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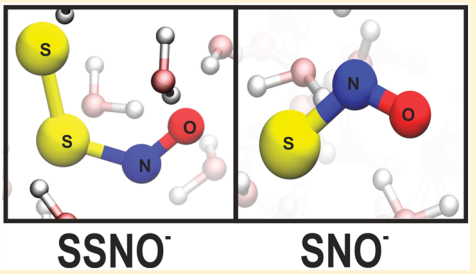
NO/H_2S "Crosstalk" Reactions. The Role of Thionitrites (SNO^-) and Perthionitrites ($SSNO^-$)

Juan P. Marcolongo,[†] Mateus F. Venâncio,[‡] Willian R. Rocha,[‡] Fabio Doctorovich,*[,†] and José A. Olabe*[,†]

[†]Departamento de Química Inorgánica, Analítica y Química Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (INQUIMAE−UBA−CONICET), Pabellón 2, 3er piso, Ciudad Universitaria, C1428EHA Ciudad Autónoma de Buenos Aires, Argentina

[‡]Laboratório de Estudos Computacionais em Sistemas Moleculares, Departamento de Química, ICEx, Universidade Federal de Minas Gerais, 31270-901 Belo Horizonte, Minas Gerais, Brazil

ABSTRACT: The redox chemistry of H_2S with NO and other oxidants containing the NO group is discussed on a mechanistic basis because of the expanding interest in their biological relevance, with an eye open to the chemical differences of H_2S and thiols RSH. We focus on the properties of two "crosstalk" intermediates, SNO^- (thionitrite) and $SSNO^-$ (perthionitrite, nitrosodisulfide) based in the largely controversial status on their identity and chemistry in aqueous/nonaqueous media, en route to the final products N_2O, NO_2^-, NH_2OH/NH_3, and S_8. Thionitrous acid, generated either in the direct reaction of NO + H_2S or through the transnitrosation of RSNO's (nitrosothiols) with H_2S at pH 7.4, is best described as a mixture of rapidly interconverting isomers, {(H)SNO}. It is reactive in different competitive modes, with a half-life of a few seconds at pH 7.4 for homolytic cleavage of the N−S bond, and could be deprotonated at pH values of up to ca. 10, giving SNO^-, a less reactive species than {(H)SNO}. The latter mixture can also react with HS^-, giving HNO and HS_2^- (hydrogen disulfide), a S^0(sulfane)-transfer reagent toward {(H)SNO}, leading to $SSNO^-$, a moderately stable species that slowly decomposes in aqueous sulfide-containing solutions in the minute−hour time scale, depending on $[O_2]$. The previous characterization of HSNO/SNO^- and $SSNO^-$ is critically discussed based on the available chemical and spectroscopic evidence (mass spectrometry, UV−vis, ^{15}N NMR, Fourier transform infrared), together with computational studies including quantum mechanics/molecular mechanics molecular dynamics simulations that provide a structural and UV−vis description of the solvatochromic properties of *cis*-$SSNO^-$ acting as an electron donor in water, alcohols, and aprotic acceptor solvents. In this way, $SSNO^-$ is confirmed as the elusive "yellow intermediate" (I_{412}) emerging in the aqueous crosstalk reactions, in contrast with its assignment to polysulfides, HS_n^-. The analysis extends to the coordination abilities of {(H)SNO}, SNO^-, and $SSNO^-$ into heme and nonheme iron centers, providing a basis for best unraveling their putative specific signaling roles.



INTRODUCTION

A recent Viewpoint entitled "Saying NO to H_2S: A Story of HNO, HSNO, and $SSNO^-$" dealt with the chemical reactions of H_2S with diverse oxidants: NO and its redox siblings NO^+/NO^- (nitrosonium/nitroxyl ions), NO_2^- (nitrite), $ONOO^-$ (peroxynitrite), RSNOs (*S*-nitrosothiols), and $\{ML_5NO\}^n$'s (metallonitrosyls in heme or nonheme coordination environments).[1] The authors addressed the significance of NO/H_2S crosstalk in chemistry and biochemistry by disclosing a retrospective story centered on their work over recent years, highlighting the chemistry of three relevant molecules, and finally posing questions in the perspective of future research directions. Most of the Viewpoint's content, as exemplified by text, charts, and figures, deals with HNO and HSNO. On the other hand, the species $SSNO^-$ has been claimed to be extremely reactive in aqueous solutions, "immediately" decomposing into N_2O and S_8.[1] For the latter reason, it has been suddenly thrown out of the main story by concluding that it cannot sustain any chemical or biologically relevant role.[1]

