Nitric Oxide 103 (2020) 31–46

Contents lists available at ScienceDirect

Nitric Oxide

journal homepage: www.elsevier.com/locate/yniox

Review

The solution chemistry of nitric oxide and other reactive nitrogen species

Peter C. Ford [a,*], Katrina M. Miranda [b]

[a] Department of Chemistry and Biochemistry, University of California, Santa Barbara, Santa Barbara, CA, 93106-9510, USA
[b] Department of Chemistry and Biochemistry, University of Arizona, Tucson, AZ, 85721, USA

ARTICLE INFO

Keywords:
Nitric oxide
Nitrogen oxides
Nitroxyl
Nitrite
Nitrogen dioxide
S-nitrosothiols
Peroxynitrite
Nitrate

ABSTRACT

In this article we discuss the fundamental chemical and physical properties of NO and related nitrogen oxides (NO_2^-, NO_2, N_2O_3, etc.) under solution conditions relevant to mammalian biology.

1. Introduction

Redox chemistry plays numerous integral roles in biological systems, and the interconversion between redox-related species is central to nitrogen-based signaling [1]. Nitric oxide (NO, aka nitrogen monoxide) [2],[1] is formed endogenously by the five-electron enzymatic oxidation of L-arginine by NO synthase (NOS). Once formed, NO can directly induce signaling processes or can be converted by further redox steps into other nitrogen oxide species, such as nitrogen dioxide (NO_2) and dinitrogen trioxide (N_2O_3) that can stimulate signaling cascades through post-translational modification. Interactions with low molecular weight thiols produce still other signaling molecules such as *S*-nitrosothiols (RSNO). Oxidations of NO to nitrite (NO_2^-) or nitrate (NO_3^-) serve as degradation pathways, but these ions can also be recycled back to NO by physiological pathways.

The goal of this review is to outline the fundamental solution phase chemistry of NO and related nitrogen oxides (NO_x) relevant to biological conditions. While NO and related NO_x species are simple molecules and ions, their chemistry is extensive and often complex. Other derivatives such as low molecular weight *S*-nitrosothiols and their protein analogues have drawn increasing attention in terms of potential roles in biological signaling. Understanding both the nature and kinetic likelihood of the chemical interactions of these species in a cellular environment is crucial to having a holistic view of their known and potential physiological roles.

The principal biological targets for NO in biology are metal centers such as heme and non-heme iron proteins. Of particular importance with regard to signaling mechanisms is the ferroheme enzyme soluble guanylyl cyclase (sGC). Indeed, it was the effort to characterize the endothelium derived relaxation factor (EDRF) activation of sGC that uncovered the importance of endogenously produced NO [3–5]. The NO synthases (NOS) are themselves ferriheme proteins [6], and the interactions of NO with hemes extends across all major protein types [7]. NO can function as an activating agent, as an inhibitor or as a substrate with these enzymes, while other heme proteins serve as NO carriers. For instance, the release of NO from salivary ferriheme proteins of certain blood-sucking insects facilitate receipt of a sufficient blood meal [8]. There is also growing interest in the interactions of NO with non-heme iron [9] and iron-sulfur clusters [10] as well as other metalloproteins and cofactors. The metal-mediated chemistry of NO and NO_x derivatives has been reviewed elsewhere [11], so the focus of the present article will largely be on the rich solution chemistry of NO and NO_x species independent of metal centers.

While the reactions of NO with metals are typically regulatory in nature, the interactions with O_2 and free radicals often lead to formation of deleterious reactive nitrogen species (RNS) such as NO_2 and N_2O_3 that induce pathophysiological effects [12,13]. Other recent developments have suggested that nitrite may serve as a source of NO, especially under

* Corresponding author.
E-mail addresses: ford@chem.ucsb.edu (P.C. Ford), kmiranda@email.arizona.edu (K.M. Miranda).

[1] The International Union of Pure and Applied Chemistry (IUPAC) recommends the name "nitrogen monoxide" for NO (also, oxidonitrogen(•) and oxoazanyl) [2], but "nitric oxide" is much more commonly used and we will do so in this review.

https://doi.org/10.1016/j.niox.2020.07.004
Received 15 May 2020; Received in revised form 14 July 2020; Accepted 16 July 2020
Available online 25 July 2020
1089-8603/

hypoxia [14,15]. The bioregulatory and therapeutic roles of HNO are also of interest [16]. Even nitrate, long considered to be a stable end-product of nitrogen metabolism, may not be so innocent, since it can be reduced to nitrite by the action of bacterial nitrate reductases in the saliva and perhaps endogenously as well [14].

2. Nitric oxide: physical and chemical properties

2.1. Structure and physical properties

Nitric oxide is a diatomic, *stable free radical* with one unpaired electron, and as such, is sometimes written as NO• ("NO dot"). However, from a chemical perspective, it is well recognized that NO has an odd number of electrons, so we will not add the extra "dot" in this article. Typically, free radicals are short-lived owing to their high reactivity, especially the propensity to dimerize. The halogens offer familiar examples; chlorine atoms (Cl•) readily dimerize, so the standard elemental form is the diatomic species Cl_2. In contrast, NO does not readily form dimers and, while it does react rapidly with many other free radicals and redox active metals, it does not display the aggressive reactivity of a species such as Cl•. This limited reactivity is a critical feature for NO's role as a signaling agent, since it allows diffusion across several cell diameters before encountering a reaction target or trap.

A more detailed understanding arises from simple molecular orbital (MO) theory. MO diagrams of heteronuclear diatomics reflect the relative energies of the atomic orbitals of the constituent atoms (Fig. 1). As the less electronegative atom, the atomic orbitals of nitrogen are higher in energy than the corresponding orbitals of oxygen. The MOs that result from overlap of these atomic orbitals are delocalized over both atoms, but to an unequal extent. The lower energy bonding orbitals of NO are polarized toward the oxygen, while the higher energy, anti-bonding orbitals have higher nitrogen character. The unpaired electron of NO is found in orbitals that are π-antibonding with respect to the N–O bond. Thus, rather than being localized on one of the atoms, the unpaired electron is delocalized over both atoms, which is one rational for the low tendency for NO to dimerize.

The molecular orbital diagram predicts a bond order of 2.5, and the N–O bond length of 1.154 Å [17] is correspondingly intermediate between those of N_2 (bond order of 3) and of O_2 (bond order of 2). The infrared stretching frequency of free NO (ν_{NO}) occurs at 1876 cm^{-1}, consistent with this 2.5 bond order [18]. A consequence of the opposing polarization effects of the highest occupied MO having more nitrogen character and the higher electronegativity of oxygen is that NO has only a very small dipole moment (0.15 Debye) [17].[2]

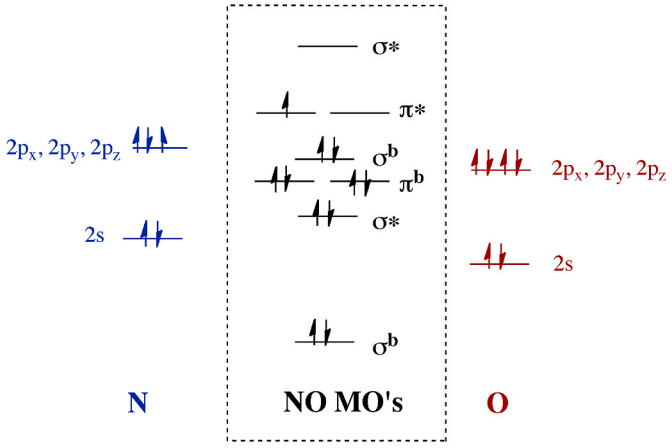


Fig. 1. Qualitative molecular orbital diagram for NO.[a]
[a]The ground and excited states of diatomic molecules can be designated by the orbital occupation of their electrons with a spectroscopic term symbol of the form $^{2S}+^{1}L$, where 2S + 1 is the spin multiplicity while L designates the net orbital angular momentum. Ground state NO has a $^{2}\Pi$ electronic configuration.

Due to its low molecular polarity, the solubility and transport properties of NO are similar to those of O_2. The aqueous solution solubility of NO is 1.9 mM atm^{-1} at 25 °C and 1.4 mM atm^{-1} at 37 °C [19]. It is more soluble in organic solvents with its solubility ranging from ~3 mM atm^{-1} in DMSO to 15.0 mM atm^{-1} in cyclohexane at 25 °C.

The low solubility of NO in water has several important biological implications. First, the higher solubility in organic solvents would lead to partitioning of NO from the cytoplasm to lipid membranes. The varied NO concentrations in the heterogeneous environment of cells impacts the types of reactions that can occur, as described below. Second, the partition coefficient of NO from water into the gas phase is even higher. At equilibrium, the NO concentration in the gas phase is nearly 20 times higher than in an aqueous medium [19]. Furthermore, the rate of partitioning is dependent on factors such as interface area, headspace volume and stirring, such that instantaneous NO concentrations depend on a complex function involving the competing dynamics of formation, diffusion, partitioning and consumption. As a consequence, NO levels will rapidly decline in Petri dishes but will remain relatively constant in sealed vessels with little headspace.

As a neutral small molecule, NO not only partitions to cell membranes, it can readily cross these barriers by passive diffusion. Along with low unusually low reactivity, the relatively fast rate of diffusion of NO in biological media, reported to be 50 μm s^{-1} [20–22], is a critical aspect to the function of NO as a paracrine signaling agent. Specifically, NO is typically produced in one cell type, then diffuses into and interacts with targets in neighboring cells of another type, such as smooth muscle cells, bacteria and cancer cells or neurons. The diffusibility of NO has been discussed extensively for cellular and vascular systems by Lancaster [21] and Liao [22] while the reactivity is described in detail below.

2.2. Preparation of NO for experimental studies

Unlike most free radicals, NO can be stored for extended periods of time, both in the gas phase and in solution. NO gas can be synthesized in the laboratory by mixing sodium nitrite and copper chloride solutions [23], or it can be purchased in high pressure tanks from commercial sources. As a toxic gas, NO must be transported, stored and used with appropriate care. Aqueous solutions of NO can be prepared by simply sparging the gas through buffered solutions, but this is often complicated by two common sources of impurities. First, while high purity NO is commercially available, the purity will decline with time, especially in the high pressure storage tanks, due to slow disproportionation via the reactions shown in eqs. (1)–(3).

$$3\ NO \rightarrow N_2O + NO_2 \quad (1)$$

$$NO + NO_2 \rightleftarrows N_2O_3 \quad (2)$$

$$2\ NO_2 \rightleftarrows N_2O_4 \quad (3)$$

Nitric oxide is a colorless gas while NO_2 is brown and liquid N_2O_3 is blue. NO also has a boiling point (1 atm) at 121 K and a melting point at 110 K, which are both somewhat higher than the boiling point of liquid N_2 (77 K) [17]. This makes it possible to purify NO by distillation on a vacuum line, and this method has been used in experimental studies where it was considered especially important to ensure the delivery of clean gaseous NO to the reaction vessel [24]. The NO_2 and N_2O_3 impurities can also be removed by passing the NO gas stream through high surface area solid KOH or concentrated KOH solutions and impurities

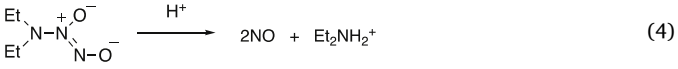
[2] Since a dipole moment is required for observation of linear molecules by infrared spectroscopy, the IR absorption band for NO in solution has a very low intensity (ε ~ 20 M^{-1} cm^{-1}) [20]. Anchoring of NO to a molecule, such as by binding to a metal, increases the intensity significantly.

such as nitrous oxide (N_2O) can be removed by distilling the NO at very low temperature.

A second potential source of impurities in NO solutions is direct oxidation by free O_2, which is difficult to remove from aqueous media. The facile reaction of O_2 with NO will be discussed in detail below, but it produces NO_2, N_2O_3, and N_2O_4 in aprotic media and NO_2^- in water [25]. The concentration of NO in aqueous solutions should then be measured rather than assumed from published solubility values. In addition, since a typical biological experiment is likely to be carried out under normoxic conditions, it is essential to understand the potential formation and reactivities of the other nitrogen oxides. This is especially true since some of the higher oxides of nitrogen are more reactive than NO, are more soluble in organic solvents, and form nitrous and nitric acids in aqueous solutions. Such species also concentrate in the solution phase, so the impacts of trace impurities are amplified when a NO gas stream is entrained through a solution. Furthermore, since nitrite formation acidifies aqueous solutions, the use of a high capacity buffer is recommended.

It is well documented that artifacts can be introduced by bolus injection of even high purity NO stock solutions, due to high local concentrations of NO. Under such conditions, the reaction with the oxygen present is accelerated leading to autoxidation products (see section 2.4.2). As an alternative, NO can be delivered using donors that release NO homogenously within a solution and at different rates [26–28]. In principle, such donors more closely replicate the intracellular production of NO. A variety of donor systems are available including organic nitrates, *S*-nitrosothiols and metal nitrosyls. Organic nitrates, such as nitroglycerin (29), and metal nitrosyls, such as sodium nitroprusside (30), have been used clinically to treat vascular conditions including angina and hypertension, but such compounds are not ideal spontaneous NO donors. Compounds such as *S*-nitroso-glutathione (GSNO) and *S*-nitroso-*N*-acetylpenicillamine (SNAP) have been used extensively in biological experiments, but these compounds are more likely to function as trans-nitrosating agents (transfer of a NO^+ moiety) rather than as NO donors.

For spontaneous release of NO under controlled conditions, the diazeniumdiolate salts (NONOates) pioneered by Keefer and coworkers [27], such as $Na[Et_2N(O)NO]$ (1,1-diethyl-2-hydroxy-2-nitrosohydrazine, commonly called "DEA/NO") (eq. (4)) and related derivatives have proved very useful for generating a steady state flux of NO production under buffered physiological-like conditions. The rates of NO release from diazeniumdiolates range seconds to many hours depending on the nature of the alkyl functional groups on the amine and on solution pH and temperature [31], and these compounds are valuable tools for mimicking acute and chronic generation of NO. Polymer based diazeniumdiolates have been developed for potential therapeutic applications [28,32]. A large variety of diazeniumdiolates that generate NO with half-lives ranging from 2 s to 24 h at physiological pH and temperature are now commercially available, and these readily facilitate analysis of both acute and chronic effects of NO.



(4)

Another approach to NO delivery to physiological sites under investigation is the photochemical activation of "caged" NO. In this case, the use of light as a trigger allows one to control the timing, location and dosage of NO release. Various metal nitrosyls and nitrito complexes [33] as well as nitro-organic compounds [34] are being explored as potential photochemical NO precursors.

2.3. Electrochemical potentials.[3]

The MO diagram (Fig. 1) predicts that one-electron reduction to the nitroxyl anion, NO^- involves addition of an electron to a π-antibonding orbital. The lowest energy state of NO^-(the ground state) is isoelectronic to that of O_2 and has one electron in each π* orbital with parallel spins to give the triplet state ion $^3NO^-$.[4] If these two π* electrons instead have opposite spins, the result would be the higher energy singlet state $^1NO^-$.

The reduction of NO to the $^3NO^-$ anion is quite unfavorable (eq. 5) [35,36].[5]

$$NO + e^- \rightarrow {}^3NO^- \; E_{1/2}^\circ = -0.81 \text{ V (vs NHE)} \quad (5)$$

The spin state of NO^- is also quite important when considering protonation of this anion to give HNO, which is commonly called "nitroxyl" (the IUPAC name is "azanone" [2]). Like most molecules, the ground state of HNO is a singlet, meaning all electrons are spin paired and the molecule is diamagnetic. Protonation of NO^- thus requires a change in the electronic spin state (eq. (6)) [37,38].

$$^3NO^- + H^+ \rightleftarrows {}^1HNO \qquad pK_a \sim 11.4\text{(est.)} \quad (6)$$

The consequence of this spin change is that deprotonation of HNO and protonation of NO^- are exceedingly slow relative to the protonation/deprotonation steps of weak acids with similar pK_a's. Deprotonation of HNO is mediated by OH^- rather than by water (eq. (7)), so that the rate is pH-dependent [39].

$$\text{rate} = k_2[OH^-][HNO] \; k_2 = 5 \times 10^4 \text{ M}^{-1} \text{ s}^{-1} \quad (7)$$

The impact of such slow interconversion is that HNO and NO^- when formed *in situ* are each sufficiently long-lived to be treated as independent species rather simply in rapid equilibrium. (For a more complete discussion, see ref 16a). Despite these complexities, the high pK_a implies that HNO will be the predominant form under biological conditions.

