Inorganic Chemistry

Low-Temperature Trapping of Intermediates in the Reaction of NO[•] with O₂

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ABSTRACT: The autoxidation of NO[•] was studied in glasslike matrices of 2-methylbutane at 110 K and in a 8:3 v/v mixture of 2,2-dimethylbutane and *n*-pentane (rigisolve) at 80-90 K, by letting gaseous NO[•] diffuse into these solvents that were saturated with O₂. In 2-methyllbutane, we observed a red compound. However, in rigisolve at 85-90 K, a bright yellow color appears that turns red when the sample is warmed by 10-20 K. The new yellow compound is a precursor of the red one and also diamagnetic. The UV-vis spectrum of the yellow compound contains a band which resembles that present in

No NONO, but ONOO

A new, yellow, EPR-silent intermediate in the autoxidation of NO' has been found below 90 K.



 $ONOO^{-}$. Because the red and yellow intermediates are not paramagnetic, we postulate that $O=N-O-O^{\bullet}$ is in close contact with NO[•], or with another $O=N-O-O^{\bullet}$. Diffusion of gaseous O_2 into rigisolve saturated with NO[•] does not produce a color; however, a weak EPR signal (g = 2.010) is observed. This signal most likely indicates the presence of ONOO[•]. These findings complement our earlier observation of a red color at low temperatures and the presence of ONOO[•] in the gas phase (Galliker, B.; Kissner, R.; Nauser, T.; Koppenol, W. H. Chem. Eur. J. 2009, 15, 6161-6168), and they indicate that the termolecular autoxidation of nitrogen monoxide proceeds via the intermediate ONOO^{\bullet} and not via N₂O₂.

INTRODUCTION

NO[•] has attracted considerable attention because of its many functions in biology, such as its role as a messenger, regulation of blood pressure, and as promotor or inhibitor of biological redox reactions.1-

The autoxidation of NO[•] is very likely a multistep process because at least three molecules are involved. Its rate law in water⁷⁻¹⁰ is the same as that in the gas phase, which has been known for nearly a century.¹¹ It is also known that the rate does not depend on pH.^{12,13} In aqueous solution, the third-order rate constant comes close to the theoretical value for a termolecular process.¹⁴ However, in the gas phase, the thermal dependence of the reaction rate¹¹ contradicts the termolecular model, and in the context of the independence of the rate law on the medium, it is not necessary to assume a change of mechanism. It should be stated clearly that irrespective of the mechanism discussed below, the overall reaction is fairly slow at physiologically low NO[•] concentrations.¹⁵ The question then arises whether intermediates, if any, might be biologically relevant.

The mechanism of autoxidation has been repeatedly discussed¹⁶⁻¹⁹ and remains a topic for speculation. Because a true termolecular process for the gas-phase reaction is most unlikely, either N_2O_2 , favored by most authors,^{16,19–21} or $ONOO^{\bullet 22}$ are the possible intermediates of the initial step, and the intermediate must be in equilibrium with the educts in order to account for the rate law. Thus, it is of great interest whether the initial reaction intermediate is N₂O₂ or ONOO[•]. If the intermediate is N_2O_2 , then it can only react with O_2 . However, if it is ONOO[•], it may react with other molecules

than NO[•]. This would be especially relevant when O_2 is in excess over NO[•], as in the atmosphere and in biological systems. The biological concentrations of NO[•] and O₂ are such that it is difficult to complete either of the reaction paths to N_2O_4 or NO_2^{\bullet} . Although NO[•] has been shown to oxidize thiols to disulfides under anaerobic conditions via the intermediate RSNO^{\bullet -,²³ in the presence of O₂, NO₂^{\bullet} generated by NO^{\bullet}} autoxidation, or N2O3 formed with excess NO[•], are thought to be the initiators of S-nitrosothiol formation.²⁴ ONOO[•] is a mildly oxidizing radical, $E^{\circ}(ONOO^{\bullet}/ONOO^{-}) = +0.51 \text{ V}_{1}^{25}$ and thus is unlikely to be the initiator of the radical processes that lead to nitrosothiols. Its half-life has been estimated at ≈ 0.1 s in water.²⁵ The electrode potential and the half-life are sufficient to oxidize ascorbate and tocopherols (Vitamin E).