Many reports on the reactions of H_2S and thiols RSH with oxidant nitrogen species, in either the gas phase or aqueous solutions, in anaerobic or aerobic media, date back to more than a century. Synthetic and mechanistic aspects have been relevant to industrial purposes and for a better understanding of geochemical, atmospheric, and biological processes associated with the origins of life and its long-term evolution.[2−8] The time elapsed for these "crosstalk" reactions to be completed from the mixing of the N/S reactants up to the irreversible formation of stable solids such as S_8, gaseous stable molecules as N_2O, N_2, or NH_3 can be minutes to hours. Many intermediates are generated during the first seconds after mixing, the nature of which can depend on the reaction conditions. Therefore, different stoichiometries and mechanisms have been frequently reported for a given system. The corresponding scientific interest has grown enormously in this

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century along with discovery of the roles of NO and H_2S in animal and plant physiology[8] and is still persistent. An enhanced mechanistic focus is now developing on the chemistry of persulfides RSSH[9−11] and polysulfides HS_n^-,[11−13] as well as on a plentiful set of the so-called reactive sulfur species (RSS).[4,9−13]

HNO (nitroxyl, azanone) is a key molecule emerging at the first instances of many of the considered redox reactions.[1] Despite its high reactivity as a precursor of N_2O and toward diverse substrates in aqueous media, it is currently accepted that it plays specific signaling roles en route to biological effects, as is also the case with NO. The properties of HNO have been comprehensively updated in a book dealing with acid−base and redox reactions, detection methods, and biochemically relevant issues.[14] The structural and chemical properties of HNO/NO^- can be analyzed in the context of reversible redox interconversions of the three redox states of the diatomic nitrosyl group: NO^+, $NO^•$, and NO^-, which have been largely analyzed in the transition-metal coordination chemistry field.[15]

On the other hand, the structure and chemistry of HSNO (thionitrous acid), SNO^- (thionitrite), and $SSNO^-$ (perthionitrite, nitrosopersulfide) are much more poorly understood in aqueous solutions, thus allowing for only limited insight into the biochemically significant issues.[16] By photolysis of *cis*-HNSO (thionylimide) at low temperatures, four isomers (HNSO, HOSN, HSNO, and HONS) were characterized by IR spectroscopy in an argon matrix at −261 °C, showing different stabilities and reactivities (ν_{NO}, 1569 cm^{-1} for HSNO).[1,16] Significantly, *only* HSNO has been obtained at room temperature by the direct mixing of gaseous NO and H_2S on metallic catalytic surfaces, with intermediate formation of N_2O_3;[17] both its cis and trans isomers have been characterized by microwave spectroscopy, providing values of 1570 and 1596 cm^{-1} for the N−O stretching frequencies, as well as notoriously high values for the N−S distances, 1.834 and 1.852 Å, suggesting a high chemical reactivity prone to either homolytic or heterolytic bond cleavage. Quantum-chemical calculations support H−S−N═O as the main resonance structure and a N−S dissociation energy of 127.1 kJ mol^{-1}, lower than that for NOSR's. Theoretical studies on HSNO isomers are highly dependent on the methodologies and basis set used.[16−18] Prohibitively high barriers for interconversions between HSNO, HONS, and HNSO in the gas phase have been calculated (125−209 kJ mol^{-1}),[18] which agree with HSNO being found as the unique isomer in the surface-catalyzed direct reaction.[17] Remarkably, however, much lower values for the *aqueous* interconversion barriers were provided (37.6 kJ mol^{-1}), arising in the polar aqueous environment and water-assisted proton shuttle.[18] Therefore, either of the three isomers could be kinetically accessible under physiological conditions.[1] We found consistent comparisons with the above results by using quantum mechanics/molecular mechanics (QM/MM) molecular dynamics (MD) simulations.[19,20] Figure 1 shows the output for HSNO and SNO^- in an aqueous medium, including the onset of hydrogen bonds. The protonation status of the aqueous solutions of HSNO/SNO^- has not been clearly defined in the literature: both species apparently display similar UV−vis properties (maxima at ca. 320 and 340 nm at pH 7.4 and 11, respectively),[21,22] in agreement with density functional theory (DFT) computations,[19] and the mass-spectral evidence does not allow an unambiguous assignment either.[21] The H−S group would be

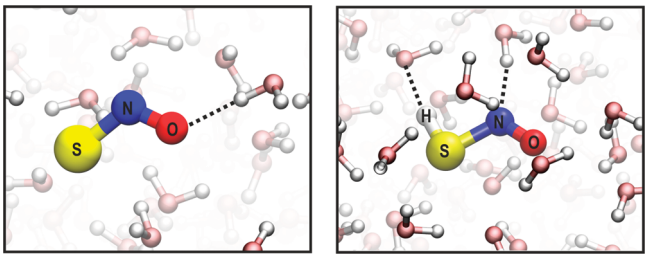

Figure 1. QM/MM MD simulations for SNO^- and HSNO in water, with dotted lines indicating hydrogen bonds. The methodology employed to perform the calculations is described in ref 19.