A combination of experimental and theoretical studies [16,35] were required to estimate both the pK_a of HNO (eq. (6)) and the potential for reduction of NO to HNO (eq. (8)). The thermodynamic favorability of NO^- protonation of to HNO results in a positive shift when comparing eqs (5) and (8), but the potential is still unfavorable overall. While a variety of oxidants such as ferricyanide can convert HNO to NO, as with deprotonation, this proton coupled electron transfer process is predicted to be unusually slow due a large reorganization energy. Whether redox reactions involving the NO/HNO couple are impeded by a small thermodynamic driving force or by a large kinetic barrier, NO and HNO do not appear to readily interconvert *in vivo* [16a].

$$NO + H^+ + e^- \rightarrow {}^1HNO \; E^\circ = -0.5 \text{ V (est., pH 7.4)} \quad (8)$$

Although the one-electron oxidation of NO to the nitrosonium ion, NO^+ involves loss of the π-antibonding electron, this process is quite unfavorable as seen by the very positive reduction potential of NO^+ in aqueous solution (eq. (9)) [40a,b]. Thus, *NO is neither readily oxidized nor reduced by one-electron processes.*

$$NO^+ + e^- \rightarrow NO \; E^\circ = 1.45 \text{ V} \quad (9)$$

In aqueous solution the nitrosonium ion is rapidly hydrolyzed to nitrous acid HNO_2, the conjugate acid of nitrite ion NO_2^-, the nitrogens

[3] Unless otherwise noted, standard reduction potentials will be cited, meaning that the reaction conditions are 1 M reactants, 25 °C and either pH 14 or 0.

[4] The nomenclature arises from the multiplicity, $2S + 1$, where S = the total electron spin and equals the sum of the individual spins of each electron or Σm_s. The spin multiplicity can also be calculated by adding1 to the total number of unpaired electrons of the same spin.

[5] Notably, the one-electron reduction of O_2 to superoxide (O_2^-) is more favorable ($E^\circ = -0.33$ V at pH 7), such that the presence of O_2 may protect NO from consumption by certain reductants.

of these three species each being in the +3 oxidation state. In acidic solution, nitrous acid is also a strong oxidant as shown by the very positive potential for the half-cell described by eq. (10) [40a,b].

$$HNO_2 + H^+ + e^- \rightarrow NO + H_2O \; E^\circ = 0.99 \text{ V} \quad (10)$$

However, as noted already, the pH and protonation state of the nitrogen species often has a major impact on reduction potentials, and that for nitrite is especially pH-sensitive. Thus, in strongly alkaline solution, reduction of nitrite to NO is relatively unfavorable (eq. (11)) [40a,b].

$$NO_2^- + H_2O + e^- \rightarrow NO + 2\ OH^- \; E^\circ = -0.46 \text{ V} \quad (11)$$

Near physiological pH, the potential for reduction of nitrite to NO is intermediate between these extremes and is somewhat positive (0.37 V at pH 7.0).

In summary, NO is neither readily oxidized nor reduced by one-electron processes, and this feature is an important factor in maintaining a lifetime sufficiently long for NO to diffuse through cells. Nonetheless, the fundamental redox relationships of NO with NO^+ and NO^- as well as the pH-dependence of these interactions are central to the chemical biology of NO and will be explored in further detail in subsequent sections.[6]

2.4. Reactivity and kinetics

In this section, thermodynamic and kinetic parameters will be presented for key reactions of NO, since such quantitative information lays the groundwork for understanding the potentially complex processes that might be occurring in an organism. While the thermodynamic favorability is certainly the critical factor dictating feasibility, the rate of a reaction will define its importance under the conditions of interest. A thermodynamically favorable reaction may have a substantial kinetic barrier that makes the reaction very slow. An example is the protonation of $^3NO^-$ (eq. (6)), a favorable process (high pK_a) that is kinetically slow with a barrier attributed to nuclear reorganization requirements. As noted above, these are due to the different spin states of the nitroxyl anion and HNO [39]. In other words, one must consider the reaction mechanism, which also identifies potential intermediates that might have physiological roles.

Reaction rates depend on concentration as well as spatial and temporal factors. A first step in considering the propensity for potentially competing reactions is to compare rate constants. However, this process is only straightforward for reactions with the same order and thus having rate constants with the same units. If a reaction displays first order kinetics (an unimolecular reaction), such as decomposition through bond cleavage, the rate constant will be labeled here as k_1 with the units s^{-1}.[7] The lifetime (τ) of that reactant is defined as $(k_1)^{-1}$ under the conditions where k_1 was measured. A second order (bimolecular) process, for example, a reaction between two species where the concentration of each directly impacts the rate, will have a rate constant k_2 with the units $M^{-1}\ s^{-1}$. However, if the concentration of one of these reactants is much larger (≥10-fold) than the other, then the kinetics become effectively (pseudo) first order, and the observed rate constant, k_{obs}, is equal to the second order rate constant k_2 times the concentration of the excess reactant. Higher order reactions are possible but relatively rare, although the chemistry of NO incorporates several such examples. One of particular importance is NO autoxidation, the reaction of NO with O_2, which will be discussed below.

The rate of a reaction depends both on the rate constant, which is a function of the energetic barrier, and on the concentrations of the reactants. For example, a bimolecular reaction depleting NO, would give a rate law such as described in eq. (12) [8]:

$$\text{rate} = -\text{d[NO]/dt} = k_2\text{[NO][reactant]} \quad (12)$$

Published rate constants are typically measured in homogeneous solutions. When defining the relevance of a particular pathway *in vivo*, the heterogeneity of location, media, concentrations, etc. of the specific reaction site must be evaluated. Furthermore, it would be quite reasonable for a reactive species like NO to be depleted via competing pathways. In that case, the reactions will partition along those pathways according to the respective rate constants and concentrations of the relevant reactants.

2.4.1. Reactions of NO with superoxide and other free radicals

The free radical nature of NO leads to facile reactivity with other reactants having unpaired electrons, examples being redox active transition metals and other free radicals. While the reactions of NO with metals are typically regulatory in nature, interactions with other free radicals can lead to either protective or deleterious effects, depending on the reactant. Important reactions in this category are described in this section.

The rate at which two free radicals react in aqueous solution often approaches the diffusion limit for bimolecular reactions ($k_d \sim 10^{10}\ M^{-1}\ s^{-1}$ at 25 °C [41]), meaning that once the two species approach each other by diffusion, the probability of reaction is very high. The literature contains numerous examples of NO reactions with free radicals produced by pulse radiolysis techniques [42,43a,b]. Although these reactions may not necessarily have physiological relevance under normal conditions, they provide reference points for understanding such interactions. For example, the k_2 rate constants for NO reactions with the $(SCN)_2^-$, Br_2^-, and ethoxy (EtO•) radicals all fall in the range of 3–5 × 10^9 $M^{-1}\ s^{-1}$ [42].

A much more biologically relevant reaction is that with the superoxide anion O_2^-. Although reduction of O_2 to O_2^- is thermodynamically unfavorable (E° (O_2/O_2^-) = −0.33 V vs NHE [46]), this deleterious species is nonetheless a side-product of respiration. In addition, the immune system harnesses the cytotoxic effects of O_2^- in response to pathogen invasion. NO reacts rapidly and irreversibly with the superoxide ion radical O_2^- to form the peroxynitrite anion ($ONOO^-$, eq. (13)) with reported second order rate constants (4.3 × 10^9 $M^{-1}\ s^{-1}$ and 1.6× 10^{10} $M^{-1}\ s^{-1}$), that approach the diffusion limit [43a,b]. In aqueous solution this rate constant proved to be independent of ionic strength and of pH over the range 6.1–10.

$$O_2^- + NO \rightarrow ONOO^- \quad (13)$$

The physiological impact of the reaction depicted in eq. (13) has received considerable attention and has been the subject of much controversy given the toxicity of O_2^- and the potential roles of $ONOO^-$ in a variety of diseases. On one side, it has been suggested that removal of O_2^- by reaction with NO is protective in nature [44]. However, others have argued that the peroxynitrite ion facilitates harmful chemical modifications different from those triggered by either NO or O_2^- [45,46]. The outcome will depend strongly on location, timing and concentration of formation. Given the numerous pathways for depleting NO and O_2^-, these species must be produced in close temporal and spatial proximity for the reaction to give $ONOO^-$ to be physiologically significant. This is the case during inflammation, indicating that $ONOO^-$ formation is most likely to be important in close proximity to immune cells and within mitochondria.

Lipid and carbon-centered radicals are also formed as the result of oxidative stress as well as of metabolism [47]. Lipid peroxidation, for

[6] NO^- and NO^+ are sometimes incorrectly referred to as different forms of NO. While these ions are related to NO by a simple gain or loss of an electron, each species is chemically unique.

[7] Rate constants are for 25 °C unless otherwise noted.

[8] In a simple rate law such as described by eq. (12), the molar concentration of a reactant in solution is usually denoted by brackets, [ ].

example, can occur as a function of oxyradical formation during inflammation [48–50]. Such modifications can ultimately lead to cell membrane compromise through perpetuation of lipid oxidation. Not surprisingly, NO reacts with organic peroxyl radicals ($ROO^{\bullet}$) (eq. (14)) with near diffusion limited k_2 values of 1–3 × 10^9 M^{-1} s^{-1} to form organic peroxynitrite intermediates [51].

$$ROO^{\bullet} + NO \rightarrow ROONO \quad (14)$$

Again, trapping of such radicals by NO may be protective in nature due to termination of the radical chain reactions involved in peroxidation of lipids [52,53], but the resulting peroxynitrite derivatives ROONO, may themselves decay to the reactive radicals $RO^{\bullet}$ and NO_2 [51].

The efficacy of radiation therapy depends on O_2 trapping of radicals induced by γ-radiation, since the resulting peroxo species are not as susceptible to natural repair mechanisms. The tendency of solid tumors to have hypoxic interiors is, thus, often a barrier to effective radiotherapy. Long-standing interest in developing drugs that enhance the effectiveness of γ-radiation in cancer treatment [54] led to analysis of the ability of NO to function as a sensitizer that may enhance radiation damage to targeted tissues [55] by the rapid reaction with radiation generated radicals. Furthermore, the vasodilatory properties of NO at nanomolar concentrations may serve to increase tissue oxygenation, and in this way also to enhance the radiation sensitivity of the targets.

NO also reacts with tyrosyl and tryptophan radicals in amino acids, peptides and proteins with very large second order rate constants (k_2 = 1-2 × 10^9 M^{-1} s^{-1}) [56]. This reactivity carries over even to the catalytically competent and well-protected tyrosine radicals produced during turnover of ribonucleotide reductase [57]. The adduct formed is no longer active, although it is unstable and can regenerate the tyrosine radical.

The very fast reactions of NO with NO_2 to form N_2O_3 (eq. (15), k_2 = 1.1 × 10^9 M^{-1} s^{-1}) [58] and of NO with thiyl radicals ($RS^{\bullet}$) to produce *S*-nitrosothiols RSNO (eq. (16), k_2 = 2-3 × 10^9 M^{-1} s^{-1} for the glutathione radical $GS^{\bullet}$) [59] are of considerable biological relevance. These reactions will be discussed further below.

$$NO_2 + NO \rightleftarrows N_2O_3 \quad (15)$$

$$RS^{\bullet} + NO \rightarrow RSNO \quad (16)$$

Although NO does react with another NO to form a dimer, the equilibrium constant K_D for this dimerization (eq. (17)) is quite small at ambient temperature owing to the very weak N–N bond (dissociation energy of ~8 kJ/mol) [60]. Thus, the dimer is typically observed only at very high NO pressure or very low temperature. Nonetheless, it has been characterized structurally [61] and has been the subject of several computational analyses [62,63]. The dimer is an acyclic, nearly planar, *cis* configuration with O═N–N bond angles of 99.6°. The N–O bond lengths in the dimer (1.161 Å) are nearly the same as in the NO monomer (1.154 Å) while the N–N bond is long (2.237 Å) [61] consistent with its very small dissociation energy. It has been suggested that aromatic solvents may stabilize the dimer (63), but to our knowledge this has not been demonstrated experimentally.

$$2NO \rightleftarrows ONNO \quad (17)$$

2.4.2. NO autoxidation

Autoxidation is the oxidation of a species by O_2. Atmospheric NO is well known to undergo autoxidation to NO_2 (eq. (18)), which leads to the characteristic brown color of smog. Since NO also reacts rapidly with nitrogen dioxide to give N_2O_3 (eq. (15)), these three nitrogen oxide species will be in equilibrium. The gas phase autoxidation of NO is often cited in chemistry texts as a rare example of an elementary ternary reaction [64], but, in fact, the mechanistic details of the interaction of these reactants to form NO_2 are not fully elucidated. An analogous autoxidation pathway and resulting equilibria of NO, NO_2 and N_2O_3 are also valid in aprotic solvents such as carbon tetrachloride [25,65].

$$2\ NO + O_2 \rightarrow 2\ NO_2 \quad (18)$$

In aqueous media the products are different; NO autoxidation leads instead to the formation of nitrite, the general stoichiometry being shown in eq. (19) [66].

$$4\ NO + O_2 + 2H_2O \rightarrow 4\ H^+ + 4\ NO_2^- \quad (19)$$

The reaction is irreversible and provides a measurable end product that aids analysis. Two independent kinetics studies in 1993 [25,67,68] demonstrated that, although the reaction in aqueous solution gives nitrite instead of nitrogen dioxide, the rate law (eq. (20)) remains third order.

$$\frac{d[NO_2^-]}{dt} = 4\ k_{aq}[NO]^2[O2] \quad (20)$$

Stopped flow kinetics analysis[9] determined a third order rate constant of k_{aq} ~2 × 10^6 M^{-2} s^{-1} at 25 °C [67,68] and that the reaction rate is relatively independent of both the solution pH and the temperature. Several subsequent studies of this reaction under analogous conditions confirmed the form of the rate law and determined similar rate constants (the reported k_{aq} values in aqueous media are in general agreement) [69–72]. However, there are, as yet, unresolved issues regarding the nature of the reactive intermediates formed during NO autoxidation (see below).

The discovery that NO is a regulatory agent in the cardiovascular system [3–5] occurred nearly concurrent with the discovery that NO elicits cytotoxicity during immune response to pathogens [73,74]. These seemingly incongruent effects of NO raises the obvious question of how NO can be essential or beneficial in certain functions yet be toxic in others. The answer is in part evident from a fundamental toxicological/pharmacological perspective in which dose response curves define the concentration ranges for deficiency, normal function and toxicity. Also crucial are the kinetics of the competing pathways depleting NO. The autoxidation reaction serves as a prime example of how both timing and concentration are critical factors in defining the effects of NO biosynthesis.

For example, if NO is consumed by autoxidation, how can it function as a signaling agent in normoxic arterial tissues? The answer lies in the third order kinetics in aqueous media, first order in [O_2] and second order in [NO] (eq. (20)). Consequently, the rate of the reaction is especially sensitive to the NO concentration. The lifetime (τ_{NO}, ~ $\tau_{1/2}$) of NO consumption by autoxidation can be approximated by assuming that under normoxic conditions the O_2 is in large stoichiometric excess. Under those conditions, τ_{NO} can be approximated from the relationship:

$$\tau_{NO} = (4k_{aq}[O_2][NO])^{-1} \quad (21)$$

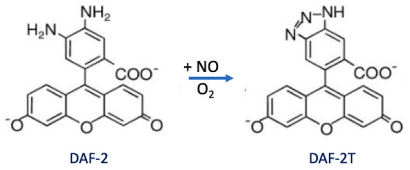
The solubility of oxygen in water is 1.3 mM/atm at 25 °C [21], and in an air saturated aqueous solution, [O_2] ~ 260 μM. Thus, using eq. (21) gives a calculated τ_{NO} for an aerobic aqueous solution initially 10 nM in NO of ~13 h, but τ_{NO} is only ~1 min for a 10 μM NO solution under ambient conditions. Notably, both calculated lifetimes would be longer under endogenous normoxic conditions since the O_2 concentrations in the fluids and cells are significantly lower than the value stated above [75].

Thus, at the l-10 nM concentrations relevant to bioregulatory processes such as blood pressure maintenance and neurotransmission [76], NO autoxidation would be very minor relative to other depletion pathways, such as diffusion into the blood stream and consumption by

[9] The stopped flow method involves rapid mixing of two (or more) solutions in a spectrophotometer cell while monitoring the temporal absorption changes once the solutions stop. flowing. Commercial stopped-flow spectrometers generally have a time resolution of 1–2 ms.

oxyhemoglobin. In other words, the effective lifetime of NO under bioregulatory conditions allows reactions with targets such as soluble-guanylyl cyclase ($k_2 = 1.4 \times 10^8\ M^{-1}\ s^{-1}$) [7a,77] a key step in blood pressure regulation. In contrast, at higher concentrations, for example, during immune response, NO autoxidation becomes increasingly feasible kinetically. Thus, the third order kinetics behavior for NO autoxidation determines how this reactive molecule can play contrasting roles in oxygenated media, bioregulatory at low concentrations, but potentially cytotoxic when generated at higher concentrations in response to pathogen invasion [78,79].

Notably, NO and O_2 are more soluble in hydrophobic media such as lipids, and as a consequence, both tend to partition into such cellular regions. Thus, NO autoxidation may preferentially occur in lipid membranes rather than the cytoplasm, producing NO_2 and N_2O_3 rather than nitrite [80]. In fact, this reactivity is often used as indirect marker of NO in that hydrophobic dyes such as 2,3-diaminofluorescein (DAF-2) are converted to the fluorescent triazole following autoxidation of NO to N_2O_3 (eq. (22)) [81].