We have already obtained evidence for the reaction intermediates ONOO[•] and an uncommon red N₂O₄ isomer from experiments at ambient conditions in the gas phase and from a gas diffusion reaction at low temperature in a glass-like hydrocarbon matrix, respectively.²² The N₂O₄ isomer is red and decays to colorless sym-N2O4 above 115 K. As the thermally more stable nitrosonium nitrate [NO⁺ NO₃⁻] has also been reported to be red,²⁶ one might ask whether the assignment of the red color to a N_2O_4 isomer is correct. In another approach, Beckers et al. attempted to characterize NO[•] autoxidation intermediates by photolysis of the final reaction product, sym- N_2O_4 , in a rigid frozen neon matrix.²¹ They found that $(NO)_2$

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and O_2 , together with an adduct, are formed and speculate that these may be involved in the autoxidation reaction.

In order to resolve the autoxidation pathway of NO^{\bullet} , we extended our investigations at low temperature by carrying out the NO^{\bullet} autoxidation in a viscous hydrocarbon matrix softer than 2-methylbutane, at lower temperatures than before. We find a yellow intermediate that irreversibly changes to a red intermediate at slightly higher temperatures.

EXPERIMENTAL SECTION

Compressed NO[•] (3 MPa, \geq 99.5%) was obtained from Linde (München, Germany). It was passed through 10 M aqueous analytical grade KOH (Siegfried, Zofingen, Switzerland) solution before use to remove any NO₂[•]. Dioxygen (\geq 99.999%) was obtained from PanGas (Lucerne, Switzerland), 2-methylbutane (Uvasol grade) from Merck (Darmstadt, Germany), analytical grade 2,2-dimethylbutane from Sigma-Aldrich (Buchs, Switzerland), and analytical grade *n*-pentane from Carlo Erba (Milano, Italy). Aqueous solutions were prepared with water purified by an Advance Unit from Millipore (Billerica, MA, U.S.A.). Liquids and gases were handled with Gastight syringes from Hamilton (Reno, NV, U.S.A.).

Optical spectra were measured with a Specord 200 double-beam spectrophotometer (Analytik Jena, Jena, Germany) in transmission mode. Samples for UV–vis spectroscopy were prepared in a vacuum-insulated variable-temperature flow cell from Specac (Bromley, U.K.). The cell and the Dewar had both quartz windows, and the optical path length was 5 mm. X-band EPR spectra were acquired with an EMX spectrometer from Bruker Biospin (Rheinstetten, Germany) equipped with a TM cavity and a thermostat from Eurotherm Germany (Limburg, Germany).

Preliminary experiments were carried out in 100 mm long, 10 mm diameter borosilicate glass tubes. The hydrocarbon (2-methylbutane or a mixture of 8:3 v/v 2,2-dimethylbutane and *n*-pentane, named rigisolve) was cooled immediately above the liquid nitrogen surface in a Dewar and simultaneously saturated with dioxygen. Afterward, it was immersed into the liquid nitrogen until the solvent became glass-like, and 2–6 mL of NO[•] were injected with a syringe. The gas forms bubbles in the viscous hydrocarbon which collapse within less than a second because of the low temperature (see Figure 1).



Figure 1. NO[•] gas bubble absorption in 2-methylbutane or rigisolve glass by thermal collapse.

Optical spectroscopy samples were prepared directly in the Specac cell by the following procedure: the hydrocarbon was saturated with dioxygen at about 253 K above liquid nitrogen. It was then transferred into a syringe and injected into the Specac cell, precooled to 253 K, through PEEK tubing. Care was taken to leave some small space in the cell top to improve compressibility. The cell was subsequently cooled to the desired reaction temperature, and another syringe was loaded with 2–6 mL NO[•] gas. When the temperature was stable at 90 or 110 K, the NO[•] gas was injected into the glass-like hydrocarbon. Color changes were observed directly; when colors were stable, the cell was placed in the spectrometer. EPR spectra were taken with a sampling assembly described before.²⁷ For experiments with O₂ excess in the reaction zone, 2-methylbutane or rigisolve was transferred into the

quartz tube, filling it to the middle. Then, N_2 was bubbled slowly through the solvent in order to remove O_2 . When the solvent had partially evaporated but still did cover the inlet tubing, the gas inlet was switched to the NO[•] supply. Bubbling was continued until the solvent had evaporated down to the gas inlet tip, and then the assembly was inserted into the precooled EPR cavity. As to the positioning of the quartz tube in the cavity, it was taken into account that the solvent volume shrinks by about 20% upon glass formation. When the solvent had become viscous, the remaining NO[•] above the glass was expelled with N₂. The measurement was started after a slow stream of O₂ was established over the glass. In total, 20–40 field scans were accumulated over a time of 30 to 60 min. The background was taken under the same conditions but by passing N₂ instead of O₂ over the glass.