expectedly more acidic than the O−H group in nitrous acid,[1,16] thus predicting that SNO^- will largely predominate around pH 7 over HSNO. However, the thermal accessibility of more reactive protonated isomers with a putative pK_a greater than that for HSNO could be onset,[20] allowing for deprotonation at higher pH values, say around 10. Under this description, we could explain the coexistence and facile interconversions of all of the protonated isomers in the pH 7−10 range, and therefore we describe the mixture as {(H)SNO} hereafter. Values of the N−O and N−S distances obtained from QM/MM simulations were 1.20 and 1.71 Å (for SNO^-) and 1.16 and 1.77 Å (for HSNO), respectively, in agreement with nearly double and single bonds for N═O and N−S. The shorter N−S distance in SNO^- (cf. 1.695 Å in the solid phase)[23] suggests that it could be less reactive toward N−S cleavage than HSNO.

Finally, $SSNO^-$ is well characterized as an iminium salt {PNP^+SSNO^-} in the solid state,[23,24] as well as a stable anion in aprotic solvents, identified by a UV−vis absorption band maximum at ca. 450 nm.[22,24−27] We highlight the available chemical, spectroscopic, and computational evidence for *aqueous* $SSNO^-$. We focus on the work that has emerged since 2012 on the properties of {(H)SNO} as an intermediate during the transnitrosation reaction of GSNO with H_2S.[21] In that study, a striking initial absorption increase and an ensuing slower decay at 412 nm suggested that $SSNO^-$ might also be formed as a transient, although this possibility was discarded in favor of an assignment to polysulfides, HS_n^-, which were also produced along with NO and HNO/N_2O. In subsequent contributions, kinetic studies and chemical methodologies confirmed {(H)SNO} as a precursor of $SSNO^-$,[22] and evidence for $SSNO^-$ was provided through UV−vis and mass spectrometry (MS).[22,26] Similar conclusions were drawn by using NO as the oxidant toward H_2S.[22,26] Advanced computational work using QM/MM MD simulations combined with time-dependent DFT (TD-DFT) analysis allowed for the best visualization of the solution's structural and UV−vis spectroscopic properties of $SSNO^-$.[19,20] Comparisons between the results in water, alcohols, and aprotic solvents allowed detection of its solvatochromic properties, as evidenced by the UV−vis spectral changes.[19,20] In complementary articles, some spectroscopic and chemical studies on $SSNO^-$ were displayed, namely, (1) a revision of the solid-state structure of *cis*-{PNP^+SSNO^-} and computational characterization of both the cis and trans isomers, as well as the preparation and characterization of the nitrate-like species $SN(O)S^-$,[24] (2) experiments with {PNP^+SSNO^-} interacting with aqueous or aqueous acetone mixtures,[24] (3) reactivity studies in nonaqueous media and in mixed solvents toward nucleophiles.[24,27] Given that arguments on the chemical irrelevance of aqueous $SSNO^-$ are currently supported,[1,28,29]

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we aim to demonstrate that such a conclusion is at least controversial, particularly in a chemical "in vitro" context. We aim to enrich the significance of aqueous $SSNO^-$ in the whole story by addressing the new unsolved questions in the area and contributing positively in this regard to a debate in the scientific community.

TRANSNITROSATION OF RSNO'S (*S*-NITROSOTHIOLS) WITH H_2S. IS THIONITROUS ACID AN INTERMEDIATE EN ROUTE TO $SSNO^-$? A ROLE FOR DISULFIDES HS_2^-