(22)

Conversion of NO into more reactive nitrogen species (RNS) has been implicated in pathophysiological effects [12,13]. In particular, both NO_2 and N_2O_3 can induce oxidative, nitrative (i.e., donation of NO_2^+) and nitrosative (i.e., donation of NO^+) modifications that are not directly accessible to NO. Nitration of tyrosine residues is enhanced in many neurodegenerative diseases [82] while nitrosation of DNA bases can lead to mutations and tumor development [78,83]. As a result, there is much interest in the role of nitrosative stress as an etiological factor for many diseases. Altogether, the second order dependence of autoxidation on NO concentration explains the dichotomy between the beneficial role of NO as a signaling agent in the cardiovascular system and as a toxic agent formed in response to infection.

While it is clear that the reactive intermediates formed during the autoxidation of aqueous NO have cytotoxic and mutagenic activities during immune response, these have not been well defined. The autoxidation mechanism is commonly assumed to parallel the gas-phase reaction, where the rate-limiting step produces NO_2, which is trapped by NO to give N_2O_3. Hydration of the latter would give nitrous acid. Wink et al. [67] attempted to identify the key autoxidation intermediates by examining relative reactivities with trapping agents such as ferrocyanide, 2-2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), azide and GSH. Comparing the results of competition studies with reported reactivities, led to the conclusion that the relevant oxidant generated during aqueous NO oxidation is not NO_2. Kinetics simulations attempted to model these results using the published rate constants for NO_2 reactions with such trapping agents came to a similar conclusion [84], although an alternative mechanism was not proposed.

2.4.3. Other reactions of NO not mediated by metal centers

Unlike more aggressive radicals, NO does not participate in H-atom abstraction from compounds having C–H, O–H, S–H or N–H bonds (eq. (23)). Such H-atom abstraction is thermodynamically unfavorable owing to the relatively weak H–NO bond (~197 kJ/mol [85]). As a result, NO tends to terminate, rather than initiate or propagate radical chain reactions.

$$R\text{-}H + {\bullet}NO \not\rightarrow R{\bullet} + HNO \quad (23)$$

There are known reactions that incorporate two molecules of NO (bimolecular in NO). An important example of such a reaction is the synthesis of the diazeniumdiolates or NONOates (eq. (24)) [86].

$$2\ RR'NH + 2NO \rightleftarrows RR'NN(O)N(O)^- + RR'NH_2^+ \quad (24)$$

Preparation of NONOates relies on use of high NO pressures and the precipitation of the product as an alkylammonium salt from the organic solvent. Two pathways were proposed for NONOate formation [86]. One involves NO addition to an amide ion formed by auto-ionization of the amine to produce a radical anion intermediate (eqs. (25) and (26)) that is then trapped by a second NO (eq. (27)).

$$2\ RR'NH \rightleftarrows RR'N^- + RR'NH_2^+ \quad (25)$$

$$RR'N^- + NO \rightleftarrows RR'N(NO^{\cdot})^- \quad (26)$$

$$RR'N(NO^{\cdot})^- + NO \rightleftarrows RR'NN(O)N(O)^- \quad (27)$$

An alternative pathway would be for the amide ion react directly with an NO dimer (eq. (28)). Although the equilibrium constant (K_D) for dimer formation (eq. (17)) is quite small, computational studies [63] argue the N_2O_2 is considerably more electrophilic than is NO itself, so the enhanced reactivity in the second step (eq. (28)) may compensate for the small K_D.

$$RR'N^- + N_2O_2 \rightleftarrows RR'NN(O)N(O)^- \quad (28)$$

Triaryl phosphines (Ar_3P) also react with two molecules of NO, although in this case, nitrous oxide and phosphine oxide are formed (eq. (29)), rather than a stabilized NONOate type product.

$$Ar_3P + 2\ NO \rightarrow Ar_3PO + N_2O \quad (29)$$

This reaction proved more amenable to kinetics analysis than the high pressure reaction of NO with amines, The rate law is third order, first order in phosphine and second order in NO (eq. (30)) [87]. The rate constant k_3 is strongly dependent on the nature of the aryl group Ar–, increasing with increasing electron donor character. The reaction also proved to be faster in more polar solvents [87,88].

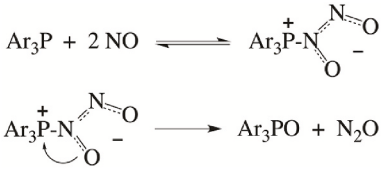
$$\frac{d[Ar_3PO]}{dt} = k_3[NO]^2[Ar_3P] \quad (30)$$

Again, there are several mechanisms that are consistent with this third order rate law. The sensitivity to solvent polarity and to the basicity of Ar_3P suggests that the oxidation proceeds through the reversible, stepwise formation of a NONOate-type adduct followed by rate limiting intramolecular oxygen atom transfer as shown in Scheme 1 [87]. If the equilibrium constant for formation of this adduct is small, as would be expected for the relatively weak Ar_3P base, the reaction would display third order kinetics.

An alternative mechanism that is also consistent with this rate law involves reaction of N_2O_2 with Ar_3P, either by a concerted oxygen atom transfer to give $Ar_3P(O)$ plus nitrous oxide or by formation of the zwitterionic intermediate $Ar_3P(N_2O_2)$ complex followed by decay to products in reactions equivalent to the last step in Scheme 1. The rate law does not differentiate these pathways: however, the computational study indicating the electrophilicity of the NO dimer [63] suggests that the latter mechanism may indeed be viable.

3. The reactivity of other nitrogen oxides

One of the key characteristics of nitrogen as an element is the large



Scheme 1. Possible mechanism for the NO oxidation of a triaryl phosphine.

range of oxidation states that occur in stable compounds [17]. Table 1 lists some simple compounds illustrating the range of the formal oxidation states of nitrogen from −3 to +5. Interconversion between these oxidation states is critical in the nitrogen cycle as well as in NO-dependent redox signaling.

This section describes the properties, structures and chemistry of nitrogen oxides as a function of the oxidation state of nitrogen and in the context primarily of the chemical biology of NO in mammalian systems. Since NO is neither readily reduced nor oxidized by simple one-electron processes, both the production of NO and the conversion of NO into other nitrogen oxides is typically driven by metalloenzymes or by reactions with other free radicals or O_2. Nitrogen-containing species that are more reduced than NO are often precursors of NO. The most common process for biosynthesis of NO begins with two-electron oxidation by nitric oxide synthase (NOS) of L-arginine to N^{ω}-hydroxy-L-arginine (NOHA), converting a guanidino nitrogen atom from the oxidation state of an amine (−3) to that of a hydroxylamine (−1). this is followed by a three-electron oxidation resulting in release of NO (+2) [1]. The primary end-products of mammalian NO biology are nitrite (+3) and nitrate (+5), so the eventual fate of NO *in vivo* is further oxidation.

3.1. Oxidation state +1: HNO, NO^- and N_2O

As noted above, the slow rate of proton transfer dictates that NO^- and HNO should effectively function independently, although of the two, HNO is expected to be the major species under physiological conditions. While there has been considerable speculation about endogenous production of nitroxyl, there are known and emerging pharmacological applications of exogenously applied HNO in treatments of alcoholism [89], sickle cell disease [90], heart failure [91], cancer [92] and pain [93]. Such studies have established that HNO can be produced by metabolic pathways [94]. The chemistry and potential biological properties of HNO have been the subject of a number of reviews, examples of which are presented in ref. [16].

Of potential biosynthetic pathways for HNO production [95], perhaps the most intriguing is by NOS under hypoxic or low cofactor conditions or by oxidative degradation of the intermediate NOHA [96]. Rather than a three-electron oxidation needed to generate NO from NOHA, HNO production would involve a two-electron oxidation of that intermediate. The possibility of an O_2-dependent trigger has interesting signaling ramifications. For instance, Colton and colleagues [97] showed that HNO and NO uniquely impact the *N*-methyl-D-aspartate (NMDA) receptor in neurons under hypoxia. NO-mediated channel activation is prolonged by low O_2 levels, resulting in enhanced neuronal toxicity. In contrast, hypoxia attenuates the HNO response, potentially offering a protective mechanism toward ischemia/reperfusion injury in the brain.

Multiple comparisons of NO or HNO have shown that in non-vasoactive assays the responses to these two nitrogen oxides are generally discrete, due to distinct chemical modifications [16]. HNO is in many cases more reactive than NO. For example, it undergoes rapid dimerization, forming hyponitrous acid, which then dehydrates irreversibly to nitrous oxide (eq. (31)). This is a major sink for HNO, and the high second order rate constant of self-consumption ($k_2 = 8 \times 10^6$ M^{-1} s^{-1} in ambient aqueous solution) [38] precludes the storage of HNO. The N_2O produced has often been cited as an indirect marker of HNO formation.

$$2\ HNO \rightarrow [HONNOH] \rightarrow N_2O + H_2O \quad (31)$$

In addition to dimerization, HNO can be consumed in a cellular environment by a number of molecular species [16,98]. In particular, reaction with a oxidizing metal center will convert HNO into NO (eq. (32)). HNO and NO can also react with each other at a near diffusion-limited rate to give the radical HN_2O_2 [39], which may provide a rapid mechanism to switch from HNO to NO signaling.

$$M^{n+} + HNO \rightarrow M^{(n-1)} + NO + H^+ \quad (32)$$

An important characteristic for HNO in terms of biological signaling is its electrophilicity.[10] Although HNO does not react appreciably with water, it is electrophilic toward Lewis bases such as thiols, amines, and phosphines. Given the high intracellular concentration of glutathione (0.5–10 mM [99]), the reaction of HNO with GSH is of particular importance. The second order rate constant for this reaction is high ($k_2 = 2 \times 10^6$ M^{-1} s^{-1}) [100]. The initial product is an *N*-hydroxy-sulfenamide (eq. (33)) [100,101], and this species can either isomerize to a more stable species (eq. (34)) or react with a second thiol (eq. (35)) to give GSSG and hydroxylamine.

$$HNO + GSH \rightarrow GSNHOH \quad (33)$$

$$GSNHOH \rightarrow GS(O)NH_2 \quad (34)$$

$$GSNHOH + GSH \rightarrow GSSG + NH_2OH \quad (35)$$

Eq. (35) results in a reversible modification while sulfinamide formation is currently assumed to be irreversible. $GS(O)NH_2$ formation is also unique to HNO [101], thus providing a specific marker. This product has been used to quantitatively detect HNO and can be visualized from cell lysates.

Given the kinetic parameters, consumption by GSH would be expected to limit the lifetime of HNO to milliseconds. However, cysteine residues in cellular proteins can be modified by HNO [102], suggesting that GSH may not be as effective a scavenger as predicted. As with NO, partitioning into cell membranes or proteins may play a role.

HNO also reacts with phosphines to give the analogous phosphine oxide and the HNO-specific phosphine aza-ylide products in equal concentrations (eq. (36)). These products presumably result from the initial formation of an HNO/R_3P adduct that then reacts with a second phosphine [103]. As with sulfinamides, the aza-ylide has been utilized for specific detection of HNO. The kinetics of this reaction have yet to be reported.

$$HNO + 2\ R_3P \rightarrow R_3P{=}NH + R_3P{=}O \quad (36)$$

Both HNO and its conjugate base NO^- react with O_2. The latter reaction is isoelectronic to that of NO with O_2^- (eq. (12)) and produces the peroxynitrite ion (eq. (37)) at a rate close to the diffusion limit ($k_2 = 2.7 \times 10^9$ M^{-1} s^{-1}). Both reactants are triplets, so this is a spin allowed reaction [38,39]. Thus, if NO^- were formed in normoxic tissue, it would be rapidly consumed by this process.

$$NO^- + O_2 \rightarrow ONOO^- \quad (37)$$

HNO reacts with O_2 much more slowly with a reported second order rate constant in aqueous media of $\sim 10^3$ M^{-1} s^{-1} [104], perhaps as the result of spin restrictions. The identity of the product has proved elusive, but it is not peroxynitrite. The HNO/O_2 product is cytotoxic, able to induce double strand DNA breaks, but given other faster pathways that deplete HNO, this product is not likely to be highly significant physiologically.

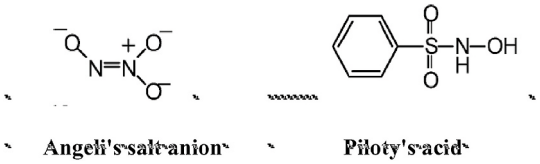
While donor systems for NO are experimentally convenient, they are mandatory for HNO, which cannot be stored. Several classes of HNO donors have been developed [105–108], with the most common being diazeniumdiolates such as Angeli's salt and hydroxylamines such as Piloty's acid (Scheme 2).

Angeli's salt ($Na_2N_2O_3$) [108] is the most frequently used HNO donor and is an oxide-rather than amine-based diazeniumdiolate. HNO formation from Angeli's dianion occurs through protonation ($pK_a(2) = 9.7$) [109] followed by dissociation of HNO (eq. (38)) at a first order rate ($k_1 = 6.8 \times 10^{-4}$ s^{-1} at 25 °C; 4–5 × 10^{-3} s^{-1} at 37 °C) that is effectively pH-independent over the range 4–8.

[10] The conjugate base, NO^-, in contrast is a nucleophile.

Table 1
Representative simple species with different formal oxidation states of nitrogen.

N(-III)	N(-II)	N(-I)	N(0)	N(I)	N(II)	N(III)	N(IV)	N(V)
NH_3 (ammonia)	N_2H_4 (hydrazine)	H_2NOH (hydroxyl amine)	N_2 (dinitrogen)	HNO (nitroxyl)	NO (nitric oxide)	NO_2^- (nitrite)	NO_2 (nitrogen dioxide)	NO_3^- (nitrate)
				N_2O (nitrous oxide)		NO^+ (nitrosonium ion)		



Scheme 2. Two common HNO donors.

$$N_2O_3^{2-} + H^+ \rightleftarrows HN_2O_3^- \rightarrow HNO + NO_2^- \quad (38)$$

An alternative HNO source is Piloty's acid ($PhS(O)_2NHOH$, Ph = phenyl). In this case, deprotonation (pK_a = 9.29) gives the conjugate base, which dissociates to give HNO (eq. (39)) and a sulfonate anion (at pH 13, $k_1 = 4.2 \times 10^{-4}$ s^{-1} at 25 °C and 1.8×10^{-3} s^{-1} at 35 °C) [110]. One complication is that Piloty's acid is readily oxidized to the corresponding nitroxide, which releases NO rather than HNO.

$$PhS(O)_2NHOH + OH^- \rightleftarrows PhS(O)_2NHO^- \rightarrow PhSO_2^- + HNO \quad (39)$$

Decomposition of Angeli's salt generates HNO and a stoichiometric equivalent of nitrite. Given the growing interest in both HNO and nitrite in terms of potential therapeutic effects, it is necessary to evaluate the effects of these species separately. This need has led to development of other HNO donors for study and possible therapeutic applications [111]. For example, primary amine diazeniumdiolates ($RN(H)N(O)NO^-$) have been found to function as HNO rather than NO donors [112]. Other approaches to HNO donors are *N*-hydroxyurea-derived acyl nitroso compounds [113], which release HNO via a thermal retro-Diels-Alder decomposition.

Nitrous oxide (N_2O), a colorless gas (bp −88.5 °C), is a linear molecule that is isoelectronic and structurally analogous to carbon dioxide. It is an atmospheric greenhouse gas and also affects stratospheric ozone concentrations [114]. Its use as an anesthetic and as a recreational drug is well known, although the mechanisms for these effects are not well understood. In addition to its formation by HNO dimerization (eq. (31)) and by NO disproportionation (eq. (1)), N_2O is a byproduct of industrial activity such as the manufacture of nylon [114] and is generated by natural processes such as bacterial NO reductase activity [115]. Although N_2O is often considered to be relatively unreactive, it will undergo oxygen atom transfer reactions to other substrates owing to the high stability of the N_2 product. N_2O is known to inactivate cobalamins (e.g., vitamin B_{12}) by oxidation [116], but is otherwise not considered to be a biologically significant nitrogen oxide.

3.2. Oxidation state +3: NO^+, NO_2^-, N_2O_3 and N-Nitrosoamines

The chemistry of this oxidation state is dominated by nitrosative modifications in which NO^+ is formally donated to a nucleophile. Under physiological conditions, NO^+ itself is at best a transient species since the one-electron reduction to NO is so highly favorable (eq. (7)) and NO^+ reacts rapidly with water to form nitrous acid (eq. (40)).

$$NO^+ + H_2O \rightleftarrows HNO_2 + H^+ \quad (40)$$

The equilibrium constant for eq. (40) has been estimated at 0.7×10^8 M [117,118], such that NO^+ is a prominent species only in highly concentrated acid (e.g., 60% sulfuric acid). Under such conditions, the nitrosonium ion has been characterized spectroscopically, and nitrosonium salts of the type $(NO^+)X^-$ have been isolated. However, it should be emphasized that the identification of NO^+ in concentrated sulfuric acid solutions is possible *only* because the activity of the acid is so high and that of H_2O is so low under these conditions.