To test whether the colored products had unpaired electrons, NO^{\bullet} was injected into hydrocarbon glasses saturated with O₂ directly in wide EPR tubes under visual control, similarly to the preliminary experiments and subsequent measured in the precooled EPR cavity.

RESULTS

In rigisolve, the reaction temperature can be lower than in the 2-methylbutane glass, because rigisolve does not crystallize even at liquid nitrogen temperature. When the injection of NO[•] is performed into rigisolve at about 80–90 K, saturated with O_2 at 250 K, a yellow compound is formed (Figure 2), instead of a red one, as seen in 2-methylbutane.²²



Figure 2. Photograph of the yellow compound obtained in rigisolve at 80–90 K.

If rigisolve is saturated with N_2 instead of $O_{2^{\prime}}$ no color appears upon injection of NO[•]. Therefore, we observe a reaction product from both O₂ and NO[•] and not from NO[•] only. If the procedure is reversed, that is, O2 is injected into NO[•]-saturated solvent, also no color appears. Furthermore, the yellow compound is EPR-silent, just as the red compound obtained at 110 K in 2-methylbutane.²² Therefore, both compounds most likely are not free radicals. The probable composition of the yellow compound can be deduced from the ratio of NO[•] to O_2 in the reaction zone. Though the preparation method does not yield a homogeneous distribution of products, we can estimate the product concentration at a semiquantitative level. The colored strips obtained (see Figure 2) are ca.10 mm long and ca. 1 mm wide, and therefore, they comprise a volume of ca. 10 μ L. A typical initial bubble has a diameter of about 4-6 mm, the initial volume is thus approximately 0.1 mL, which corresponds to 4 μ mol of NO[•] at room temperature. After collapse of the bubble, [NO[•]] is about 0.1 M in the strip. The $[O_2]$ after saturation at 250 K is ca. 25 mM.²⁸ The concentration of the yellow material, under the assumption that only one O₂ molecule becomes part of the product, is therefore ≤ 25 mM. Because the gas in the bubble, during its collapse at low temperature, cannot diffuse very far into the glass, we conclude that the yellow material is

composed of one O_2 and two NO[•] molecules, since NO[•] must be in excess over O_2 . The combination of a diradical, O_2 , with two simple radicals, NO[•], also explains why the products are diamagnetic. When the temperature is increased from 90 to 110 K, the yellow color changes to red, then to blue (N₂O₃) upon further warming—the glass is now more fluid—and finally to colorless (N₂O₄). This sequence cannot be reversed, at any point, by lowering the temperature; the yellow and red compounds must be steps in a kinetically driven chain. The optical spectrum of the yellow compound shows three bands at 275, 350, and 400 nm after subtraction of the light-scattering background (Figure 3). There is no strong band that reaches far



Figure 3. Spectra of products. Yellow: optical spectrum of the yellow compound at 90 K in rigisolve glass; red: red compound at 110 K in 2-methylbutane glass; gray: N_2O_4 at 120 K in liquid 2-methylbutane. The glass spectra were corrected for the scattering background by taking records with solvent only under the same conditions.

into the visible range, and thus, our product cannot be NO₂[•]. Because it is colored, it cannot be N₂O₄ either; it must be a previously unknown compound. We obtain ε_{400} (yellow product) $\approx 32 \text{ M}^{-1} \text{ cm}^{-1}$ from the concentration estimate given above and a strip cross section of 1 mm. This extinction coefficient is larger if the conversion of O₂ were incomplete, or if the reaction had partially proceeded beyond the yellow product toward colorless compounds. The second possibility is unlikely because we know that with increasing temperature, we obtain the red compound. Furthermore, a 1:1 compound of O₂ and NO[•] would be paramagnetic and show an EPR response.

As mentioned above, no color is observed when O_2 is injected into hydrocarbon glasses containing NO[•]. However, when O_2 is passed over a 2-methylbutane glass saturated with NO[•], a weak EPR signal can be detected, but only if the liquid– gas interface in a setup as described earlier²⁷ is positioned at the center of the spectrometer cavity (Figure 4). The response is so weak that accumulation of spectra is required to improve the signal-to-noise ratio. Accumulation can be explored for about 30 min, when the signal distinctly begins to fade. Increasing the microwave power is of limited use because above 2 mW, the signal does not improve in intensity anymore.