Filipovic and co-workers reported on the chemistry of HSNO aimed at studying its biological performance, particularly its ability to cross membranes and effect the transnitrosation of proteins.[21] To best characterize HSNO, 0.5 mM *S*-nitrosoglutathione (GSNO) and H_2S were mixed at pH 7.4. "H_2S" is a volatile species that dissolves in water by setting equilibrium mixtures, with the good nucleophile HS^- as the main species at pH 7.4 (pK_{a1} = 7.0; pK_{a2} = 12−17).[2,30] The successive UV−vis spectral changes showed decay of the main band of GSNO at 334 nm, along with emergence of a band at 412 nm, I_{412}; the net conversion involved well-defined isosbestic points at 300 and 375 nm (Figure S2 in ref 21). I_{412} reached a maximum absorbance value in 1 min. No UV−vis spectral evidence of {(H)SNO} could be obtained through the kinetic runs, although positive MS evidence was reported.[21] The spectral features of the transnitrosation reactions have been reproduced in subsequent works,[22,26] which also revealed that I_{412} remained stable for more than 30 min, although decaying faster in aerobic media.[31] Pioneering reports by Seel and Wagner[32] and Munro and Williams[33] already suggested that $SSNO^-$ could be present in aqueous media, showing maxima at 409−410 nm. We describe the initial events upon mixing of the reactants through reaction (1), comprising an initial *equilibrium* step, with adequate speciation, and in a generalized way; other *S*-nitrosothiols, *S*-nitrosopenicillamine, *S*-nitroso-*N*-acetylpenicillamine (SNAP), and *S*-nitrosocysteine (CysNO), behave similarly.[22,26] The latter reactions are similar to the transnitrosation of *S*-nitrosothiols (RSNO + R′SH ⇄ R′SNO + RSH).[34] We remark that there is an interchange of the nitroso N═O group among the RS/R′S (or the RS/HS) moieties and avoid the expression "formal NO^+-transfer" currently used for transnitrosation.[1] IR values of ν_{NO} in the 1450−1530 cm^{-1} range reflect the properties of a wide number of RSNO's,[34] strongly supporting a dominant R—S—N═O resonance structure over that for R—S═N—O, with a negligible contribution of the ionic NO^+RS^- form containing the triple-bond-order nitrosonium species.

$$RSNO + HS^- + H^+ \rightleftarrows \{(H)SNO\} + RSH \quad (1)$$

Considering the nucleophilic attack of HS^- at the N atom of RSNO, we expect reaction (1) to be displaced in favor of the accumulation of {(H)SNO} for 1:1 [HS^-]/[RSNO] because the (mainly) protonated thiol (pK_a = 8.8 for GSH) is a very poor nucleophile for the reverse step, in contrast with HS^- for the direct one. For reactions with SNAP, electronic spectral changes and NO-releasing profiles were monitored by using time-resolved UV−vis spectrometry and gas-phase chemiluminescence, at variable values of [HS^-]/[SNAP]: from substoichiometric sulfide up to a 10-fold excess.[22,26] The measurements were done after two incubation periods for the reactants, 1 and 10 min. We highlight some remarkable features, mainly described in Figure 2A−F in ref 26: (i) For 1:1 up to 1:10 [HS^-]/[SNAP], a transient band emerged at 320 nm (overlapped with the decreasing band of SNAP at 340 nm), simultaneously with the onset of I_{412}. Importantly, both bands at 320 and 412 nm attained greater absorbance values for greater [HS^-], for 1 min of reaction advancement, with further absorbance decay at 412 nm. The new features for SNAP provide strong evidence for the intermediacy of HSNO/SNO^- as a precursor of I_{412}.[22,26] (ii) With a high excess of sulfide (2 mM HS^- and 0.2 mM SNAP), a sigmoidal increase of the 412 nm absorption was observed, reflecting a pronounced *induction period*.[26] (iii) The induction period could be gradually suppressed by adding increasing concentrations of polysulfides, HS_n^-, before mixing the reactants (12.5−200 μM); for the greatest [HS_n^-], a true exponential absorption increase for I_{412} could be achieved. It is apparent that, without the addition of polysulfides, sulfide alone reacts slowly with SNAP to build-up I_{412} (even under a 10-fold excess) and that polysulfides need to be generated along the main reaction course (in an autocatalytical way) for allowing the I_{412} display. (iv) The complete spectrum measured 10 min after mixing (1 mM SNAP, 10 mM HS^-, and no external HS_n^-)[26] shows a well-defined band centered at 412 nm attributed to $SSNO^-$, with shoulders at 280 and 259 nm, assigned to HS_n^- and dinitrososulfite, respectively.[26] All of the results described highlight the putative role of HS_2^- or other HS_n^- in the generation of $SSNO^-$ (see below). (v) A *biphasic NO release profile* indicated ongoing decomposition of NO-generating entities, with a fast one reaching a maximum of NO production at 3 min after incubation and a long-lasting NO-releasing component being observed only at high sulfide excess. Because SNAP absorption rapidly disappeared upon incubation under high sulfide excess, the delayed NO release could not have originated from SNAP itself; thus, other NO-releasing products must have been formed in the course of the reaction, viz., $SSNO^-$.[22] (vi) In aerobic experiments, oxygen consumption has been detected at the initial stages of the reaction,[22] suggesting the onset of sulfur radicals. (vii) The nature, yield, rate of formation, and stability of the above cited intermediates were strictly dependent on [HS^-]/[RSNO].[22,26] (viii) Similar spectral changes appeared by using CysNO or GSNO, although with different rates.