Nitrous acid is a relatively weak acid (eq. (41)). The most recent reevaluation gave the pK_a of HNO_2 as 3.16 at 25 °C (3.11 at 37 °C) [119].[11] Therefore, at physiological pH, the conjugate base nitrite ion is ~99.99% of the total distribution. The equilibrium constants noted above can be used to estimate the concentration of NO^+ in a 1 mM NO_2^- solution at pH 6 to be $\sim 10^{-22}$ M. Thus, despite occasional suggestions in the literature that free NO^+ is participating in nitrosation reactions, the direct involvement of NO^+ seems unlikely to have mechanistic validity in an aqueous environment, except in concentrated acids.

$$HNO_2 \rightleftarrows H^+ + NO_2^- \quad (41)$$

Nonetheless, solutions of nitrite in dilute aqueous acid are well-known to promote nitrosation. Currently, there are several scenarios under consideration for this chemistry [117]. The first involves a protonated nitrous acid, $H_2NO_2^+$, while the second involves dehydration of HNO_2 to produce the powerful nitrosating reagent N_2O_3 (eq. (42)).

$$2\ HNO_2 \rightleftarrows\ H_2O + N_2O_3 \quad (42)$$

The most accepted value for the equilibrium constant K_{N2O3} of eq. (42) is 3×10^{-3} M^{-1} as measured in dilute acidic aqueous solutions (0.1 M HCl) at 22 °C [120]. The N_2O_3 anhydride of nitrous acid can dissociate to NO and NO_2 (eq. (43)). The net result of eqs. (42) and (43) is the acid promoted disproportionation of nitrite to nitric oxide and nitrogen dioxide.

$$N_2O_3 \underset{kb}{\overset{kf}{\rightleftarrows}} NO + NO_2 \quad (43)$$

The equilibrium constant for eq. (43) in ambient aqueous solution is 2×10^{-5} M [121]. Since $K = k_f/k_b$ and given that the back reaction has a rate constant k_b of 1.1×10^9 M^{-1} s^{-1}, k_f, the first order rate constant for N_2O_3 dissociation would be 1.8×10^4 s^{-1}. Therefore, acid promoted nitrite disproportionation has been suggested to be a viable pathway toward NO formation from NO_2^-, especially in the acidic fluids of the stomach [14]. In fact, there has long been a concern that nitrate and nitrite in the diet promotes colorectal and other cancers owing to amine nitrosation to give *N*-nitroso compounds (NOCs) [122]. The type of amine impacts the outcome in that secondary amines are often stably nitrosated, while *N*-nitrosated primary amines undergo facile deamination (see Ref. [123]).

The rate of NO production and the NO concentration in equilibrium with dissolved NO_2^- is a complex function of pH and nitrite concentration. For example, at equilibrium, a 10 μM NO_2^- solution at pH 6 would contain ~5 nM NO and an equal concentration of NO_2, *if there are no other pathways depleting one or the other of these species.* At pH 7.4 this number drops to ~200 pM. However, these are dynamic equilibria, so if for example NO_2 were removed by a trapping agent, higher concentrations of NO would result (Le Chatelier's principle). Thus, in evaluating the potential biological impact of HNO_2 disproportionation on steady state concentrations of NO, one must consider not only these equilibria,

[11] This is a somewhat lower value than that listed in the standard chemistry handbook (3.37 at 13 °C) [21].

but also the holistic dynamics of all individual processes that deplete or enhance the concentrations of the key species involved.

N_2O_3 not only is a potential source of NO and NO_2 from nitrite disproportionation, but also is a powerful nitrosating reagent, hence it is a RNS in its own right. Again, the word "nitrosation" represents a chemical reaction where (formally) NO^+ is added to a nucleophile (X^-) or replaces an H^+ of a compound XH. Given the extremely short lifetime of NO^+ under biological conditions, NO^+ must be directly transferred in nitrosation reactions, rather than released and captured in separate steps. A particularly valuable resource on this topic is a monograph by Williams [117].

Biologically, the two most important targets for N_2O_3 would be thiols and amines (eqs. (44) and (45)), and it is likely that nitrosation by N_2O_3 occurs by direct transfer of NO^+ to a nucleophile from N_2O_3. However, it should be emphasized that, in addition to such metal-free nitrosation processes, NO in the presence of a redox active metal center will also nitrosate such nucleophiles [124].

$$N_2O_3 + RSH \rightarrow RSNO + H^+ + NO_2^- \quad (44)$$

$$N_2O_3 + RR'NH \rightarrow RR'N(NO) + H^+ + NO_2^- \quad (45)$$

When initiated by nitrite, the kinetics for nitrosation of nucleophiles such as amines and thiols follow the rate law described in eq. (46) [117]. The second order dependence on nitrous acid concentration is interpreted as reflecting the formation of N_2O_3 according to eq. (42). According to this interpretation, $k' = k_X \times K_{N2O3}$, with k_X being the rate constant for the direct reaction of N_2O_3 with X^- and K_{N2O3} being the equilibrium constant for eq. (42) (3×10^{-3} M^{-1}). The value of k_X depends on the identity of the nucleophile. For amines, k_X is in the 10^7-10^9 $M^{-1}s^{-1}$ range [125] while values ranging from 3×10^5 to 7×10^7 M^{-1} s^{-1} have been reported for GSH [126]. Although the values for k_x are large, one must keep in mind that the equilibrium concentration of HNO_2 ($[HNO_2] = K_a^{-1}[NO_2^-][H^+]$) is very low at physiological pH. For example, in a 1 mM nitrite solution at pH 7, the concentration of HNO_2 would be $< 10^{-7}$ M, so significant nitrosation promoted by equilibrium concentrations of N_2O_3 formed by nitrite disproportionation would not be expected near neutral pH.[12] On the other hand, N_2O_3 generated transiently in a chemical or biological process could contribute to nucleophile nitrosation pathways occurring in competition with hydrolysis to nitrous acid (see below).

$$\text{rate} = k' [HNO_2]^2[X^-] \quad (46)$$

Addition of NO to aerated solutions containing such nucleophiles can also lead to nitrosation and this can be attributed to the generation of N_2O_3 intermediates formed during NO autoxidation [25,67–72]. For example, alkyl nitrites dissolved in aerated organic solvents are formed when alcohols are treated with NO [127]. Such reactions of N_2O_3 generated by NO autoxidation are likely to be a major source of amine and thiol nitrosations and subsequent transformations during immune response (78, 83, 128–130). Furthermore, since biological experiments with endogenously generated or exogenously added NO are typically carried out under normoxic conditions, it is important to consider nitrosation as a possible outcome. Hydrolysis of N_2O_3 to nitrous acid (eq. (47), the reverse of eq. (42)) occurs with a rate constant of 2×10^4 s^{-1} [121], so a lifetime of ~50 μs might be expected for N_2O_3 that is generated in aqueous solution. The time frame is likely to be much longer in a hydrophobic cellular environment where the activity of water would be much lower. Thus, nitrosation of thiols and amines by NO autoxidation may have biological relevance particularly under conditions where the concentration of NO is high enough that autoxidation is a significant sink for NO.

$$N_2O_3 + H_2O \rightarrow 2H^+ + 2\ NO_2^- \quad (47)$$

An interesting feature of N_2O_3 is that it has several isomers that may be chemically important (131,132) (Scheme 3). Computational studies indicate that the asymmetric isomer ON-NO_2 is the most stable, but the other two species depicted are only slightly less so, and the three can interchange readily. Each isomer has been argued to be capable of nitrosation reactions (132), although it is quite possible that the selectivities might differ, as has been suggested (130).

Amine nitrosations have long been of interest owing to concerns about the potentially carcinogenic character of *N*-nitrosoamines when meats are preserved by adding nitrite or nitrate salts [122]. Such *N*-nitrosation can also be mutagenic, for example upon deamination of nitrosated exocyclic primary amines in DNA [133]. Again, *N*-nitrosoamines are most likely formed endogenously under conditions of immune response or in the acid conditions of the stomach. A recent development was the findings in 2018 and 2019 that certain commonly used medications contained *N*-nitrosodimethylamine (NDMA) and related nitrosoamines as impurities at levels sometimes significantly exceeding the Federal Drug Administration limits. The likely sources of this impurity lay in changes to the manufacturing protocols (134).

3.3. *S*-nitrosothiols

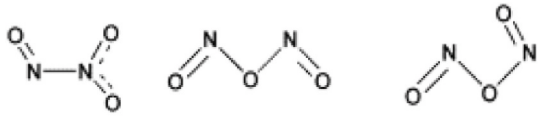
RSNOs have long been part of the overall story of nitric oxide physiology [135], and there is continuing attention to the roles of these species, especially *S*-nitrosated proteins, in biological signaling [136–138]. For example, *S*-nitrosothiols play roles in the cross-talk between the bioregulatory roles of NO and of H_2S and persulfides [139–143]. These topics are too large to cover adequately within the scope of the present review, so the focus here will be largely on the formation and fundamental chemical properties of such species.

Although *S*-nitrosothiols are decomposed readily by traces of metals, especially copper [144], they are stable in aqueous media at physiological temperatures and pH in the absence of such catalysts. Thiol (RSH) nitrosation is formally the replacement of H^+ by NO^+. However, theoretical analysis suggests that the predominant resonance structure is the neutral species R-S-N=O with smaller contributions from the ($R\text{-}S^-$) (NO^+) and ($R\text{–}S^+\text{=N–}O^-$) resonance forms (145). The ionic contribution elongates and weakens the S–N bond while the partial double bond character from the zwitterionic form somewhat restricts rotation about the S–N bond such that both the cis (syn) and trans (anti) forms (Scheme 4) can be considered separate, although easily converted, isomers.

Pure RSNOs are often colored owing to weak $n_N \rightarrow \pi^*$ transitions in the 550–600 nm range, and this leads to photochemical instability to visible light [146,147]. In solution, the photochemical pathway leads to formation of NO and the disulfide via homolytic cleavage of the RS-NO bond to give NO and the thiyl radical (eq. (48)), which subsequently dimerizes. This photoreaction has been used to liberate NO from a RSNO functionalized silica xerogel film [148], and analogous photolability of the RS-NO bond is one likely source of NO release upon the irradiation of various tissues with light [149,150].

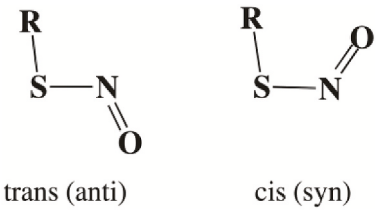
$$RS\text{-}NO + h\nu \rightarrow RS^\bullet + NO \rightarrow \tfrac{1}{2}\ RSSR + NO \quad (48)$$

The thermochemical bond dissociation energy of the RS-NO is



Scheme 3. Three different N_2O_3 isomers: (1) *asym*-N_2O_3. (2) *sym*-N_2O_3. (3) *cis-trans*-N_2O_3 [132].

[12] At near neutral pH, the equilibrium concentration of N_2O_3 would be $K_{N2O3}[HNO_2]^2 = K_{N2O3}\ K_a^{-2}[NO_2^-]^2[H^+]^2$ with $K_a = 10^{-3.11}$ and $K_{N2O3} = 10^{-2.52}$. Thus, at pH 7 and $[NO_2^-] = 1$ mM, the equilibrium $[N_2O_3]$ would be $< 10^{-16}$ M.f



Scheme 4. Isomers of RSNO.

~110–130 kJ/mol, if R^- is an alkyl group or otherwise does not stabilize the radical by resonance [151,152]. So, despite their photochemical lability, RS-NO bonds are not prone to spontaneous homolysis. Note, however, that if $RS^\bullet$ is resonance stabilized, for example if is R is a phenyl group, the bond dissociation energy is about 40 kJ/mol smaller [151].

Of particular relevance is the reaction of *S*-nitrosothiols with other nucleophiles, leading to reversible transnitrosation (eq. (49)). This process represents a pathway by which a mobile species such as GSNO or *S*-nitrosocysteine can nitrosate a protein thiol and thereby may be a mechanism for signal transduction [153–156]. There is an emerging interest as well in protein-protein transnitrosylation in cell signaling pathways [137]. However, transnitrosations are not particularly fast reactions (k_2 values of ~0.1–500 M^{-1} s^{-1} depending on the reactants [154]). The reactions are faster at higher pH, indicating that the thiolate anion is a much stronger nucleophile than the thiol conjugate [117,154], as has been observed for reactions with other electrophiles (157).

$$RS-NO + R'S-H \rightleftarrows RS-H + R'S-NO \quad (49)$$

Interestingly, the reaction of *S*-nitrosothiols with thiols has also been reported to produce HNO [100] (eq. (50)). It is thus possible that the interaction of a mobile *S*-nitrosothiols such as GSNO with a protein thiol might result in *S*-thiolation rather than transnitrosation. The factors that may determine these potentially competitive pathways have not been elucidated (158).

$$RSNO + RSH \rightleftarrows RSSR + HNO \quad (50)$$

RSNOs are not formed from the direct reaction of thiols with NO under physiological conditions but must be mediated by some other redox process. H-atom abstraction from RSH (eq. (23)) would be unfavorable by more than 100 kJ mol^{-1} given that the H–NO bond is much weaker than typical RS-H bonds (85,159). Nitrosation of RSH by an NO^+ transfer agent such as N_2O_3 generated by NO autoxidation is one such pathway. Another would be mediation by a redox active metal center (124a). For example, the formation of dinitrosyl iron complexes (DNICs) via the reaction of free Fe^{2+}, NO and glutathione was found to be accompanied by the formation of the GS• radical (detected), which was then be trapped by NO to give GSNO (160).

3.4. Oxidation state +4: NO_2 and N_2O_4

Although, acidic nitrite solutions are a means to NO formation via the disproportionation reactions depicted in eqs. (42) and (43), the coproduct of this reaction is nitrogen dioxide. This free radical is also produced from NO autoxidation, at least in hydrophobic media (eq. (18)), from decomposition of peroxynitrite (see below) [161–163] and by metal catalyzed oxidation of nitrite. The chemical and physiological roles of NO_2 have not received the attention given to those of nitric oxide, but may be quite significant [164].

NO_2 is a very strong one-electron oxidant with a one electron reduction potential of +1.04 V in aqueous media (eq. (51)). [40a,b]. As a consequence, it readily undergoes redox reactions with various biological reductants such as ascorbate (k_2 = 1.8 × 10^7 M^{-1} s^{-1} at pH 6.5 [58]), ferrocytochrome *c* (6.6 × 10^7 M^{-1} s^{-1}) [165] and thiols and thiolates (5 × 10^7 M^{-1} s^{-1} for cysteine at pH 7.4, the reaction being largely attributed to the thiolate form) [166]. The reversible one-electron oxidation of tyrosine by NO_2 [167] is of particular interest since elevated levels of 3-nitrotyrosine have been detected in a variety of inflammatory disease states [168].

$$NO_2 + e^- \rightarrow NO_2^- \; E^\circ = +0.895 \text{ V (in aq. solution)} \quad (51)$$

The second order pH-dependent rate constants for the reaction of NO_2 with tyrosine have been reported (3.2 × 10^5 M^{-1} s^{-1} at pH 7.5 and 2.0 × 10^7 M^{-1} s^{-1} at pH 11.3) [58,167]. Tyrosine reacts even more rapidly with the carbonate radical at physiological pH (k_2 = 4.5 × 10^7 M^{-1} s^{-1}). In this context, subsequent reaction of the resulting tyrosine radical with NO_2 (k_2 = 1.3 × 10^9 M^{-1} s^{-1}) would be a potential pathway for the formation of 3-nitrotyrosine (see below) [169].

Unlike NO, the free radical NO_2 is prone to dimerization (eq. (52)).

$$2\ NO_2 \rightleftarrows\ N_2O_4 \quad (52)$$

The second order rate constant for dimerization is 4.5 × 10^8 M^{-1} s^{-1}, and the equilibrium constant is 7.7 × 10^4 M^{-1} in water [58]. The NO_2/N_2O_4 mixture has been used extensively to modify organic compounds. The dimer can serve as a nitrating (NO_2^+) or nitrosating (NO^+) agent in relatively polar, non-aqueous media or in highly concentrated acid solutions (eqs. (53) and (54)) [117].

$$N_2O_4 \rightleftarrows NO_2^+ + NO_2^- \quad (53)$$

$$N_2O_4 \rightleftarrows NO^+ + NO_3^- \quad (54)$$

The equilibrium between NO_2, N_2O_4 and the related ions is dependent on solvent, with heterolytic cleavage being significant only in polar solvents. In nonpolar media, the modifications are assumed to arise from direct reaction with N_2O_4 in a similar fashion to that for N_2O_3. Exposure to NO_2/N_2O_4 often leads to a mixture of nitrated products, with nitrosated and oxidized species also possible [117].

In aqueous solution, the dimer undergoes fairly rapid disproportionative hydrolysis to nitric and nitrous acid (eq. (56), $k = 1 \times 10^3$ s^{-1}) [169], such that its inherent lifetime in water is short, ~1 ms. Given the second order dependence of the concentration of N_2O_4 on NO_2, which is highly reactive, and the sink represented by the hydrolysis pathway (eq. (55)), the role of the dimer in physiological nitration pathways may be limited. However, *N*-nitrosation of both primary and secondary amines in neutral or alkaline conditions has been observed to outcompete hydrolysis [169], although the yields are typically quite poor compared to organic media [170].

$$N_2O_4 + H_2O \rightleftarrows 2\ H^+ + NO_3^- + NO_2^- \quad (55)$$

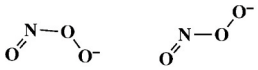
3.5. Oxidation state +5: NO_3^-, NO_2^+ and $ONOO^-$

Nitrate (NO_3^-) is a planar ion that is isoelectronic and structurally analogous to carbonate (CO_3^{2-}). HNO_3 is a very strong acid ($pK_a = -1.3$), so, unlike nitrite, nitrate is never protonated under physiologically relevant conditions. Nitrate is often viewed as a physiological end-product of nitrogen metabolism and is excreted in the urine. Notably, the higher nitrate concentrations in the urine of patients with bacterial infections were an important clue to Tannenbaum, Hibbs and others that elevated NO concentrations are generated during the immune response [73,74].