It consists of a wide single line, $\Delta B_{p-p} = 0.8 \text{ mT}$, and its isotropic g-factor is 2.010. The line width suggests that it is composed of multiple lines but with a splitting constant too small to be resolved with the high modulation amplitude that was necessary to obtain sufficient sensitivity to measure the small concentration. The observed radical is most likely composed of one O₂ and one NO[•] molecule because of the



Figure 4. EPR spectrum obtained during diffusion of O₂ into NO[•]saturated 2-methylbutane at 108 K, ν = 9.502 GHz; microwave power: 2 mW; modulation amplitude: 0.2 mT.

concentration ratio of O_2 over NO[•] at the gas-liquid interface. When the EPR tube is lifted after the radical detection such that only the bottom part of the solvent is now at the center of the cavity, no EPR signal is found. The reason is that O_2 cannot diffuse down to this region in the glass during the chosen signal accumulation time. We also applied the gas injection approach in EPR tubes; in tubes of 3 mm diameter, the heat capacity of the small amount of 2-methylbutane they hold is insufficient to prevent melting, but in 5 mm tubes, the yellow and the red compound could be obtained, though not on every attempt. All preparations, colored or not, produced no EPR signal.

DISCUSSION

During our studies of the mechanism of the autoxidation of NO[•] under thermal conditions in the gas phase and in a glassy hydrocarbon matrix, we found a yellow intermediate (Figure 2) at 80–90 K. Previously,²² we reported the formation of a red compound upon injection of NO[•] into O₂-saturated 2methylbutane at a slightly higher temperature. Indeed, the yellow color changes to red when the temperature is increased, and at even higher temperatures, stable sym-N₂O₄ is obtained (Figure 3). The temperature at which rigisolve becomes a glass is lower than for 2-methylbutane, 90 K compared to 110 K, and it does not crystallize even at liquid N2 temperature. Both colored products do not show signals in EPR spectroscopy, and it is thus unlikely, but not entirely excluded, that they are radicals. Upon injection of NO• at room temperature into the cold O₂-saturated hydrocarbon, the bubble collapses, and the resulting local NO^{\bullet} concentration will be larger than that of O_{2} , whether or not NO[•] liquefies during collapse. On the basis of the diffusion distance estimated from the width of the color streaks, we assume that liquefaction does not happen. Under these conditions, colored products are observed, and we conclude that they both have the composition N_2O_4 .

If the mixing is carried out in reverse mode, namely, by letting O_2 diffuse into a NO[•]-containing hydrocarbon, which leads to a local excess of O_2 over NO[•], no colors develop. However, traces of a radical with $g_{iso} = 2.010$ are detected by EPR spectroscopy (Figure 4). This observation allows us to exclude the following species: NO[•], NO[•], N₂O₃, and N₂O₄. NO[•] itself has a *g*-factor that is much smaller than 2 because of strong spin–orbit coupling; NO[•] therefore produces no EPR signal in the normal X-band range.^{29,30} NO[•] would recombine with itself or NO[•] at 110 K, producing diamagnetic N₂O₄ and

Scheme 1



 N_2O_3 . NO[•] and NO₂[•] would display distinct hyperfine splitting caused by the ¹⁴N nucleus (I = 1) because there is substantial spin population on the N atom.^{29,31} ¹⁴N hyperfine splitting would also occur in ONOO[•], although one would expect the coupling constant to be smaller than in compounds with highspin population on the nitrogen atom. Indeed, the spectra of ONOO[•] obtained by irradiation of nitrates show coupling constants of less than 0.2 mT,³² and even in the HOO[•] radical, the hyperfine coupling constant does not exceed 0.5 mT in noncrystalline environment.³³ The weak signal cannot be assigned to a 2-methylbutane radical. Only NO2 would be able to generate it; however, NO2 can only evolve from N2O4, which would not dissociate at 110 K. We conclude that the signal most likely originates from a 1:1 compound between O_2 and NO[•], because the combination of a radical with a diradical yields a radical. Since the O–O bond is unlikely to break at low temperatures, and the g_{iso} factor of symmetrical NO₃[•] is 2.02^{34-36} the signal must stem from a peroxyl radical, an assignment that is supported by its g_{iso} value of 2.010.^{37,38} This value also comes close to the that of 2.014 observed during the gas phase reaction-at room temperature-of NO[•] with O₂ in excess, which was assigned to ONOO^{•.22} Although the circumstances under which the two observations were made are very different, it is not unreasonable to suppose that they concern the same species. The concentration of ONOO[•], present in the gas phase in steady-state concentration, must be very small.²² This observation implies that the equilibrium, eq 1