Fourier transform infrared (FTIR) and ^{15}N NMR results have been obtained along the onset of reaction (1) with GSNO.[21] A shift in the IR stretching frequency for 1 min after mixing (1515 → 1568 cm^{-1}) was reasonably assigned to the GSNO → HSNO conversion, consistent with a recent HSNO characterization in the gas phase.[17] Additional weaker bands centered at 1615 and 1750 cm^{-1} can be observed in that first spectrum, not assigned by the authors (cf. Figure 3B in ref 21), which increase in the intensity along with the reaction progress, up to 10 min. With respect to NMR, the assignment of the ^{15}N signal at 322 ppm to HSNO/SNO^-[21] needs to be reconsidered in light of other experimental evidence. In fact, chemical shift values at 334/332 ppm have been assigned to $SSNO^-$ in acetone[25] and tetrahydrofuran (THF),[35] respectively. That small difference in the parts per million suggests that we are dealing with the same molecular species in different solvents, namely, $SSNO^-$. By considering that the ^{15}N NMR relevant chemical shifts might span a wide range of ca. 1000 ppm, we aimed to compare the available experimental results with new theoretical calculations on both SNO^- and $SSNO^-$.

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Table 1. Experimental and Theoretical MS, UV−Vis, ^{15}N NMR, and FTIR Parameters Assigned in This Work to $SSNO^-$ and $HSNO/SNO^-$

technique	$SSNO^-$	$HSNO/SNO^-$
MS	93.9427 (exp); 93.94268 (theor)[a]	63.9902 (exp); 63.9852 (theor)[b]
UV−vis (λ_{max}, nm)	409−412 (exp, H_2O);[c] 411 (calcd, H_2O);[d] 422 (exp, MeOH);[e] 448 (exp, acetone);[e,f,g] 458 (calcd, acetone);[d] 446 (exp, THF);[h] 448 (exp, DMF);[i] 447 (exp, acetonitrile);[g] 448 (exp, DMSO)[j]	340 (H_2O, pH 11);[b] 320 (H_2O, pH 7.4);[k] 315,360 (H_2O, theor);[d] 323 (exp, acetonitrile);[g] 315 (calcd, acetonitrile);[d] 325 (exp, DMSO)[j]
FTIR (ν_{NO}, cm^{-1})	1311, 1286, 1270, 1254 (exp); 1311, 1281, 1273, 1251 (calcd)[f]	1569 (exp, gas);[l] 1568 (exp, H_2O, pH 7.4)[b]
^{15}N NMR (ppm, vs NO_2CH_3)	322 (exp, pH 7.4);[m] 314 (exp, 10% H_2O/90% acetone);[n] 350 (exp, 90% H_2O/10% acetone);[o] 334 (exp, acetone);[l] 323 (calcd., gas);[p] 317 (calcd., H_2O and acetone);[p] 332 (THF)[h]	539 (exp, acetone);[l] 540 (calcd, acetone);[p] 550 (calcd, H_2O);[p] 558 (calcd, gas)[p]

[a]Reference 26. [b]Reference 21. [c]References 22, 26, and 31−33. [d]Reference 19. QM/MM MD/TD-DFT calculations. [e]Reference 27. [f]Reference 24. Broad band centered at 1300 cm^{-1} for $SS^{14}NO^-$ in acetone; exp (acetone) and calcd values for $SS^{15}NO^-$. [g]Reference 23. [h]Reference 35. [i]Reference 25. [j]Reference 27. [k]Reference 22. [l]Reference 17. [m]Reference 21. Assigned to $HSNO/SNO^-$ and reassigned in this work. [n]References 24 and 27. Assigned to SNO^- and reassigned in this work. [o]Reference 24. Assigned to SNO^- and reassigned in this work. [p]This work and ref 41. ^{15}N NMR calculations for SNO^- and *cis*-$SSNO^-$ were performed at the TPSSh/aug-pcSseg-4 level of theory,[42−44] validated in ref 45, and checked with appropriate substances. The auxiliary aug-cc-pV5Z/JK basis set[46] for Coulomb and exchange fitting was also employed. These calculations were performed on fully optimized RI-MP2/aug-cc-pVTZ[47] employing the gauge-independent atomic orbital method[48] for evaluation of the ^{15}N NMR isotropic shielding constants, which were converted into the chemical shift scale based on the chemical shifts of nitromethane (CH_3NO_2), also calculated under the same level of theory. All solvent effects for water and acetone presented in this work were calculated using the solvation model density (SMD) of Truhlar et al.[49,50] All quantum-chemical calculations were performed with the *ORCA* program.[51] Chemical shifts, in ppm, refer to $CH_3{}^{15}NO_2$ (exp/calcd): for $^{15}NH_3$, −382/−412; for $^{15}NOSCH_3$, 390/413.[41]

We show below our conclusions on the FTIR and NMR interpretations (see the text and Table 1).