Although certain mammalian enzymes such as xanthine oxidoreductase have been shown to exhibit nitrate reductase activity [171,172], such reactions do not appear to play a major role in mammalian systems. As noted above, excessive dietary nitrate was generally considered to be a harmful component of food that caused infantile methemoglobinemia and carcinogenesis [173,174]. However, more recent discoveries that certain high nitrate foods such as beets and green leafy vegetables have a positive effect in lowering blood pressure and that nitrated fatty acids are endogenous anti-inflammatory signaling mediators (NO_2-FA) have generated reevaluations of those concerns [14,175–178]. An

explanation of the former effect has been that nitrate in circulating fluids is transported to the mouth where it concentrates in the saliva. Oral bacteria have nitrate reductase activity that converts nitrate to nitrite, which is then swallowed. The acidity of the stomach fluids then results in disproportionation of the resulting nitrous acid, and the NO equivalents generated may be transferred to the cardiovascular system leading to vasodilatory effects. The mechanism of such transfer is uncertain, but it has been proposed that antihypertensive effects of orally administered nitrite or nitrate result from formation of gastric *S*-nitrosothiols [179].

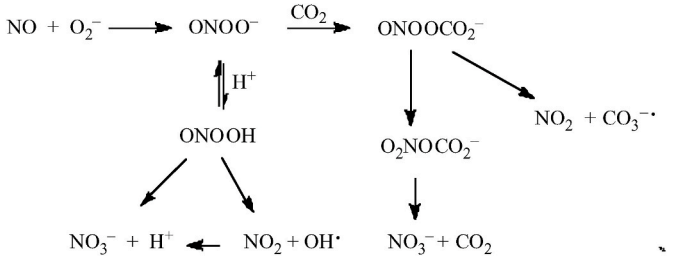
Peroxynitrite: The peroxynitrite ion is an isomer of nitrate, but formation of $ONOO^-$ has been related to the pathogenesis of multiple diseases [163]. The principal source of $ONOO^-$ is the very fast reaction of NO with O_2^- (eq. (13)). Both NO and O_2^- are produced during the inflammatory immune response. For this reason, the chemistry, biochemistry and biology of $ONOO^-$ have drawn considerable attention and has been reviewed extensively elsewhere [45,46,163] and mentioned above. The brief discussion here will focus on reactions of $ONOO^-$ in aqueous media.



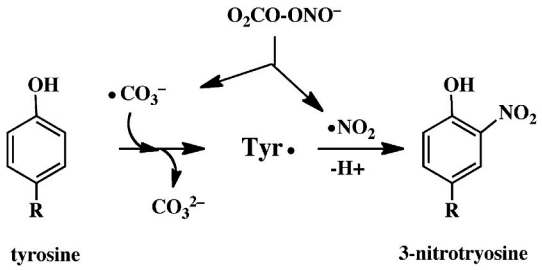
***cis*- and *trans*-peroxynitrite ion**

The pK_a of $ONOO^-$ is reported to be ~6.8 [180], so it is significantly protonated at normal physiological pH values. Peroxynitrous acid (ONOOH) is very reactive, and one pathway for decomposition is via unimolecular isomerization to nitrate. A secondary process involves decay through homolytic cleavage of the peroxide bond to give NO_2 and the hydroxyl radical (Scheme 5). Cage recombination of these two radicals would provide another mechanism for isomerization to nitrate, but escape from the solvent cage of these species could lead to significant damage. The extent of this process has been reviewed elsewhere [163c,d].

Another pathway to $ONOO^-$ decomposition is the reaction with carbon dioxide ($k_2 = 3 \times 10^4$ M^{-1} s^{-1}) to form the nitrosoperoxycarbonate ion $ONOOCO_2^-$ (Scheme 5) [162,163]. Given the millimolar concentrations of CO_2 in the cytosol, this reaction rate is sufficient to significantly consume $ONOO^-$ formed during inflammation. One decay pathway is to undergo rearrangement to the nitrocarbonate ion $O_2NOCO_2^-$, followed by hydrolysis to nitrate and bicarbonate. The CO_2 adduct is also inherently unstable toward homolytic cleavage of the peroxo O–O bond to NO_2 and the carbonate radical anion, $CO_3^{\bullet-}$, and this pathway accounts for much of the overall decay of $ONOO^-$ [163]. Both NO_2 and $CO_3^{\bullet-}$ are strong oxidants and while they are readily trapped by GSH ($k_2 = 2 \times 10^7$ and 5×10^6 M^{-1} s^{-1} at pH 7.4, respectively) [166,180], they react at comparable rates with tyrosine to give $Tyr^{\bullet}$ radicals. The very fast reaction of $Tyr^{\bullet}$ with NO_2 ($k = 1.3 \times 10^9$ M^{-1} s^{-1}) is a likely predecessor to the formation of nitrotyrosine residues (Scheme 6). Protein nitration is a marker for several diseases, such as amyotrophic lateral sclerosis (ALS or Lou Gerig's disease) [181]. Lim et al. have published an extensive kinetics analysis of the reactions of $ONOO^-$ under physiologically relevant conditions [182] and concluded that the radical scavenging ability of ascorbate and GSH left



Scheme 5. Formation of $ONOO^-$ and selected decomposition pathways.



Scheme 6. Proposed pathway for tyrosine nitration by the CO_2 adduct of $ONOO^-$ [163].

3-nitrotyrosine as the only tyrosine derivative that is likely to be formed at a significant rate.

Notably, the concept that $ONOO^-$ is a particularly dangerous species formed during inflammation is not universally held [183]. One argument is that the reaction of O_2^- with NO removes the more cytotoxic O_2^- and that the rapid reaction of $ONOO^-$ with CO_2 to form the $ONOOCO_2^-$ adduct leads mostly to nitrate. In this context, it is further noteworthy that the rate constants for the reactions of $ONOO^-$ itself with various common reductants such as ascorbate ($k_2 = 2.4 \times 10^2$ M^{-1} s^{-1}) and GSH ($k_2 = 7 \times 10^2$ M^{-1} s^{-1}) at physiological pH are rather small [180b,184]. While direct modifications of biological species by $ONOO^-$ are unlikely, $ONOO^-$ can function as a donor of reactive radical. However, given the large concentrations of the physiological antioxidant GSH, the carbonate and NO_2 radicals should be rapidly trapped under most circumstances. By comparison, the reaction of O_2^- with GSH is surprisingly slow ($k_2 = 2 \times 10^2$ M^{-1} s^{-1}) [185], thus the trapping of O_2^- by NO may provide several additional pathways for decreasing the overall impact of ROS generated during inflammation. Nonetheless, there is little doubt that the generation of RNS at certain locations, regardless of whether it results from $ONOO^-$ or $ONOOCO_2^-$ decomposition or from NO autoxidation, could have deleterious physiological effects.

4. Summary

In this article we have discussed some fundamental reactions of NO and simple NO_x species in aqueous media in order to lay the ground work for understanding the roles these simple compounds play in the chemical biology of mammalian organisms. This background is important in trying to interpret the reactions with biologically relevant metals. In brief overview, NO is a stable free radical (one unpaired electron) that is reactive with other radicals and with redox active metals; however it is neither a strong oxidant nor a strong reductant. NO is relatively unreactive in processes requiring two-electron oxidations or reductions. In such processes, it is not uncommon for the kinetics to require two NO molecules in the rate limiting step, hence giving kinetics that are second order in NO. Although, not discussed in this review, the chemical reactivity of NO is strongly mediated by coordination to redox active metal centers (11).

Among the derivatives of NO, NO_2 is also a free radical but is also a very strong oxidant and also may play a role in protein nitration. The anhydride of nitrous acid N_2O_3, which can be formed by combining these two radicals, is a strong oxidant that can also promote protein nitrosation. With nitrite much of the relevant chemistry is again dependent on the interactions with metal centers. However, the relatively facile acid disproportionation provides a mechanism by which the pool of NO represented by the much higher concentration of endogenous nitrite in tissues and fluids might be accessed. Even nitrate may be involved in mammalian generation of NO; although, in this case the key step is apparently the reduction of nitrate to nitrite by the nitrate reductase activity of bacteria. In sum, the chemical biology and reactivity of the various nitrogen oxides are interrelated. This often complicates analysis of the specific roles of individual species but is critical to the breadth of nitrogen-based signaling processes in a dynamic

environment.

List of abbreviations

ABTS	2-2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid)
atm	atmosphere
DEA/NO	the diazeniumdiolate salt $Na[Et_2N(O)NO]$ ()
DFT	density functional theory
EDRF	endothelium derived relaxation factor
est	estimated
GSH	glutathione
Hb	hemoglobin
Mb	myoglobin
mM	millimolar
nM	nanomolar
NHE	normal hydrogen electrode
NOS	nitric oxide synthase
RNS	reactive nitrogen species
ROS	reactive oxygen species
RSNO	*S*-nitrosothiol
sGC	soluble guanylyl cyclase
SOMO	singly occupied molecular orbital

References

[1] L.J. Ignarro, B. Freeman (Eds.), Nitric Oxide: Biology and Pathobiology, third ed., Academic Press, Burlington, MA, 2017.

[2] W.H. Koppenol, Names for inorganic radicals (IUPAC Recommendations 2000), Pure Appl. Chem. 72 (2000) 437–446.

[3] L.J. Ignarro, Nitric oxide : a unique endogenous signaling molecule in vascular biology (Nobel lecture), Angew. Chem. Int. Ed. 38 (1999) 1882–1892.

[4] R.F. Furchgott, Endothelium-derived relaxing factor: discovery, early studies, and identification as nitric oxide (Nobel lecture), Angew. Chem. Int. Ed. 38 (1999) 1870–1880.

[5] F. Murad, Discovery of some of the biological effects of nitric oxide and its role in cell signaling (Nobel lecture), Angew. Chem. Int. Ed. 38 (1999) 1856–1868.

[6] (a) O.W. Griffith, D.J. Stuehr, Nitric oxide synthases: properties and catalytic mechanism, Annu. Rev. Physiol. 57 (1995) 707–736;
(b) D.J. Stuehr, M.M. Haque, Nitric oxide synthase enzymology in the 20 years after the Nobel Prize, Br. J. Pharmacol. 176 (2019) 177–188.

[7] (a) P.C. Ford, Reactions of NO and nitrite with heme models and proteins, Inorg. Chem. 49 (2010) 6226–6239;
(b) (2) J.C. Toledo Jr., O. Augusto, Connecting the chemical and biological properties of nitric oxide, Chem. Res. Toxicol. 25 (5) (2012) 975–989;
(c) D.E. Williams, L.-M. Nisbett, B. Bacon, E. Boon, Bacterial heme-based sensors of nitric oxide, Antioxidants Redox Signal. 29 (2018) 1872–1887.

[8] F.A. Walker, W.R. Montfort, The nitric oxide-releasing heme proteins from the saliva of the blood-sucking insect Rhodnius prolixus, Adv. Inorg. Chem. 51 (2001) 295–358.

[9] (a) J.C. Toledo, C.A. Bosworth, S.W. Hennon, H.A. Mahtani, H.A. Bergonia, J. R. Lancaster, Nitric oxide-induced conversion of cellular chelatable iron into macromolecule-bound paramagnetic dinitrosyliron complexes, J. Biol. Chem. 283 (2008) 28926–28933;
(b) J.R. Hickok, S. Sahni, H. Shen, A. Arvind, C. Antoniou, L.W.M. Fung, D. D. Thomas, Dinitrosyliron complexes are the most abundant nitric oxide-derived cellular adduct: biological parameters of assembly and disappearance, Free Radic. Biol. Med. 51 (8) (2011) 1558–1566;
(c) A.F. Vanin, Dinitrosyl iron complexes with thiol-containing ligands as a "working form, of endogenous nitric oxide *Nitric Oxide- Biology and Chemistry* 54 (2016) 15–29;
(d) D.R. Truzzi, O. Augusto, A.V. Iretskii, P.C. Ford, Dynamics of dinitrosyl iron complex (DNIC) formation with low molecular weight thiols, Inorg. Chem. 58 (2019).

[10] (a) A.R. Butler, I.L. Megson, Non-heme iron nitrosyls in biology, Chem. Rev. 102 (2002) 1155–1165;
(b) L.A. Ekanger, P.H. Oyala, A. Moradian, M.J. Sweredoski, J.K. Barton, Nitric oxide modulates endonuclease III redox activity by a 800 MV negative shift upon [Fe4S4] cluster nitrosylation, J. Am. Chem. Soc. 140 (2018) 11800–11810;
(c) J.C. Crack, N.E. Le Brun, Mass spectrometric identification of [4Fe-4S](NO) (x) intermediates of nitric oxide sensing by regulatory iron-sulfur cluster proteins, Chem. Eur J. 25 (2019) 3675–3684.

[11] (a) P.C. Ford, I.M. Lorkovic, Mechanistic aspects of the reactions of nitric oxide with transition-metal complexes, Chem. Rev. 102 (2002) 993–1017;
(b) P. Ford, J.C. Melo Pereira, K. Miranda, Mechanisms of nitric oxide reactions mediated by biologically relevant metal centers, Struct. Bond 154 (2013) 99–135.

[12] (a) D.D. Thomas, L.A. Ridnour, J.S. Isenberg, W. Flores -Santana, C.H. Switzer, S. Donzelli, P. Hussain, C. Vecoli, N. Paolocci, S. Ambs, C.A. Colton, C.C. Harris, D.D. Roberts, D.A. Wink, The chemical biology of nitric oxide: implications in cellular signaling *Free Rad*, Biol. 45 (2008) 18–31;
(b) V. Somasundaram, D. Basudhar, G. Bharadwaj, J.H. No, L.A. Ridnour, R.Y. S. Cheng, M. Fujita, D.D. Thomas, S.K. Anderson, D.W. McVicar, D.A. Wink, Molecular mechanisms of nitric oxide in cancer progression, signal transduction, and metabolism, Antioxidants Redox Signal. 30 (2018) 1124–1143.

[13] (a) M.C. Martinez, R. Andriantsitohaina, Reactive nitrogen species: molecular mechanisms and potential significance in health and disease, Antioxidants Redox Signal. 11 (2009) 669–702;
(b) J. K Kundu, Y.J. Surh, Emerging avenues linking inflammation and cancer, Free Rad. Biol. 52 (2012) 2013–2037, 2012;
(c) C. Fionda, M.P. Abruzzese, A. Santoni, M. Cippitelli, Immunoregulatory and effector activities of nitric oxide and reactive nitrogen species in cancer, Curr. Med. Chem. 23 (2016) 2618–2636.

[14] (a) J.O. Lundberg, E. Weitzberg, M.T. Gladwin, The nitrate -nitrite-nitric oxide pathway in physiology and therapeutics, Nat. Rev. Drug Discov. 7 (2008) 156–167;
(b) A.W. DeMartino, D.B. Kim-Shapiro, R.P. Patel, M.T. Gladwin, Nitrite and nitrate chemical biology and signalling, Br. J. Pharmacol. 176 (2019) 228–245.

[15] (a) M. Feelisch, B.O. Fernandez, N.S. Bryan, M.F. Garcia-Saura, S. Bauer, D. R. Whitlock, P.C. Ford, D.R. Janero, J. Rodriguez, H. Ashrafian, Tissue Processing of Nitrite in Hypoxia: an intricate interplay of nitric oxide-generating and -scavenging systems, J. Biol. Chem. 283 (2008) 33927–33934;
(b) J.L. Zweier, H. Li, A. Samouilov, X. Liu, Mechanisms of nitrite reduction to nitric oxide in the heart and vessel wall, Nitric Oxide-Biol. Chem. 22 (2010) 83–90.

[16] (a) K.M. Miranda, The chemistry of nitroxyl (HNO) and implications in biology, Coord. Chem. Rev. 249 (2005) 433–455;
(b) Z. Miao, S.B. King, Recent advances in the chemical biology of nitroxyl (HNO) detection and generation, Nitric Oxide-Biology and Chemistry 57 (2016) 1–14;
(c) J.M. Fukuto, A recent history of nitroxyl chemistry, pharmacology and therapeutic potential, Br. J. Pharmacol. 176 (2019) 135–146.

[17] N.N. Greenwood, A. Earnshaw, Nitrogen, Chpt 11 in "Chemistry of the Elements, Pergamon Press, Oxford, 1984.

[18] J. Laane, J.R. Ohlsen, Characterization of nitrogen oxides by vibrational spectroscopy, Prog. Inorg. Chem. 27 (1980) 465.