$$NO^{\bullet} + O_2 \rightleftharpoons ONOO^{\bullet}$$
 (1)

will not shift extensively to the product side at low temperatures. Additionally, NO[•] will dimerize to some extent when precooled to temperatures below 150 K,^{39,40} in the hydrocarbon glass, and probably be unavailable for reactions with O₂. A mechanism via (NO)₂ would require insertion of O₂ into the dimer bond, which has to be activated by thermal motion. However, the dimer is actually stabilized by the absence of such motion, as follows from the result that a weak low-temperature adduct of ONNO with O₂ starts to react only upon infrared irradiation.²¹ In the color-producing experiments, NO[•] of ambient temperature is added to the cold O₂-containing glass. Then, locally, NO[•] is in excess and hardly dimerized, such that the chance of two subsequent reactions is increased.

It could be argued that the colored products are only minor contributions or even byproducts with high extinction coefficients. The experience with nitrogen—oxygen compounds tells us that this is very unlikely, at least in the visible part of the spectrum. The highest reported coefficient is that of ONOO⁻, $\varepsilon_{302} = 1705 \text{ M}^{-1} \text{ cm}^{-1}$ in water.⁴¹ Even if the yellow compound had a similar value, and not $\varepsilon_{400} = 32 \text{ M}^{-1} \text{ cm}^{-1}$ as estimated, its concentration would be 0.5 mM, which, although not negligible, is small compared to the estimated maximum yield of 25 mM. In our previous report,²² we had assigned the red color to the chain-like isomer of N₂O₄, ONOONO. In the context of the results presented here, this interpretation can only be maintained if we assume that the yellow material is not a molecule with a covalent bond between the terminal O of ONOO[•] and the nitrogen of NO[•], but a radical pair complex of ONOO[•] with NO[•] (path A in Scheme 1), or, possibly, between two ONOO[•] molecules. The yellow color is certainly not caused by (NO)₂ because in liquid NO[•] this dimer is present to an extent of ca. 90%,⁴⁰ but the liquid is colorless.

An alternative interpretation is shown in Scheme 1, path B, where the yellow color is assigned to ONOONO and the red color to ONONO₂.

It is quite likely that, indirectly, the yellow color was observed before. In 1911, Raschig reported that he obtained a green paste when he led NO[•] into a double-walled glass container-a predecessor of the Dewar flask-with liquid O2. He then let excess O2 evaporate. At temperatures slightly above the boiling temperature of O₂, 90 K, the compound started to decompose, whereby O₂ was generated, and a deep-blue solution remained with white crystals of N2O4. On the basis of an elemental analysis, Raschig proposed the formula NO₃.⁴² Our interpretation is that the green color resulted from the yellow compound described here and blue N₂O₃. Raschig's publication turned out to be controversial: a few years later, Müller argued that the compound was not NO₃. He argued that the green color came from blue N_2O_3 and yellow NO_2^\bullet that was trapped before it could dimerize. 43 Given the observation of a yellow intermediate at the temperatures used by these workers, we prefer the interpretation by Raschig.

Is there thermochemical support for ONOO[•]? Given an estimate of +117 kJ/mol for $\Delta_f G^\circ$ of ONOO[•] in water and of +102 kJ/mol in the gas phase,²² a solvation energy of +16.4 kJ/mol for O₂⁴⁴ and a $\Delta_f G^\circ$ of +102 kJ/mol for NO[•] in water,⁴⁵ formation of ONOO[•] from NO[•] and O₂ is favorable by 1 kJ/mol in water at 25 °C and 1 molal concentrations. In the gas phase, the same reaction is uphill by 15 kJ/mol. Not knowing the solvation energies of all components in 2-methylbutane and rigisolve, we cannot calculate whether the same reaction is endergonic or exergonic in these media.