The MS signal at m/z 63.9902 (positive-ion mode) was consistent with an $[HSNO + H]^+$ species.[21] Cortese-Krott and co-workers were unable to obtain any corresponding MS signal under their experimental setup conditions[26] and questioned the significance of the previous value because of the large deviation error of 78 ppm from the theoretical m/z 63.9852.[16] Although the observed HSNO peak could be due (all or in part) to $SSNO^-$ fragmentation (S−SNO bond breakage), we appreciate that Filipovic and Ivanovic-Burmazovic have provided good arguments for their results being correctly obtained and interpreted, together with a sound criticism of the negative results obtained by Cortese-Krott and co-workers, supposedly because of the use of poorly sensitive detection conditions.[1,28] Given the UV−vis and FTIR evidence for {(H)SNO} intermediacy, we conclude that reaction (1) can be accepted as a plausible first step in the transnitrosation processes with HS^- and that {(H)SNO} survives sufficiently for detection before consumption (a half-life of 6 s has been estimated for HSNO, by measuring its unimolecular homolytic decomposition rate through the detection of NO).[21]

In summary, the controversy about thionitrous acid acting as a first intermediate in the transnitrosation reaction seems to be closed, whatever its isomeric description or protonation state considered. In addition, $SSNO^-$ has gained credit for remaining inside the aqueous story, with both {(H)SNO} and HS_n^- appearing as necessary precursors for attaining a fast buildup. We go further into the mechanistic details in the next sections.

DIRECT REACTION, NO + HS^- (NITROSATION OF SULFIDE)

Studies on the direct reaction between NO and H_2S in aqueous media showed a diversity of products such as NH_3, N_2O, N_2, and S_8 among others, depending on the conditions. One of most plausible stoichiometries for the first step has been described as $H_2S + 2NO \rightarrow N_2O + S + H_2O$, with $\Delta G^\circ = -70$ kJ mol^{-1}.[1] The formation of N_2O cannot be explained as a single-step process. In 2014 Eberhardt and co-workers reported a very fast formation of HNO when the NO and H_2S precursors are produced in a colocalized way,[36] at pH 7.4, under anaerobic or aerobic conditions, providing evidence for specific signaling effects of HNO reacting with protein sulfides to form persulfides.[29] Given that direct outer-sphere electron transfer in a possible first step appears to be endergonic ($HS^- + NO \rightleftarrows HNO + S^{\bullet-}$; $\Delta G^\circ = +142$ kJ mol^{-1}), other associative routes have been proposed to favor a lower initial barrier, followed by the subsequent fast removal of products.[1] A mechanistic proposal under anaerobic conditions has been raised, comprising reactions (2)−(7), starting with a first proton-coupled nucleophilic addition (PCNA) step, forming $\{HSNO\}^{\bullet-}$, seemingly a very reducing intermediate. No direct evidence has been provided for characterizing this transient radical,[1,29] although the proposal is consistent with the recently reported reaction process between NO and thiolates, giving the thionitroxide radical $\{RSNOH\}^\bullet$ in the first step,[37] and with the reactions of NO with other biologically significant reductants (tyrosine, ascorbic acid, and hydroquinone) also involving radical intermediates.[38] We published more recently the direct reduction of NO to HNO through a PCET reaction, with a (new) computed redox potential of $E^\circ(NO,H^+/HNO) = -0.16$ V at pH 7.[39] Moreover, on the basis of a previous computational work predicting that NO can be in equilibrium with the $^2HONO^{\bullet-}$ radical in aqueous solutions,[40] we also showed a competitive route by which $^2HONO^{\bullet-}$ could be reduced to HNO in a one-electron step, with $E^\circ = 0.989$ V at pH 7. Both reductive routes are thermodynamically accessible to the current biological reductants, and the one involving the $^2HONO^{\bullet-}$ radical gives further support to reaction (2), with HSNO, HNO, and HS_2^- appearing as follow-up intermediates en route to the irreversible generation of N_2O, NO_2^-, and S_8.