[19] C.L. Young (Ed.), IUPAC Solubility Series: Oxides of Nitrogen, Pergamon Press, Oxford, UK, 1983.

[20] J.A. Gally, P.R. Montague, G.N. Reeke, G.M. Edelman, The nitric oxide hypothesis: possible effects of a short-lived, rapidly diffusible signal in the development and function of the nervous system, Proc. Natl. Acad. Sci. Unit. States Am. 87 (1990) 3547.

[21] J.R. Lancaster Jr., Simulation of the diffusion and reaction of endogenously produced nitric oxide, Proc. Natl. Acad. Sci. Unit. States Am. 91 (1994) 8137–8141.

[22] M.W. Vaughn, L. Kuo, J.C. Liao, Effective diffusion distance of nitric oxide in the microcirculation, Am. J. Physiol. 274 (5) (1998) H1705–H1714. Pt. 2.

[23] M.G. Suryaraman, A. Viswanathan, Preparation of nitric oxide: some laboratory methods, J. Chem. Educ. 26 (1949) 594.

[24] M.D. Lim, I.M. Lorkovic, P.C. Ford, The preparation of anaerobic nitric oxide solutions for the study of heme model systems in aqueous and nonaqueous media: some consequences of NOx impurities, Methods Enzymol. 396 (2005) 3–17. Nitric Oxide, Part E.

[25] P.C. Ford, D.A. Wink, D.M. Stanbury, Autoxidation kinetics of aqueous nitric oxide, FEBS Lett. 326 (1993) 1–3.

[26] (a) P.G. Wang, M. Xian, X.P. Tang, X.J. Wu, Z. Wen, T.W. Cai, A.J. Janczuk, Nitric oxide donors: chemical activities and biological applications, Chem. Rev. 102 (2002) 1091–1134;
(b) M.R. Miller, I.L. Megson, Recent developments in nitric oxide donor drugs, Br. J. Pharmacol. 151 (2007) 305–321;
(c) S. Huerta, S. Chilka, B. Bonavida, Nitric oxide donors: novel cancer. therapeutics (Review), Int. J. Oncol. 33 (2008) 909–927;
(d) A.B. Seabra, N. Duran, Nanoparticulated nitric oxide donors and their biomedical applications, Mini Rev. Med. Chem. 17 (2017) 216–223.

[27] (a) J.A. Hrabie, L.K. Keefer, Chemistry of the nitric oxide-releasing diazeniumdiolate ("Nitrosohydroxyl-amine") functional group and its oxygen-substituted derivatives, *Chem. Rev.* 102 (2002) 1135–1154;
(b) L.K. Keefer, Nitric oxide (NO)- and nitroxyl (HNO)-generating diazeniumdiolates (NONOates): emerging commercial opportunities, Curr. Top. Med. Chem. 5 (2005) 625–636;
(c) Larry K. Keefer, Fifty years of diazeniumdiolate research. From laboratory curiosity to broad-spectrum biomedical advances, ACS Chem. Biol. 6 (2011) 1147–1155.

[28] (a) D.A. Riccio, M.H. Schoenfisch, Nitric oxide release: Part I. Macromolecular scaffolds, Chem. Soc. Rev. 41 (2012) 3731–3741;
(b) A.W. Carpenter, M.H. Schoenfisch, Nitric oxide release: Part II. Therapeutic applications, Chem. Soc. Rev. 41 (2012) 3742–3752;
(c) P.N. Coneski, M.H. Schoenfisch, Nitric oxide release: Part III. Measurement and reporting, Chem. Soc. Rev. 41 (2012) 3753–3758.

[29] S. Divakaran, J. Loscalzo, The role of nitroglycerin and other nitrogen oxides in cardiovascular therapeutics, J. Am. Coll. Cardiol. 70 (2017) 2393–2410.

[30] D.G. Hottinger, D.S. Beebe, T. Koxhimannil, R.C. Prielipp, K.G. Belani, Sodium nitroprusside in 2014: a clinical concepts review, J. Anaesthesiol. Clin. Pharmacol. 30 (2014) 462–471.

[31] K.M. Davies, D.A. Wink, J.E. Saavedra, L.K. Keefer, Chemistry of the diazeniumdiolates. 2. Kinetics and mechanism of dissociation to nitric oxide in aqueous solution, J. Am. Chem. Soc. 123 (2001) 5473–5481.

[32] C. Yanga, S. Jeonga, S. Kub, K. Leea, M.H. Parka, Use of gasotransmitters for the controlled release of polymer-based nitric oxide carriers in medical applications, J. Contr. Release 279 (2018) 157–170.

[33] (a) P.C. Ford, Photochemical delivery of nitric oxide, Nitric Oxide-Biology & Chemistry 34 (2013) 56–64;
(b) P.C. Ford, Metal complex strategies for photo-uncaging the small molecule bioregulators nitric oxide and carbon monoxide, Coord. Chem. Rev. 376 (2018) 548–564.

[34] (a) T. Suzuki, O. Nagae, Y. Kato, H. Nakagawa, K. Fukuhara, N. Miyata, Photoinduced nitric oxide release from nitrobenzene derivatives, J. Am. Chem. Soc. 127 (2005) 11720–11726;
(b) K. Hishikawa, H. Nakagawa, T. Furuta, K. Fukuhara, H. Tsumoto, T. Suzuki, N. Miyata, Photoinduced nitric oxide release from a hindered nitrobenzene derivative by two-photon excitation, J. Am. Chem. Soc. 131 (2009) 7488–7489, update.

[35] M.D. Bartberger, W. Liu, E. Ford, K.M. Miranda, C. Switzer, J.M. Fukuto, P. J. Farmer, D.A. Wink, K.N. Houk, The reduction potential of nitric oxide (NO) and its importance to NO biochemistry, Proc. Natl. Acad. Sci. Unit. States Am. 99 (2002) 10958–10963.

[36] A.S. Dutton, J.M. Fukuto, K.N. Houk, Theoretical reduction potentials for nitrogen oxides from CBS-QB3 energetics and (C)PCM solvation calculations, Inorg. Chem. 44 (2005) 4024–4028.

[37] G.A. Janaway, J.I. Brauman, Direct observation of spin forbidden proton-transfer reactions: $^{3}NO^{-} + HA \rightarrow {}^{1}HNO + A^{-}$, J. Phys. Chem. 104 (2000) 1795–1798.

[38] V. Shafirovich, S.V. Lymar, Nitroxyl and its anion in aqueous solutions: spin states, protic equilibria, and reactivities toward oxygen and nitric oxide, Proc. Natl. Acad. Sci. Unit. States Am. 99 (2002) 7340–7345.

[39] V. Shafirovich, S.V. Lymar, Spin-forbidden deprotonation of aqueous nitroxyl (HNO), J. Am. Chem. Soc. 125 (2003) 6547–6552.

[40] (a) A.J. Bard, R. Parsons, J. Jordan (Eds.), Standard Potentials in Aqueous Solutions, Marcel Dekker, New York, 1985, pp. 127–139;
(b) D.A. Armstrong, R.E. Huie, W.H. Koppenol, S.V. Lymar, G. Merényi, P. Neta, B. Ruscic, D.M. Stanbury, S. Steenken, Standard electrode potentials involving radicals in aqueous solution: Inorganic radicals, Pure Appl.Chem 87 (2015) 1139–1150;
D.A. Armstrong, R.E. Huie, W.H. Koppenol, S.V. Lymar, G. Merényi, P. Neta, B. Ruscic, D.M. Stanbury, S. Steenken, Standard electrode potentials involving radicals in aqueous solution: Inorganic radicals, Pure Appl.Chem 87 (2015) 1139–1150.

[41] E.F. Caldin, Fast Reactions in Solution, J. Wiley & Sons, Inc, 1964, p. 12.

[42] G. Czapski, J. Holcman, B.H.J. Bielski, Reactivity of nitric oxide with simple short-lived radicals in aqueous solutions, J. Am. Chem. Soc. 116 (1994) 11465–11469.

[43] S. Goldstein, G. Czapski, The reaction of NO with O_2^- and HO_2, a pulse radiolysis study, Free Radic. Biol. Med. 19 (1995) 505–510;
(b) T. Nauser, W.H. Koppenol, The rate constant of the reaction of superoxide with nitrogen monoxide: Approaching the diffusion limit, J.Phys.Chem. A 106 (2002) 4084–4086.

[44] A.M. Miles, D.S. Bohle, P.A. Glassbrenner, B. Hansert, D.A. Wink, M.B. Grisham, Modulation of superoxide-dependent oxidation and hydroxylation reactions by nitric oxide, J. *Biol. Chem.* 271 (1996) 40–47.

[45] J.S. Beckman, W.H. Koppenol, Nitric oxide, superoxide, and peroxynitrite : the good, the bad, and the ugly, Am. J. Physiol. 271 (5) (1996) C1424–C1437. Pt. 1.

[46] (a) C. Szabo, H. Ischiropoulos, R. Radi, Peroxynitrite: biochemistry, pathophysiology and development of therapeutics, Nat. Rev. Drug Discov. 6 (2007) 662–680;
(b) S. Bartesaghi, R. Radi, Fundamentals on the biochemistry of peroxynitrite and protein tyrosine nitration, Redox Biology 14 (2018) 618–625.

[47] A. Negre-Salvayre, et al., Pathological aspects of lipid peroxidation, Free Radic. Res. 44 (2010) 1125–1171.

[48] V.B. O'Donnell, P.H. Chumley, N. Hogg, A. Bloodsworth, V.M. Darley-Usmar, B. A. Freeman, Nitric oxide inhibition of lipid peroxidation: kinetics of reaction with lipid peroxyl radicals and comparison with alpha-tocopherol, Biochemistry 36 (1997) 15216–15223.

[49] N. Hogg, B. Kalyanaraman, Nitric oxide and lipid peroxidation, Biochim. Biophys. Acta Bioenerg. 1411 (1999) 378–384.

[50] V.B. O'Donnell, B.A. Freeman, Interactions between nitric oxide and lipid oxidation pathways. Implications for vascular disease, Circ. Res. 88 (2001) 12–21.

[51] S. Padmaja, R.E. Huie, The reaction of nitric oxide with organic peroxyl radicals, Biochem. Biophys. Res. Commun. 195 (1993) 539–544.

[52] D.A. Wink, J.A. Cook, M.C. Krishna, I. Hanbauer, W. DeGraff, J. Gamson, J. B. Mitchell, NO inhibition of lipid peroxidation, A*rch. Biochem. Biophys* 319 (1995) 402–407.

[53] H. Rubbo, H. Botti, C. Batthyany, A. Trostchansky, A. Denicola, R. Radi, Antioxidant and diffusion properties of nitric oxide in low-density lipoprotein, Methods Enzymol. 359 (2002) 200–209.

[54] (a) P. Wardman, Chemical radiosensitizers for use in radiotherapy, Clin. Oncol. 19 (2007) 397–417;
(b) J.E. Moulder, Chemical radiosensitizers: the Journal history, IJRB (Int. J. Radiat. Biol.) 95 (2019) 940–944.

[55] (a) J.B. Mitchell, D.A. Wink, W. DeGraff, J. Gamson, Hypoxic Mammalian Cell Radiosensitization by Nitric Oxide *Cancer Research*, vol. 53, 1993, p. 5845;
(b) J. Bourassa, W. DeGraff, S. Kudo, D.A. Wink, J.B. Mitchell, P.C. Ford, In situ nitric oxide generation to sensitize γ-radiation induced cell death, J. Am. Chem. Soc. 119 (1997) 2853;
(c) Bryan T. Oronsky, Susan J. Knox, Jan J. Scicinski, Is nitric oxide (NO) the last word in radiosensitization? A review, Translational Oncology 5 (2012) 66–71.

[56] J.P. Eiserich, J. Butler, A. Vanderliet, C.E. Cross, B. Halliwell, Nitric oxide rapidly scavenges tyrosine and tryptophan radicals, Biochem. J. 310 (1995) 745–749.

[57] O. Guittet, B. Roy, M. Lepoivre, Nitric oxide: a radical molecule in quest of free radicals in proteins, Cell. Mol. Life Sci. 55 (1999) 1054–1067.

[58] P. Neta, R.E. Huie, Rate constants for reactions of inorganic radicals in aqueous solutions, J. Phys. Chem. Ref. Data 17 (1988) 1027–1284.

[59] E. Madej, L.K. Folkes, P. Wardman, G. Czapski, S. Goldstein, Thiyl radicals react with nitric oxide to form S-nitrosothiols with rate constants near the diffusion-controlled limit, Free Radic. Biol. Med. 44 (2008) 2013–2018.

[60] E.A. Wade, J.I. Cline, K.T. Lorenz, C. Hayden, D.W. Chandler, Direct measurement of the binding energy of the NO dimer, J. Chem. Phys. 116 (2002) 4755–4757.

[61] (a) S.G. Kukolich, Structure of the NO dimer, J. Am. Chem. Soc. 104 (1982) 4715–4716;
(b) M.D. Brookes, A.R.W. McKellar, T. Amano, Millimeter wave spectrum of the nitric oxide dimer, J. Mol. Spectrosc. 185 (1997) 153–157.

[62] N. Taguchi, Y. Mochizuki, T. Ishikawa, K. Tanaka, Multi-reference calculations of nitric oxide dimer, Chem. Phys. Lett. 451 (2008) 31–36.

[63] Y.-L. Zhao, M. Bartberger, K.N. Houk, K. Goto, Theoretical evidence for enhanced NO dimerization in aromatic hosts: implications for the role of the electrophile (NO)(2) in nitric oxide chemistry, J. Am. Chem. Soc. 127 (2005) 7964–7965, and refs therein.

[64] Tsukahara, H.; Ishida, T.; Mayumi, M. (1999)Gas-phase oxidation of nitric oxide: chemical kinetics and rate constant. Nitric Oxide, 3, 191-198..

[65] W.C. Nottingham, J.R. Sutter, Kinetics of the oxidation of nitric oxide by chlorine and oxygen in nonaqueous media, Int. J. Chem. Kinet. 18 (1986) 1289–1302.

[66] V.L. Pogrebnaya, A.P. Usov, A.V. Baranov, A.I. Nesterenko, P.I. Bez'yazychnyi, Liquid-phase oxidation of nitric oxide by oxygen, Zh. Prikl. Khim. 48 (1975) 954–958.

[67] D.A. Wink, J.F. Darbyshire, R.W. Nims, J.E. Saveedra, P.C. Ford, Reactions of the bioregulatory agent nitric oxide in oxygenated aqueous media: determination of the kinetics for oxidation and nitrosation by intermediates generated in the nitric oxide/oxygen reaction, Chem. Res. Toxicol. 6 (1993) 23.

[68] H.H. Awad, D.M. Stanbury, Autoxidation of nitrogen oxide (NO) in aqueous solution, Int. J. Chem. Kinet. 25 (1993) 375.

[69] R.S. Lewis, W.M. Deen, Kinetics of the reaction of nitric oxide with oxygen in aqueous solutions, Chem. Res. Toxicol. 7 (1994) 568.

[70] M. Pires, D.S. Ross, M.J. Rossi, Kinetic and mechanistic aspects of the NO oxidation by O_2 in aqueous phase, Int. J. Chem. Kinet. 26 (1994) 1207.

[71] V.G. Kharitonov, A.R. Sundquist, V.S. Sharma, Kinetics of nitric oxide autoxidation in aqueous solution, J. Biol. Chem. 269 (1994) 5881.

[72] S. Goldstein, G. Czapski, 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. 117 (1995) 12078.

[73] J.B. Hibbs Jr., R.R. Taintor, Z. Vavrin, E.M. Rachlin, Nitric oxide : a cytotoxic activated macrophage effector molecule, Biochem. Biophys. Res. Commun. 157 (1988) 87–94.

[74] C.D. Leaf, J.S. Wishnok, S.R. Tannenbaum, L-Arginine is a precursor for nitrate biosynthesis in humans, Biochem. Biophys. Res. Commun. 1*63* (1989) 1032–1037.

[75] R.H. Wenger, V. Kurtcuoglu, C.C. Scholz, H.H. Marti, D. Hoogewijs, Frequently asked questions in hypoxia research, Hypo*xia* 3 (2015) 35–43.

[76] J. Garthwaite, New insight into the functioning of nitric oxide receptive guanylyl cyclase: physiological and pharmacological implications, Mol. Cell. Biochem. 334 (2010) 221–232.

[77] V.G. Kharitonov, M. Russwurm, D. Madge, V.S. Sharma, D. Koesling, Dissociation of nitric oxide from soluble guanylate cyclase, Biochem. Biophys. Res. Commun. 239 (1997) 284.

[78] D.A. Wink, K.S. Kasprzak, C.M. Maragos, R.K. Elsepuru, M. Misra, T.M. Dunams, T.A. Cebula, W.H. Koch, A.W. Andrews, J.S. Allen, L.K. Keefer, DNA deaminating ability and genotoxicity on nitric oxide and its progenitors, Science 254 (1991) 1001–1002.

[79] M. Lee, K. Rey, K. Besler, C. Wang, J. Choy, Immunobiology of nitric oxide and regulation of inducible nitric oxide synthase, Results Probl. Cell Differ. 62 (2017) 181–207.