There are two previous reports of red products that involve NO[•] in cold media. A red color has been observed during the

reaction of 1-2% solutions of NO[•] in an ethane-propane mixture exposed to a pO_2 of up to 1.3 kPa and is thought to be, in spite of the low dielectric constant of the solvent, NO⁺NO₃^{-.26} [NO⁺NO₃⁻] has been detected by infrared spectroscopy; however, it has been detected in thin films that contain sym-N₂O₄ on CsBr⁴⁶ or Cu metal,⁴⁷ substrates which may promote ionization. A nitrosonium nitrate can also be made by decomposing sym-N₂O₄ with 18-crown-6 present,⁴⁸ and this compound is colorless. The asymmetric N-O bonded isomer of N_2O_4 might account for the red color; unfortunately, its optical spectrum has never been recorded. Another type of red compound derived from NO[•] requires the presence of a Lewis acid, but not of O_2 , and is stable at temperatures distinctly above 110 K.49 The red color is usually assigned to ONON, the asymmetric dimer of NO[•], attached to a Lewis acid.⁵⁰ Formation of the red color is reversible in that case: after it has disappeared upon warming, it can be brought back by cooling. In contrast, our preparation loses its red color irreversibly with increasing temperature. We are of the opinion that the red color we observe does not stem from $NO^+NO_3^-$, or from a $(NO)_2$ -Lewis acid complex.

Our mechanistic proposal, autoxidation via formation of ONOO[•], represents only one possible pathway of NO[•] autoxidation. Another report suggests that autoxidation could proceed via NO[•] dimerization to *cis*-ONNO followed by formation of a loose complex with O₂ (Scheme 1, path C), which then collapses to *sym*-N₂O₄ when exposed to infrared radiation of 400–8000 cm⁻¹ in a frozen Ne matrix.²¹ We believe that this well-developed mechanism does not represent the major course of reaction at ambient temperatures and in an environment less rigid than that of a frozen noble gas matrix. Given that the infrared absorption band of *cis*-ONNO disappears above 150 K,³⁹ we conclude that *cis*-ONNO is not an important intermediate in the NO[•]-O₂ reaction at higher temperatures. We thus prefer ONOO[•] as an intermediate in the NO[•] autoxidation at room temperature.²²

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All authors contributed to the writing of the manuscript and have given approval to its final version.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Kanner, J.; Harel, S.; Rina, G. Nitric oxide as an antioxidant. *Arch. Biochem. Biophys.* **1991**, 289, 130–136.

(2) Eich, R. F.; Li, T.; Lemon, D. D.; Doherty, D. H.; Curry, S. R.; Aitken, J. F.; Mathews, A. J.; Johnson, K. A.; Smith, R. D.; Phillips, G. N.; Olson, J. S. Mechanism of NO-induced oxidation of myoglobin and hemoglobin. *Biochemistry* **1996**, *35*, 6976–6983.

(3) Espey, M. G.; Miranda, K. M.; Thomas, D. D.; Wink, D. A. Distinction between nitrosating mechanisms within human cells and aqueous solution. *J. Biol. Chem.* **2001**, *276*, 30085–30091.

(4) Jourd'heuil, D.; Jourd'heuil, F. L.; Feelisch, M. Oxidation and nitrosation of thiols at low micromolar exposure to nitric oxide - Evidence for a free radical mechanism. *J. Biol. Chem.* **2003**, *278*, 15720–15726.

(5) Hummel, S. G.; Fischer, A. J.; Martin, S. M.; Schafer, F. Q.; Buettner, G. R. Nitric oxide as a cellular antioxidant: A little goes a long way. *Free Radical Biol. Med.* **2006**, *40*, 501–506.

(6) Hill, B. G.; Dranka, B. P.; Bailey, S. M.; Lancaster, J. R., Jr.; Darley-Usmar, V. M. What part of NO don't you understand? Some answers to the cardinal question in nitric oxide biology. *J. Biol. Chem.* **2010**, *285*, 19699–19704.

(7) Pogrebnaya, V. L.; Usov, A. P.; Baranov, A. V.; Nesterenko, A. I.; Bez''yazychnyi, P. I. Oxidation of nitric oxide by oxygen in the liquid phase. *J. Appl. Chem. USSR* **1975**, *48*, 1004–1007.

(8) Awad, H. H.; Stanbury, D. M. Autoxidation of NO in aqueous solution. *Int. J. Chem. Kinet.* **1993**, 25, 375–381.

(9) Pires, M.; Rossi, M. J.; Ross, D. S. Kinetics and mechanistic aspects of the NO oxidation by O_2 in aqueous phase. *Int. J. Chem. Kinet.* **1994**, 26, 1207–1227.

(10) Kharitonov, V. G.; Sundquist, A. R.; Sharma, V. S. Kinetics of nitric oxide autoxidation in aqueous solution. *J. Biol. Chem.* **1994**, *269*, 5881–5883.

(11) Bodenstein, M. Die Geschwindigkeit der Reaktion zwischen Stickoxyd und Sauerstoff. Z. Elektrochem. **1918**, 24, 183–220.