$$NO + HS^- \rightleftarrows \{HSNO\}^{\bullet-} \quad (2)$$

$$\{HSNO\}^{\bullet-} + NO + H^+ \rightleftarrows \{(H)SNO\} + HNO \quad (3)$$

$$\{(H)SNO\} + HS^- \rightleftarrows HNO + HS_2^- \quad (4)$$

$$HS_2^- \rightarrow HS^- + \frac{1}{8}S_8 \quad (5)$$

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Inorg. Chem. XXXX, XXX, XXX−XXX

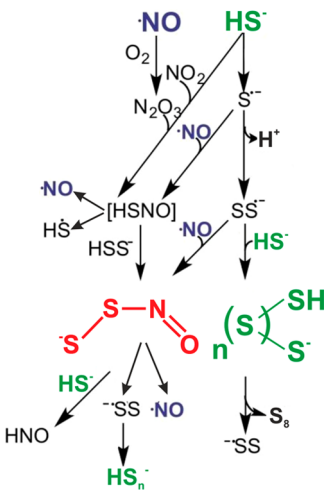
$$HNO + NO \rightleftarrows [N_2O_2]^{\bullet -} + H^+ \quad (6)$$

$$[N_2O_2]^{\bullet -} + NO \rightleftarrows [N_3O_3]^- \rightarrow N_2O + NO_2^- \quad (7)$$

$$2NO + 0.5O_2 \rightleftarrows N_2O_3 \xrightarrow{2HS^-,2H^+} 2HSNO + H_2O \quad (8)$$

Studies on the aqueous direct reaction have also been addressed by Cortese-Krott and co-workers, in both anaerobic and aerobic conditions (with NO or with a diazeniumdiolate NO donor, DEA/NO, respectively).[26] Let us first analyze the aerobic results, as described in Scheme 1, which contains the proposal of $SSNO^-$ generation. This was supported by the absorption increase at 412 nm, with {(H)SNO} as the precursor. {(H)SNO} is proposed to be formed through the intermediacy of N_2O_3, as resumed in reaction (8). Detailed mechanistic studies of the NO/O_2 reaction in diverse media can be found elsewhere and particularly in a recent work by Nava and co-workers reporting the catalytic reactivity of a NO/O_2 gas mixture on a metal surface; this work also includes the new differential mechanistic features appearing when a drop of liquid water is added to the gas mixture.[17]

Scheme 1. Chemical Reaction Cascade Depicting Pathways of Formation and Decomposition of HSNO, $SSNO^-$, and HS_n^- Intermediates in Aerated and Anaerobic Media (Adapted from Reference 26. Copyright 2015 National Academy of Sciences)



For the anaerobic studies, an absorbance increase at 412 nm has also been detected, in a minute time scale.[26] The yield of I_{412} formation depends on both $[HS^-]$ and the rate of NO release, as stated by the authors by showing the successive spectra taken 10 min after mixing. By using Scheme 1, the authors indicated a necessary one-electron oxidation step for HS^-, leading to $S^{\bullet -}$ in order to open the reaction process, although stating that the transient role of a radical N/S species could be speculative; instead, they admitted that the anhydrous Na_2S solid used could contain oxidizing polysulfide impurities.[26]

To tie up the kinetic analysis of both research groups,[1,26] we consider that the successive reactions (2)−(7) account well for the putative reaction progress.[1] However, no mention exists in ref 1 of the I_{412} absorptions eventually corresponding to $SSNO^-$. A possible {(H)SNO} → $SSNO^-$ conversion mediated by HS_2^- has not been considered either,[1] given that the authors assign to HS_2^- a unique role of engaging in a very fast irreversible disproportionation [reaction (5)].[1] Identification of the elusive $SSNO^-$ has been sustained by electrospray ionization high-resolution MS (ESI-HRMS) measurements in the negative-ion mode, under anaerobic/aerobic conditions,[26] together with polysulfides and other species. As shown in this Viewpoint, maxima at 408−412 nm have been systematically found at the initial stages of the reactions of HS^- with RSNO's and other oxidants as well. We describe below the complex nature and role of polysulfide mixtures in the crosstalk scenario.

Because the mechanism advanced through the proposal of reactions (2)−(7) still reflects unpublished results,[1,29] the scene remains open for a complete mechanistic proposal, including the species evolving in the short-, medium-, and long-time scales, with due consideration of the new feature on the possible $SSNO^-$ generation.[26] Such a goal will then provide a basis for elucidating or better understanding the putative biological signaling roles of NO, HNO, {(H)SNO}, and $SSNO^-$.