[80] M.N. Moeller, Q. Li, D.A. Vitturi, J.M. Robinson, J.R. Lancaster Jr., A. Denicola, Membrane "lens" effect: focusing the formation of reactive nitrogen oxides from the NO/O_2 reaction, Chem. Res. Toxicol. 20 (2007) 709–714.

[81] H. Kojima, K. Sakurai, K. Kikuchi, S. Kawahara, Y. Kirino, H. Nagoshi, Y. Hirata, T. Nagano, Development of a fluorescent indicator for nitric oxide based on the fluorescein chromophore, Chem. Pharmaceut. Bull. 46 (1998) 373–375.

[82] (a) S. Goldstein, G. Czapski, J. Lind, G. Merenyi, Tyrosine nitration by simultaneous generation of NO and O_2 under physiological conditions How the radicals do the job, J. Biol. Chem. 275 (2000) 3031–3036;
(b) P. Picon-Pages, J. Garcia-Buendia, F.J. Munoz, Functions and dysfunction of nitric oxide in brain, Biochim. Biophys. Acta (BBA) - Mol. Basis Dis. 1865 (2019) 1949–1967.

[83] T. deRojas-Walker, S. Tamir, H. J, J.S. Wishnok, S.R. Tannenbaum, Nitric oxide induces oxidative damage in addition to deamination in macrophage DNA, Chem. Res. Toxicol. 8 (1995) 473.

[84] J.C. Patterson, Computational Studies of Some Metalloporphyrins and of the Kinetics of the Aqueous Autoxidation of Nitric Oxide, Ph.D. Dissertation, Univ. Calif., Santa Barbara, 2004.

[85] R.N. Dixon, Heats of formation of HNO and DNO, J. Chem. Phys. 104 (1996) 6905–6906.

[86] (a) R.S. Drago, R.O. Ragsdale, D.P. Eyman, A mechanism for the reaction of diethylamine with nitric oxide, J. Am. Chem. Soc. 83 (1961) 4337–4339; (b) R. Longhi, R.O. Ragsdale, R.S. Drago, Reactions of nitrogen(II) oxide with miscellaneous Lewis bases, Inorg. Chem. 1 (1962) 768–770.

[87] M.D. Lim, I.M. Lorkovic, P.C. Ford, Kinetics of the oxidation of triphenylphosphine by nitric oxide, Inorg. Chem. 41 (2002) 1026–1028.

[88] A. Bakac, M. Schouten, A. Johnson, W. Song, O. Pestovsky, E. Szajna-Fuller, Oxidation of a water-soluble phosphine and some spectroscopic probes with nitric oxide and nitrous acid in aqueous solutions *inorg*, Inside Chem. 48 (2009) 6979–6985.

[89] J.M. Fukuto, S.J. Carrington, HNO signaling mechanisms, Antioxidants Redox Signal. 14 (2011) 1649–1657.

[90] (a) I. Akinsheye, E.S. Klings, Sickle cell anemia and vascular dysfunction: the nitric oxide connection, J. Cell. Physiol. 224 (2010) 620–625; (b) S.B. Solomon, L. Bellavia, D. Sweeney, B. Piknova, A. Perlegas, Christine C. Helms, G.A. Ferreyra, S.B. King, N.J.H. Raat, S.J. Kern, J. Sun, L.C. McPhail, A. N. Schechter, C. Natanson, M.T. Gladwin, D.B. Kim-Shapiro, Angeli's salt counteracts the vasoactive effects of elevated plasma hemoglobin, Free Radic. Biol. Med. 53 (2011) 2229–2239.

[91] (a) N. Paolocci, T. Katori, H.C. Champion, M.E. St John, K.M. Miranda, J. M. Fukuto, D.A. Wink, D.A. Kass, Positive inotropic and lusitropic effects of HNO/NO- in failing hearts: independence from beta-adrenergic signaling, Proc. Nat. Acad. Sci. USA 100 (2003) 5537–5542; (b) R.H. Ritchie, J.L. Favaloro, K.L. Andrews, R.E. Widdop, B.K. Kemp-Harper, Nitroxyl (HNO): the Cinderella of the nitric oxide story Irvine, J. C, Trends Pharmacol. Sci. 29 (2008) 601–608; (c) C.G. Tocchetti, B.A. Stanley, C.I. Murray, V. Sivakumaran, S. Donzelli, D. Mancardi, P. Pagliaro, W.D. Gao, J. van Eyk, D.A. Kass, D.A. Wink, N. Paolocci, Playing with cardiac "redox switches'': the ''HNO way'' to modulate cardiac function, Antioxidants Redox Signal. 14 (2011) 1687–1698.

[92] (a) M.E. Shoman, O.M. Aly, Nitroxyl (HNO): a possible strategy for fighting cancer, Curr. Top. Med. Chem. 16 (2016) 2464–2470; (b) H.-J. Sun, W.-T. Lee, B. Leng, Z.-Y. Wu, Y. Yang, J.-S. Bian, Nitroxyl as a potential theranostic in the cancer arena, Antioxidants Redox Signal. 32 (2020) 331–349.

[93] D.T. Longhi-Balbinot, A.C. Rossaneis, F.A. Pinho-Ribeiro, M.M. Bertozzi, F. Q. Cunha, J.C. Alves-Filho, T.M. Cunha, J.P.S. Peron, K.M. Miranda, R. Casagrande, W.A. Verri Jr., The nitroxyl donor, Angeli's salt, reduces chronic constriction injury induced neuropathic pain, Chem. Biol. Interact. 256 (2016) 1–8.

[94] D.D. Thomas, J.L. Heinecke, L.A. Ridnour, R. Cheng, A.H. Kesarwala, C. H. Switzer, D.W. McVicar, D.D. Roberts, S. Glynn, J.M. Fukuto, D.A. Wink, K. M. Miranda, Signaling and stress: the redox landscape in NOS2 biology, Free Radic. Biol. Med. 87 (2015) 204–225.

[95] M.A. Marti, L. Alvarez, S.A. Suarez, F. Doctorovich, Is azanone endogenously produced in mammals? in: F. Doctorovich, P.J. Farmer, Marti (Eds.), "Chemistry and Biology of Nitroxyl (HNO) Elsevier, 2017, pp. 337–351.

[96] (a) S. Adak, Q. Wang, D.J. Stuehr, Arginine conversion to nitroxide by tetrahydro-biopterin-free neuronal nitric-oxide synthase: implications for mechanism, J. Biol. Chem. 275 (2000) 33554–33561; (b) J.M. Fukuto, G.C. Wallace, R. Hszieh, G. Chaudhuri, Chemical oxidation of N-hydroxyguanidine compounds: release of nitric oxide, nitroxyl and possible relationship to the mechanism of biological nitric oxide generation, Biochem. Pharmacol. 43 (1992) 607–613; (c) K.M. Rusche, M.M. Spiering, M.A. Marletta, Reactions catalyzed by tetrahydrobiopterin-free nitric oxide synthase, Biochemistry 37 (1998) 15503–15512, other HNO from NOS refs?

[97] (a) M. Gbadegesin, S. Vicini, S.J. Hewett, D.A. Wink, M. Espey, R.M. Pluta, C. A. Colton, Hypoxia modulates nitric oxide-induced regulation of NMDA receptor currents and neuronal cell death, Am. J. Physiol. Cell Physiol. 277 (1999) C673–C683; (b) C.A. Colton, M. Gbadegesin, D.A. Wink, K.M. Miranda, M.G. Espey, S. Vicini, Nitroxyl anion regulation of the NMDA receptor, J. Neurochem. 78 (2001) 1126–1134.

[98] F. Doctorovich, D. Bikiel, J. Pellegrino, S.A. Suárez, A. Larsen, M.A. Martí, Nitroxyl (azanone) trapping by metalloporphyrins, Coord. Chem. Rev. 255 (2011) 2764–2784.

[99] (a) G. Wu, Y.-Z. Fang, S. Yang, J.R. Lupton, N.D. Turner, Glutathione metabolism and its implications for health, J. Nutr. 134 (2004) 489–492; (b) D. Montero, C. Tachibana, J.R. Winther, C. Appenzeller-Herzog, Intracellular glutathione pools are heterogeneously concentrated, Redox Biol 1 (2013) 508–513.

[100] P.S.-Y. Wong, J. Hyun, J.M. Fukuto, F.N. Shirota, E.G. DeMaster, D.W. Shoeman, H.T. Nagasawa, Reaction between S - nitrosothiols and thiols: generation of nitroxyl (HNO) and subsequent chemistry, Biochem 37 (1998) 5362–5371.

[101] S. Donzelli, M.G. Espey, D.D. Thomas, D. Mancardi, C.G. Tocchetti, L.A. Ridnour, N. Paolocci, S.B. King, K.M. Miranda, G. Lazzarino, J.M. Fukuto, D.A. Wink, Discriminating formation of HNO from other reactive nitrogen oxide species, Free Radic. Biol. Med. 40 (2006) 1056–1066.

[102] G. Keceli, A. Majumdar, C.N. Thorpe, S. Jun, C.G. Tocchetti, D. Lee, J.E. Mahaney, Nazareno Paolocci, J.P. Toscano, Nitroxyl (HNO) targets phospholamban cysteines 41 and 46 to enhance cardiac function, J. Gen. Physiol. 151 (2019) 758–770.

[103] (a) J.A. Reisz, C.N. Zink, S.B. King, Rapid and selective nitroxyl (HNO) trapping by phosphines: kinetics and new aqueous ligations for HNO detection and quantitation, J. Am. Chem. Soc. 133 (2011) 11675–11685; (b) Z. Miao, S.B. King, Phosphine-based HNO detection, in: F. Doctorovich, P. J. Farmer, Marti (Eds.), "Chemistry and Biology of Nitroxyl (HNO), Elsevier, 2017, pp. 225–238.

[104] J.H. Jorolan, L.A. Buttitta, C. Cheah, K.M. Miranda, Comparison of the chemical reactivity of synthetic peroxynitrite with that of the autoxidation products of nitroxyl or its anion, Nitric Oxide-Biology & Chemistry 44 (2015) 39–46.

[105] K.M. Miranda, H.T. Nagasawa, J.P. Toscano, Donors of HNO, Curr. Top. Med. Chem. 5 (2005) 647–664.

[106] H. Nakagawa, Controlled release of HNO from chemical donors for biological applications, J. Inorg. Biochem. 118 (2013) 187–190.

[107] D. Basudhar, G. Bharadwaj, D.J. Salmon, K.M. Miranda, in: F. Doctorovich, P. J. Farmer, Marti (Eds.), HNO Donors: Angeli's Salt and Related Diazeniumdiolates in "Chemistry and Biology of Nitroxyl (HNO), Elsevier, 2017, pp. 11–36.

[108] M.N. Hughes, R. Cammack, Synthesis, chemistry, and applications of nitroxyl ion releasers sodium trioxodinitrate or Angeli's salt and Piloty's acid, Methods Enzymol. 301 (1999) 279–287.

[109] P.E. Sturrock, J.D. Ray, J. McDowell, H.R. Hunt, Dissociation constants of α -oxyhyponitrous acid, Inorg. Chem. 2 (1963) 649–650.

[110] F. Seel, C. Bliefert, Mechanism of the decomposition of sodium benzenesulfohydroxamate in aqueous solution, Z. Anorg. Allg. Chem. 394 (1972) 187–196.

[111] J.F. DuMond, S.B. King, The chemistry of nitroxyl-releasing compounds, Antioxidants Redox Signal. 14 (2011) 1637–1648.

[112] (a) K.M. Miranda, T. Katori, C.L.T. de Holding, L. Thomas, L.A. Ridnour, W. J. MeLendon, S.M. Cologna, A.S. Dutton, H.C. Champion, D. Mancardi, C. G. Tocchetti, J.E. Saavedra, L.K. Keefer, K.N. Houk, J.M. Fukuto, D.A. Kass, N. Paolocci, D.A. Wink, Comparison of the NO and HNO donating properties of diazeniumdiolates: primary amine adducts release HNO in vivo, J. Med. Chem. 48 (2005) 8220–8228; (b) D. Andrei, D.J. Salmon, S. Donzelli, A. Wahab, J.R. Klose, M.L. Citro, J. E. Saavedra, D.A. Wink, K.M. Miranda, L.K. Keefer, Dual mechanisms of HNO generation by a nitroxyl prodrug of the diazeniumdiolate (NONOate) class, J. Am. Chem. Soc. 132 (2010) 16526–16532.

[113] (a) J.A. Reisz, E.B. Klorig, M.W. Wright, S.B. King, Reductive phosphine-mediated ligation of nitroxyl (HNO), Org. Lett. 11 (2009) 2719–2721; (b) J.A. Reisz, C.N. Zink, S.B. King, Rapid and selective nitroxyl (HNO) trapping by phosphines: kinetics and new aqueous ligations for HNO detection and quantitation, J. Am. Chem. Soc. 133 (2011) 11675–11685.

[114] M.H. Thiemens, W.C. Trogler, Nylon production: an unknown source of atmospheric nitrous oxide, Science 251 (1991) 932–934.

[115] I.M. Wasser, S. de Vries, P. Moeenne-Loccoz, I. Schroeder, K.D. Karlin, Nitric oxide in biological denitrification: Fe/Cu metalloenzyme and metal complex NOx redox chemistry, Chem. Rev. 102 (2002) 1201–1234.

[116] D. Deleu, Y. Hanssens, Nitrous oxide -induced cobalamin deficiency, Arch. Neurol. 58 (2001) 134–135.

[117] D.L.H. Williams, Nitrosation Reactions and the Chemistry of Nitric Oxide, Elsevier, Amsterdam, 2004, 2004.

[118] K.H. Becker, J. Kleffmann, R. Kurtenbach, P. Wiesen, Solubility of nitrous acid (HONO) in sulfuric acid solutions, J. Phys. Chem. 100 (1996), 14984. also Seel, F.; Winkler, R. (1960) Z. Phys. Chem. NF , 25, 217.

[119] G. da Silva, E.M. Kennedy, B.Z. Dlugogorski, Ab initio procedure for aqueous-phase pKa calculation: the acidity of nitrous acid, J. Phys. Chem. 110 (2006) 11371–11376.

[120] G.Y. Markovits, S.E. Schwartz, L. Newman, Hydrolysis equilibrium of dinitrogen trioxides in dilute acid solution, Inorg. Chem. 20 (1981) 445–450.

[121] A. Treinin, E. Hayon, Absorption spectra and reaction kinetics of NO_2, N_2O_3, and N_2O_4 in aqueous solution, J. Am. Chem. Soc. 92 (1970) 5821–5828.

[122] (a) P. Issenberg, Nitrite , nitrosamines, and cancer, Fed. Proc. 35 (1976) 1322–1326; (b) H. Bartsch, R. Montesano, Relevance of nitrosoamines to human cancer, Carcinogenisis 5 (1984) 1381–1393; (c) S.S. Mirvish, Role of N-nitroso compounds (NOC) and N-nitrosation in etiology of gastric, esophageal, nasopharygeal and bladder cancer, Cancer Lets 93 (1995) 17–48; (d) W. Crowe, C.T. Elliott, B.D. Green, A review of the in vivo evidence investigating the role of nitrite exposure from processed meat consumption in the development of colorectal cancer, Nutrients 11 (2019) 2673.

[123] L.A. Ridnour, D.D. Thomas, D. Mancardi, M.G. Espey, K.M. Miranda, N. Paolocci, M. Feelisch, J. Fukuto, D.A. Wink, The chemistry of nitrosative stress induced by nitric oxide and reactive nitrogen oxide species. Putting perspective on stressful biological situations, Biol. Chem. 385 (2004) 1–10.

[124] (a) P.C. Ford, B.O. Fernandez, M.D. Lim, Mechanisms of reductive nitrosylation in iron and copper models relevant to biological systems, Chem. Rev. 105 (2005) 2439–2455; (b) A.F. Vanin, What is the mechanism of nitric oxide conversion into nitrosonium ions ensuring S-nitrosating processes in living organisms, Cell Biochem. Biophys. 77 (2019) 279–292.

[125] R.S. Lewis, S.R. Tannenbaum, W.M. Deen, 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. 117 (1995) 3933–3939.

[126] (a) V.G. Kharitonov, A.R. Sundquist, V.S. Sharma, Kinetics of nitrosation of thiols by nitric oxide in the presence of oxygen, J. Biol. Chem. 270 (1995) 28158–28164;
(b) M. Keshive, S. Singh, J.S. Wishnok, S.R. Tannenbaum, W.M. Deen, Kinetics of S-nitrosation of thiols in nitric oxide solutions, Chem. Res. Toxicol. 9 (1996) 988–993;
(c) C.H. Lim, P.C. Dedon, W.M. Deen, Kinetic analysis of intracellular concentrations of reactive nitrogen species, Chem. Res. Toxicol. 21 (2008) 2134–2147.

[127] L. Grossi, S. Strazzari, A new synthesis of alkyl nitrites: the reaction of alkyl alcohols with nitric oxide in organic solvents, J. Org. Chem. 64 (1999) 8076–8079.

[128] A.A. Nedospasov, Is N_2O_3 the Main Nitrosating intermediate in aerated nitric oxide solutions in vivo, J. Biochem. Mol. Toxicol. 16 (2002) 109–120.