(12) Lewis, R. S.; Tannenbaum, S. R.; Deen, W. M. Kinetics of *N*nitrosation in oxygenated nitric oxide solutions at physiological pH: Role of nitrous anhydride and effects of phosphate and chloride. *J. Am. Chem. Soc.* **1995**, *117*, 3933–3939.

(13) Goldstein, S.; Czapski, G. Kinetics of nitric oxide autoxidation in aqueous solution in the absence and presence of various reductants. The nature of the oxidizing intermediates. *J. Am. Chem. Soc.* **1995**, *117*, 12078–12084.

(14) Gabricevic, M.; Lente, G.; Fabian, I. Hydrogen isotope exchange of chlorinated ethylenes in aqueous solution: Possibly a termolecular liquid phase reaction. *J. Phys. Chem. A* **2015**, *119*, 12627–12634.

(15) Koppenol, W. H. In Nitric Oxide and Radicals in the Pulmonary Vasculature; Weir, E. K., Archer, S. L., Reeves, J. T., Eds.; Futura Publishing Co., Inc.: Armonk, NY, 1996; pp 355–362.

(16) Solc, M. Kinetics of the reaction of nitric oxide with molecular oxygen. *Nature* **1966**, 209, 706.

(17) Lindqvist, O.; Ljungström, E.; Svensson, R. Low temperature thermal oxidation of nitric oxide in polluted air. *Atmos. Environ.* **1982**, *16*, 1957–1972.

(18) McKee, M. L. Ab initio study of the N_2O_4 potential energy surface. Computational evidence for a new N_2O_4 isomer. J. Am. Chem. Soc. **1995**, 117, 1629–1637.

(19) Eisfeld, W.; Morokuma, K. Theoretical study of the potential stability of the peroxo nitrate radical. *J. Chem. Phys.* **2003**, *119*, 4682–4688.

(20) Bodenstein, M. Eine Bemerkung zur Theorie der Reaktionen dritter Ordnung. Z. physik. Chemie **1922**, 100, 118–123.

(21) Beckers, H.; Zeng, X. Q.; Willner, H. Intermediates involved in the oxidation of nitrogen monoxide: Photochemistry of the *cis*- $N_2O_2 \bullet O_2$ complex and of *sym*- N_2O_4 in solid Ne matrices. *Chem. - Eur. J.* **2010**, *16*, 1506–1520.

(22) Galliker, B.; Kissner, R.; Nauser, T.; Koppenol, W. H. Intermediates in the autoxidation of nitrogen monoxide. *Chem. -Eur. J.* **2009**, *15*, 6161–6168.

(23) Aravindakumar, C. T.; De Ley, M.; Ceulemans, J. Kinetics of the anaerobic reaction of nitric oxide with cysteine, glutathione and cysteine-containing proteins: implications for *in vivo S*-nitrosation. *J. Chem. Soc., Perkin Trans.* 2 2002, 663–669.

(24) Folkes, L. K.; Wardman, P. Kinetics of the reaction between nitric oxide and glutathione: Implications for thiol depletion in cells. *Free Radical Biol. Med.* **2004**, *37*, 549–556.

(25) Amatore, C.; Arbault, S.; Bruce, D.; de Oliveira, P.; Erard, M.; Vuillaume, M. Characterization of the electrochemical oxidation of peroxynitrite: Relevance to oxidative stress bursts measured at the single cell level. *Chem. - Eur. J.* **2001**, *7*, 4171–4179.

(26) Parts, L.; Miller, J. T. Nitrosonium nitrate. Isolation at 79 Degrees - 205 degrees K and infrared spectra of polymorphic compound. J. Chem. Phys. **1965**, 43, 136.

(27) Kissner, R. In Advances in Inorganic Chemistry. NOx Related Chemistry, Vol. 67; van Eldik, R., Hubbard, C. D., Eds.; Academic Press: San Diego, 2015; pp 335–354.

(28) Jakubicek, J. Bestimmung der Löslichkeit des Sauerstoffs in Isopren, Hexan und Isopentan. *Collect. Czech. Chem. Commun.* **1963**, 28, 3430.

(29) Ashford, N. A.; Jarke, F. H.; Solomon, I. J. Gas-phase electron paramagnetic resonance absorption in a $N^{16}O$, $N^{17}O$ and $N^{18}O$ mixture. *J. Chem. Phys.* **1972**, *57*, 3867–3870.

