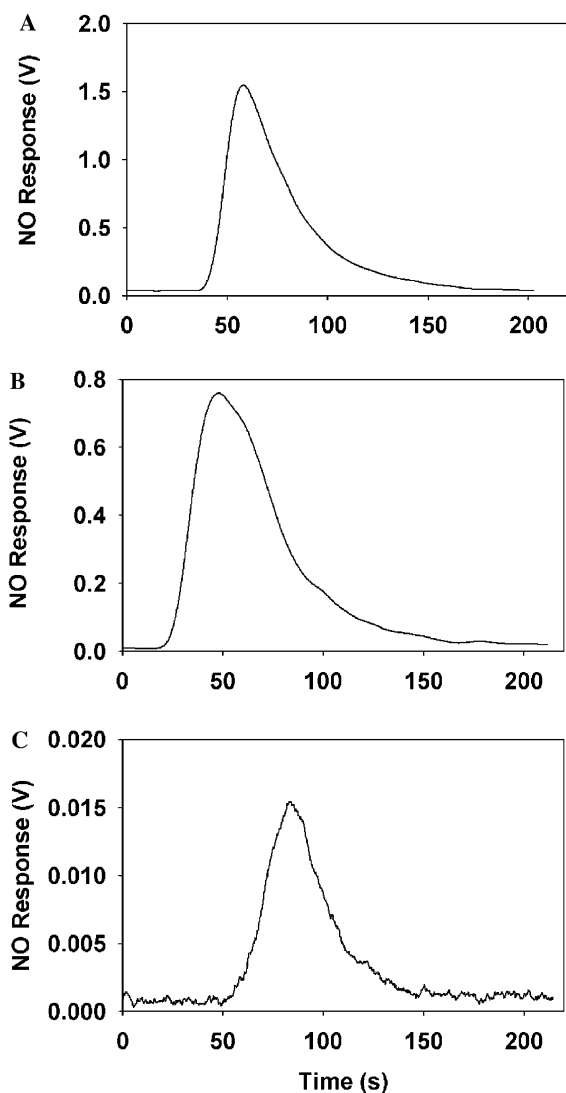


Fig. 6. NO release from metal nitrosyl porphyrins and nitrosyl myoglobin (MbNO). (A) The release of NO from the five-coordinate iron nitrosyl porphyrin (TTP)Fe(NO) (3.7 mM in CH_2Cl_2) after addition of AgBF_4 (12 mM) at $t = 37$ s. (B) The release of NO from the six-coordinate manganese nitrosyl porphyrin (TTP)Mn(NO)(1-MeIm) (1.7 mM in CH_2Cl_2) after addition of AgBF_4 (10 mM) at $t = 20$ s. (C) The release of NO from MbNO (6 mg/mL in Tris-HCl buffer at pH 7.4) after addition of $\text{K}_3[\text{Fe}(\text{CN})_6]$ (4.2 mM) at $t = 55$ s.

detected. The spectroscopic signal for ^{15}NO is lower than that for the ^{14}NO , but this is an inherent feature of the relatively weaker vibrational-rotational absorption feature at 1909.41 cm^{-1} being monitored (Fig. 2). Higher sensitivities for ^{15}NO , similar to those seen with ^{14}NO , can be obtained using different infrared absorption marker bands. However, our choice of the 1909.41 cm^{-1} feature used for our current studies was to enable us to simultaneously detect ^{14}NO and ^{15}NO using the same laser. We have also demonstrated ^{15}NO release upon ferricyanide oxidation of Mb ^{15}NO , and the result is displayed in Fig. 7B.

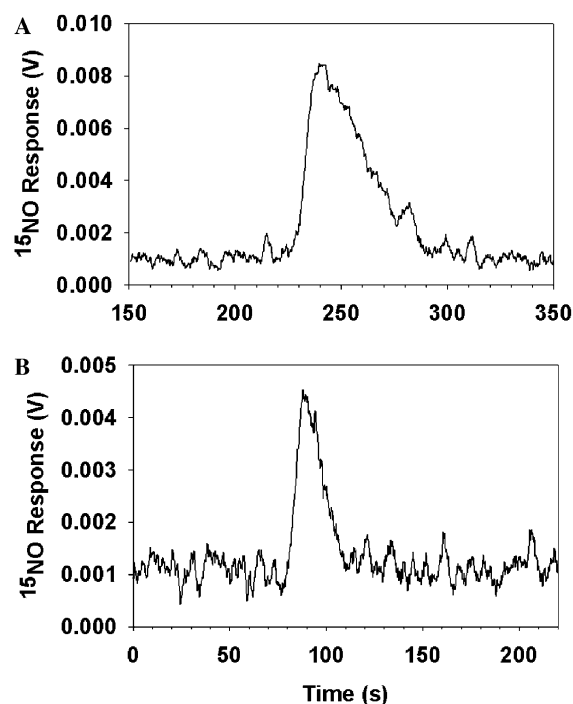


Fig. 7. Detection of ^{15}N labeled NO. (A) The release of ^{15}NO from GS^{15}NO in aqueous solution catalyzed by copper ions under anaerobic conditions. Initial $[\text{GS}^{15}\text{NO}]$ was 1.8 mM and $[\text{EDTA}]$ was 6.5 μM . $\text{Cu}(\text{II})$ (0.7 mM) was added at $t = 227$ s. (B) The release of ^{15}NO from Mb ^{15}NO (12 mg/mL in Tris-HCl buffer at pH 7.4) after addition of $\text{K}_3[\text{Fe}(\text{CN})_6]$ (15 mM) at $t = 80$ s.

Summary

In the manuscript, we report the successful application of mid-infrared spectroscopy for the direct and specific detection of ^{14}NO and ^{15}NO from decomposition of NO-containing organic, heme model, and protein compounds. Several advantages of this new application are evident: (i) the absorption features monitored are suitable for NO-releasing reactions in both aqueous (with or without buffer) solutions and in organic mixtures, (ii) it is specific for NO without contamination from signals due to the presence of other gaseous species, (iii) the NO produced in solution does not need to be derivatized before detection (e.g., oxidation to nitrite as with the Greiss reaction, ozonolysis for chemiluminescence measurements, or heme-nitrosyl formation for EPR spectroscopic detection), and (iv) impurities such as nitrite do not interfere with the NO detection methodology. An added advantage is that this method is readily applicable for the specific detection of ^{15}NO without the need to resort to mass spectrometric methods. With the capability of detecting both ^{14}NO and ^{15}NO , we should be able, using the appropriate isotopically labeled NO-generating precursors, to determine the source(s) of NO from reaction mixtures containing multiple NO-donor compounds.

While this is only qualitative at this stage, we are currently engaged in efforts to configure the system for precise

quantitative measurements over a wide range of experimental conditions (chemical and biochemical).

Acknowledgments

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