[129] M.N. Moller, Q. Li, J.R. Lancaster Jr., A. Denicola, Acceleration of nitric oxide autoxidation and nitrosation by membranes, IUBMB Life 59 (2007) 243–248.

[130] D.A. Vitturi, L. Minarrieta, S.R. Salvatore, E.M. Postlethwait, M. Fazzari, G. Ferrer-Sueta, J.R. Lancaster Jr., B.A. Freeman, F.J. Schopfer, Convergence of biological nitration and nitrosation via symmetrical nitrous anhydride, Nat. Chem. Biol. 11 (2015) 504.

[131] X. Wang, Q.-Z. Qin, Photoisomerization of N_2O_3 in an Ar matrix, J. Photochem. Photobiol. Chem. 122 (1999) 1–5.

[132] Z. Sun, Yong D. Liu, Chun L. Lv, R.G. Zhong, Theoretical investigation of the isomerization of N_2O_3 and the N-nitrosation of dimethylamine by asym-N_2O_3, sym-N_2O_3, and trans-cis N_2O_3 isomers, J. Mol. Structure. Theochem 908 (2009) 107–113.

[133] M.N. Routledge, D.A. Wink, L.K. Keefer, A. Dipple, Mutations induced by saturated aqueous nitric oxide in the pSP189 supF gene in human Ad293 and E. coli MBM7070 cells, Carcinogenesis 14 (1993) 1251–1254.

[134] L.K. Boerner, The lurking contaminant, Chemistry and Engineering News (2020) 27–31, 4/20/2020.

[135] (a) L.J. Ignarro, H. Lippton, J.C. Edwards, W.H. Baricos, A.L. Hyman, P. J. Kadowitz, C.A. Gruetter, Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide: evidence for the involvement of S-nitrosothiols as active intermediates, J. Pharmacol. Exp. Therapeut. 218 (1981) 739–749;
(b) N. Hogg, The biochemistry and physiology of S-nitrosothiols, Annu. Rev. Pharmacol. Toxicol. 42 (2002) 585–600;
(c) K.A. Broniowska, N. Hogg, The chemical biology of S-nitrosothiols, Antioxidants Redox Signal. 17 (2012) 969–980.

[136] P. Anand, J.S. Stamler, Enzymatic mechanisms regulating protein S-nitrosylation: implications in health and disease, J. Mol. Medicine 90 (2012) 233–244.

[137] T. Nakamura, S.A. Lipton, Emerging Role of Protein-Protein Transnitrosylation in Cell Signaling Pathways Antioxidants & Redox Signaling, vol. 18, 2013, pp. 239–249.

[138] V. Fernando, X. Zheng, Y. Walia, V. Sharma, J. Letson, S. Furuta, S -Nitrosylation, An Emerging Paradigm of Redox Signaling Antioxidants 8 (2019) article 404.

[139] M.R. Filipovic, J.L. Miljkovic, T. Nauser, M. Royzen, K. Klos, T. Shubina, W. H. Koppenol, S.J. Lippard, I. Ivanovic-Burmazovic, Chemical characterization of the smallest S-nitrosothiol, HSNO; cellular cross-talk of H2S and S-nitrosothiols, J. Am. Chem. Soc. 134 (2012) 12016–12027.

[140] M.M. Cortese-Krott, B.O. Fernandez, J.L.T. Santos, E. Mergia, M. Grman, P. Nagy, M. Kelm, A.R. Butler, M. Feelisch, Nitrosopersulfide (SSNO-) accounts for sustained NO bioactivity of S-nitrosothiols following reaction with sulfide, Redox Biol 2 (2014) 234–244.

[141] T.S. Bailey, H.A. Henthorn, M.D. Pluth, The intersection of NO and H_2S: persulfides generate NO from nitrite through polysulfide formation, Inorg. Chem. 55 (2016) 12618–12625.

[142] M. Nava, M.-A. Martin-Drumel, C.A. Lopez, K.N. Crabtree, C.C. Womack, T. L. Nguyen, S. Thorwirth, C.C. Cummins, J.F. Stanton, M.C. McCarthy, Spontaneous and selective formation of HSNO, a crucial intermediate linking H_2S and nitroso chemistries, J. Am. Chem. Soc. 138 (2016) 11441–11444.

[143] J.P. Marcolongo, M.F. Venancio, W.R. Rocha, F. Doctorovich, J.A. Olabe, NO/H2S "crosstalk" reactions. The role of thionitrites (SNO-) and perthionitrites (SSNO-), Inorg. Chem. 58 (2019) 14981–14997.

[144] A.P. Dicks, H.R. Swift, D.L.H. Williams, A.R. Butler, Identification of Cu+ as the effective reagent in nitric oxide formation from S-nitrosothiols (RSNO), J. Chem. Soc. Perkin Trans. 481–487 (1996).

[145] Q.K. Timerghazin, G.H. Peslherbe, A.M. English, Structure and stability of HSNO, the simplest S-nitrosothiol, Phys. Chem. Chem. Phys. 10 (2008) 1532–1539.

[146] L.V. Andreasen, I.M. Lorkovic, G.B. Richter-Addo, P.C. Ford, Kinetics studies of the reaction of the ruthenium porphyrin Ru(OEP)(CO) with the S-nitrosothiol N-acetyl-1-amino-2-methylpropyl-2-thionitrite, Nitric Oxide-Biol. & Chem. 6 (2002) 228–235.

[147] R.J. Singh, N. Hogg, J. Joseph, B. Kalyanaraman, Mechanism of nitric oxide release from S-nitrosothiols, J. Biol. Chem. 271 (1996) 18596–18603.

[148] D.A. Riccio, P.N. Coneski, S.P. Nichols, A.D. Broadnax, M.H. Schoenfisch, Photoinitiated nitric oxide-releasing tertiary S-Nitrosothiol-Modified xerogels, ACS Appl. Mater. Interfaces 4 (2012) 796–804.

[149] A.N. Paunel, A. Dejam, S. Thelen, M. Kirsch, M. Horstjann, P. Gharini, M. Murtz, M. Kelm, H. de Groot, V. Kolb-Bachofen, C.V. Suschek, Enzyme-independent nitric oxide formation during UVA challenge of human skin: characterization, molecular sources, and mechanisms, Free Radic. Biol. Med. 38 (2005) 606–615.

[150] A. Keszler, B. Lindemer, N. Hogg, N.L. Lohr, Ascorbate attenuates red light mediated vasodilation: potential role of S-nitrosothiols, Redox Biology 20 (2019) 13–18.

[151] X.H. Li, R.-Z. Zhang, X.-D. Yang, Theoretical studies of bond dissociation energies of S–NO for S-nitrosothiols, J. Mol. Struct. 817 (2007) 43–47.

[152] W.H. Koppenol, Nitrosation, thiols, and hemoglobin: energetics and kinetics, Inorg. Chem. 51 (2012) 5637–5641.

[153] D.J. Barnett, J. McAninly, D.L.H. Williams, Transnitrosation between nitrosothiols and thiols, J. Chem. Soc. Perkin Trans. 2 (1994) 1131–1133.

[154] N. Hogg, The kinetics of S-transnitrosation, A reversible second-order reaction, Anal. Biochem. 272 (1999) 257–262.

[155] H.E. Marshall, K. Merchant, J.S. Stamler, Nitrosation and oxidation in the regulation of gene expression, Faseb. J. 14 (2000) 1889–1900.

[156] J.R. Hickok, D. Vasudevan, G.R.J. Thatcher, D.D. Thomas, S-nitrosocysteine a true surrogate for nitric oxide? Antioxid, Redox Signal 17 (2012) 962–968.

[157] M.L. Souza, A.W. DeMartino, P.C. Ford, Biological thiols and carbon disulfide: the Formation and decay of trithiocarbonates under physiologically relevant conditions, ACS Omega 2 (2017) 6535–6543.

[158] J.M. Fukuto, S.J. Carrington, D.J. Tantillo, J.G. Harrison, L.J. Ignarro, B. A. Freeman, A. Chen, D.A. Wink, Small molecule signaling agents: the integrated chemistry and biochemistry of nitrogen oxides, oxides of carbon, dioxygen, hydrogen sulfide, and their derived species, Chem. Res. Toxicol. 25 (2012) 769–793.

[159] D. Shriver, M. Weller, T. Overton, J. Rourke, F. Armstrong, Inorganic Chemistry, sixth ed., W. H. Freeman & Co., NY, NY, 2014, p. 301.

[160] (a) D.R. Truzzi, O. Augusto, P.C. Ford, Thiyl radicals are Co-products of dinitrosyl iron complexes (DNICs) formation, Chem. Commun. 55 (2019) 9156–9159;
(b) D.R. Truzzi, O. Augusto, A.V. Iretskii, P.C. Ford, Dynamics of dinitrosyl iron complex (DNIC) formation with low molecular weight thiols, Inorg. Chem. 58 (2019) 13446–13456, 2019.

[161] G.L. Squadrito, W.A. Pryor, Oxidative chemistry of nitric oxide: the roles of superoxide, peroxynitrite , and carbon dioxide, Free Radic. Biol. Med. 25 (1998) 392–403.

[162] S.V. Lymar, J.K. Hurst, Carbon dioxide: physiological catalyst for peroxynitrite -mediated cellular damage or cellular protectant? Chem. Res. Toxicol. 9 (1996) 845–850.

[163] (a) G. Ferrer-Sueta, N. Campolo, M. Trujillo, S. Bartesaghi, S. Carballa, N. Romero, B. Alvarez, R. Radi, Biochemistry of peroxynitrite and protein tyrosine nitration, Chem. Rev. 118 (2018) 462;
(b) O. Augusto, S. Goldstein, J.K. Hurst, J. Lind, S.V. Lymar, G. Merenyi, R. Radi, Carbon dioxide-catalyzed peroxynitrite reactivity - the resilience of the radical mechanism after two decades of research, Free Radic. Biol. Med. 135 (2019) 210–215;
(c) W.H. Koppenol, P.L. Bounds, T. Nauser, R. Kissner, H. Rüegger, Peroxynitrous acid: Controversy and consensus surrounding an enigmatic oxidant, Dalton Trans. 41 (2012) 13779–13787;
(d) W.H. Koppenol, S. Serrano-Luginbuehl, T. Nauser, R. Kissner, Thinking outside the cage: A new hypothesis that accounts for variable yields of radicals from the reaction of CO_2 with $ONOO^-$, Chem.Res.Toxicol. 33 (2020) 1516–1527.

[164] M. Kirsch, H.-G. Korth, R. Sustmann, H. De Groot, The pathobiochemistry of nitrogen dioxide, Biol. Chem. 383 (2002) 389–399.

[165] A.S. Domazou, L. Gebicka, J. Didik, J.L. Gebicki, B. van der Meijden, W. H. Koppenol, The kineticsof the reaction of nitrogen dioxide with iron(II)- and iron(III) cytochrome c, Free Radic. Biol. Med. 69 (2014) 172–180.

[166] E. Ford, M.N. Hughes, P. Wardman, Kinetics of the reactions of nitrogen dioxide with glutathione, cysteine, and uric acid at physiological pH, Free Radic. Biol. Med. 32 (2002) 1314–1323.

[167] W.A. Pruetz, H. Moenig, J. Butler, E.J. Land, Reactions of nitrogen dioxide in aqueous model systems: oxidation of tyrosine units in peptides and proteins, Arch. Biochem. Biophys. 243 (1985) 125–134.

[168] N. Abello, H.A.M. Kerstiens, D.S. Postma, R. Bischoff, Protein tyrosine nitration: selectivity, physicochemical and biological consequences, denitration, and proteomics methods for the identification of tyrosine-nitrated proteins, J. Proteome Res. 7 (2009) 3222–3238.

[169] S. Goldstein, G. Czapski, J. Lind, G. Merenyi, Tyrosine nitration by simultaneous generation of NO and O_2^- under physiological conditions How the radicals do the job, J. Biol. Chem. 275 (2000) 3031–3036.

[170] M. Shiri, M.A. Zolfigol, H.G. Kruger, Z. Tanbakouchian, Advances in the application of N_2O_4/NO_2 in organic reactions, Tetrahedron 66 (2010) 9077–9106.

[171] C.E. Berry, J.M. Hare, Xanthine oxicloreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications, J. Physiology-London 555 (2004) 589–606.

[172] E.A. Jansson, L. Huang, R. Malkey, M. Govoni, C. Nihlen, A. Olsson, M. Stensdotter, J. Petersson, L. Holm, E. Weitzberg, J.O. Lundberg, A mammalian functional nitrate reductase that regulates nitrite and nitric oxide homeostasis, Nat. Chem. Biol. 4 (2008) 411–417.

[173] C.D. Leaf, S.R. Tannenbaum, The role of dietary nitrate and nitrite in human cancer, Nutrition and Cancer Prevention (1996) 317–324.

[174] G.M. McKnight, C.W. Duncan, C. Leifert, M.H. Golden, Dietary nitrate in man: friend or foe? Br. J. Nutr. 81 (1999) 349–358.

[175] H.G. Classen, C. Stein-Hammer, H. Thoni, Hypothesis: the effect of oral nitrite on blood pressure in the spontaneously hypertensive rat. Does dietary nitrate mitigate hypertension after conversion to nitrite? J. Amer. Col. Nutrition 9 (1990) 500–502.

[176] (a) J.O. Lundberg, E. Weitzberg, J.A. Cole, N. Benjamin, Opinion: nitrate, bacteria and human health, Nat. Rev. Microbiol. 2 (2004) 593–602;
(b) S.A. Omar, A.J. Webb, J.O. Lundberg, E. Weitzberg, Therapeutic effects of

inorganic nitrate and nitrite in cardiovascular and metabolic diseases, J. Intern. Med. 279 (2016) 315–336.
[177] A.J. Webb, N. Patel, S. Loukogeorgakis, M. Okorie, Z. Aboud, S. Misra, R. Rashid, P. Miall, J. Deanfield, N. Benjamin, R. MacAllister, A.J. Hobbs, A. Ahluwalia, Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite, Hypertension 51 (2008) 784–790.
[178] (a) T. Cui, F.J. Schopfer, J. Zhang, K. Chen, T. Ichikawa, P.R.S. Baker, C. Batthyany, B.K. Chacko, X. Feng, R.P. Patel, A. Agarwal, B.A. Freeman, Y. E. Chen, Nitrated fatty acids: endogenous anti-inflammatory signaling mediators, J. Biol. Chem. 281 (2006) 35686–35698;
(b) M. Delmastro-Greenwood, K.S. Hughan, D.A. Vitturi, S.R. Salvatore, G. Grimes, G. Potti, S. Shiva, F.J. Schopfer, M.T. Gladwin, B.A. Freeman, S. G. Wendell, Nitrite and nitrate-dependent generation of anti-inflammatory fatty acid nitroalkenes, Free Radic. Biol. Med. 89 (2015) 333–341.
[179] L.C. Pinheiro, J.H. Amaral, G.C. Ferreira, R.L. Portella, C.S. Ceron, M. F. Montenegro, J.C. Toledo Jr., J.E. Tanus-Santos, Gastric S-nitrosothiol formation drives the antihypertensive effects of oral sodium nitrite and ntrate n a rat model of renovascular hypertension, Free Radical Biol. Med. 87 (2015) 252–262.
[180] (a) S.N. Chen, M.Z. Hoffman, Rate Constants for the reaction of the carbonate radical with compounds of biochemical interest in neutral aqueous solution, Radiat. Res. 56 (1973) 40–48;
(b) S. Goldstein, J. Lind, G. Mere'nyi, The chemistry of peroxynitrite : implications for biological activity, Methods Enzymol. 436 (2008) 49–61. Part A.
[181] M.F. Beal, Oxidatively modified proteins in aging and disease, Free Radic. Biol. Med. 32 (2002) 797–803.
[182] C.H. Lim, P.C. Dedon, W.M. Deen, Kinetic analysis of intracellular concentrations of reactive nitrogen species, Chem. Res. Toxicol. 21 (2008) 2134–2147.
[183] (a) S. Pfeiffer, B. Mayer, Lack of tyrosine nitration by peroxynitrite generated at physiological pH, J. Biol. Chem. 273 (1998) 27280–27285;
(b) M. Gr Espey, K.M. Miranda, M. Feelisch, J. Fukuto, M.B. Grisham, M.P. Vitek, D.A. Wink, Mechanisms of cell death governed by the balance between nitrosative and oxidative stress, Annals. N. Y. Acad. Sci. 899 (2000) 209–221. Reactive Oxygen Species.
[184] G.L. Squadrito, X. Jin, W.A. Pryor, Stopped-flow kinetic study of the reaction of ascorbic acid with peroxynitrite, Arch. Biochem. Biophys. 322 (1995) 53–59, 184) Bonini, M. G., and Augusto, O. (2001) Carbon dioxide stimulates the production of thiyl, sulfinyl and disulfide radical anion from thiol oxidation by peroxynitrite. J. Biol. Chem. 276, 9749–9754.
[185] C.M. Jones, A. Lawrence, P. Wardman, M.J. Burkitt, Kinetics of superoxide scavenging by glutathione; an evaluation of its role in the removal of mitochondrial superoxide, Biochem. Soc. Trans. 31 (2003) 1337–1339.