(30) Carrington, A.; Levy, D. H.; Miller, T. A.; Hyde, J. S. Double quantum transitions in gas-phase electron resonance. *J. Chem. Phys.* **1967**, 47, 4859–4860.

(31) Burch, D. S.; Tanttila, W. H.; Mizushima, M. X-band ESR spectrum of nitrogen dioxide. J. Chem. Phys. 1974, 61, 1607–1612.

(32) Bannov, S. I.; Nevostruev, V. A. Formation and properties of $NO_3^{2^{-}}NO_3$ and ONOO radicals in nitrate-containing matrices. *Radiat. Phys. Chem.* **2003**, *68*, 917–924.

(33) Bosnjakovic, A.; Schlick, S. Nafion perfluorinated membranes treated in Fenton media: Radical species detected by ESR spectroscopy. J. Phys. Chem. B 2004, 108, 4332–4337.

(34) Kevan, L. Radiolysis of frozen solutions. I. Formation of nitrogen dioxide in sodium nitrate ices. *J. Phys. Chem.* **1964**, *68*, 2590–2594.

(35) Martin, T. W.; Swift, L. L.; Venable, J. H. NO₃ Radical: "Soft" lattice preparation and pure ESR spectrum at 88 K. *J. Chem. Phys.* **1970**, *52*, 2138–2143.

(36) Golding, R. M.; Henchman, M. ESR study of NO_2 and NO_3 in irradiated lead nitrate. J. Chem. Phys. **1964**, 40, 1554–1564.

(37) Ingold, K. U. Peroxy radicals. Acc. Chem. Res. 1969, 2, 1-9.

(38) Atkins, P. W.; Symons, M. C. R. The Structure of Inorganic Radicals; Elsevier Scientific Publishing Co.: Amsterdam, 1967.

(39) Dinerman, C. E.; Ewing, G. E. Infrared spectrum, structure, and heat of formation of gaseous $(NO)_2$. J. Chem. Phys. **1970**, 53, 626–631.

(40) Temleitner, L.; Pusztai, L. Oriental correlations in liquid carbon monoxide and nitric oxide. *J. Phys.: Condens. Matter* **2005**, *17*, S47–S57.

(41) Bohle, D. S.; Glassbrenner, P. A.; Hansert, B. Synthesis of pure tetramethylammonium peroxynitrite. *Methods Enzymol.* **1996**, *269*, 302–311.

(42) Raschig, F. Über Stickstoffhexoxyd NO₃. *Chemiker-Zeitung* **1911**, *118*, 1096.

(43) Müller, E. Über das Stickstoffhexoxyd und Isostickstofftetroxyd von F. Raschig. Z. anorg. Chemie 1914, 86, 230-238.

(44) Wagman, D. D.; Evans, W. H.; Parker, V. B.; Schumm, R. H.; Halow, I.; Bailey, S. M.; Churney, K. L.; Nuttal, R. L. Selected values for inorganic and C_1 and C_2 organic substances in SI units. *J. Phys. Chem. Ref. Data* **1982**, *11* (Suppl. 2), 37–38.

(45) Armstrong, D. A.; Huie, R. E.; Koppenol, W. H.; Lymar, S. V.; Merényi, G.; Neta, P.; Ruscic, B.; Stanbury, D. M.; Steenken, S.; Wardman, P. Standard electrode potentials involving radicals in aqueous solution: Inorganic radicals. *Pure Appl. Chem.* **2015**, *87*, 1139–1150.

(46) Jones, L. H.; Swanson, B. I.; Agnew, S. F. Infrared studies of autoionization of thin films of dinitrogen tetraoxide. *J. Chem. Phys.* **1985**, *82*, 4389–4390.

(47) Bolduan, F.; Jodl, H. J. Raman spectroscopy on matrix-isolated NO⁺, NO₃ and N₂O₄ in Ne. *Chem. Phys. Lett.* **1982**, 85, 283–286.

(48) Ricard, S.; Audet, P.; Savoie, R. Ionic complexation of N_2O_4 by 18-crown-6. J. Mol. Struct. **1988**, 178, 135–140.

(49) Ohlsen, J. R.; Laane, J. Characterization of the asymmetric nitric oxide dimer O=N=O=N by resonance Raman and infrared spectroscopy. J. Am. Chem. Soc. **1978**, 100, 6948–6955.

(50) Sluyts, E. J.; van der Veken, B. J. On the behaviour of nitrogen oxides in liquefied argon and krypton. Dimerisation of nitric oxide. *J. Mol. Struct.* **1994**, 320, 249–267.