EVIDENCE ON THE IDENTITY OF $SSNO^-$ AS A FOLLOW-UP INTERMEDIATE TO {(H)SNO}

Table 1 shows the results of different relevant indicators (MS, UV−vis, FTIR, and ^{15}N NMR) that allow one to obtain insight into the properties of $SSNO^-$ and $HSNO/SNO^-$. We detail for each experiment the medium conditions (viz., solvent, pH, etc.), as well as the corresponding theoretical approach when available. A comprehensive discussion ensues on the significance of the measured and calculated values.

MS Results. Significant ESI-HRMS evidence in the negative-ion mode exists for $SSNO^-$ through studies with SNAP reacting with H_2S, showing that it is a main reaction intermediate. This was also found for the direct reaction. Table 1 shows the excellent agreement of exp/theor values, as well as convincing fragmentation spectral patterns and ion chromatographic evidence for the identification of $SSNO^-$, polysulfides, and other products as well.[26] Although these new MS results were briefly mentioned in the previous Viewpoint,[1] the authors made a surprisingly long follow-up criticism, which seems irrelevant to us, based on the measurements being performed with an "NO mix", i.e., an aqueous solution mixture that contained eventually $SSNO^-$ together with other byproduct/intermediates.

^{15}N NMR Results. Table 1 shows the assignments to $SSNO^-$ of the values measured in pure water and 10% water/acetone solutions at 322 and 314 ppm, respectively. These results and assignments show minor shifts with respect to the value measured at 354 ppm in pure acetone. On the other hand, the reaction of $\{PNP^+S_2NO^-\}$ with PPh_3 in an acetone solution led to the onset of a signal at 529 ppm,[25] reasonably assigned by the authors to PNP^+SNO^- because of an S-atom transfer to the phosphine. The assignment was supported by timely reference data stating that SNO^- signals should lie in the range 430−550 ppm.[52] New theoretical evidence shown in Table 1 strongly confirms the latter results, for both $SSNO^-$ and SNO^-, with very close values for the gas-phase calculations as well as those in water and acetone, with a 10−20 ppm dispersion.[41] The differences are relatively small considering that ^{15}N NMR covers around 1000 ppm. In summary, according to the strong experimental and theoretical evidence, the ^{15}N NMR signals corresponding to SNO^- and $SSNO^-$ are separated by ca. 200 ppm, so a 40 ppm shift upon going from acetone to water is produced by a solvent change and/or by

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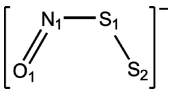
Inorg. Chem. XXXX, XXX, XXX−XXX

Table 2. Selected Distances and Angles with Standard Deviation for solid [PNP][SSNO] and for the Calculated *cis*-SSNO$^-$ Species in Different Media, According to Different Methodologies[19,23,24,41,54]



	ref	level of theory	$d(N_1-O_1)$/Å	$d(N_1-S_1)$/Å	$d(S_1-S_1)$/Å	$\theta(O_1-N_1-S_1)$/deg	$\theta(N_1-S_1-S_1)$/deg
X-ray Diffraction	23		1.222(12)	1.672(9)	1.989(9)	116.1(6)	115.8(4)
	24		1.25(1)	1.70(1)	1.97(1)	117.8(2)	115.1(2)
Geometry Optimization	24	SCS-MP3/aug-cc-pVTZ	1.23	1.67	1.99	119.5	113.4
	54	CCSD(T)-F12/CBS	1.21	1.67	1.95	119.4	113.8
	41	RI-MP2/aug-ccpVTZ	1.25	1.66	1.97	118.8	113.5
Molecular Dynamics	19 (water)	PBE/dzvp	1.24 (0.03)	1.79 (0.01)	2.07 (0.06)	118.9 (0.5)	111.6 (0.5)
	19 (acetone)	PBE/dzvp	1.24 (0.02)	1.80 (0.07)	2.03 (0.04)	119.7 (0.4)	116.1 (0.6)

the presence of an equilibrium between species (probably involving SNO$^-$ and SSNO$^-$) but with a clear predominance of SSNO$^-$, taking into account that the observed shifts are close to the 300 ppm region. It becomes clear now that, during the transnitrosation of GSNO with HS$^-$,[21] the observed value of the NMR signal in water at 322 ppm (the authors have not specified the time elapsed after mixing; cf. Figure 3C in ref 21) was assigned incorrectly to SNO$^-$ by the authors and should correspond to SSNO$^-$, consistent with the proper UV−vis spectral assignment for I_{412}. The SNO$^-$ intermediate has been described as "stable for less than an hour" after mixing the transnitrosation reactants;[21] we have already analyzed that HSNO/SNO$^-$ could not be observed in the successive initial runs with GSNO, consistent with its very low steady-state concentration in the underlying reaction conditions, related to its fast reactivity, giving SSNO$^-$ and other products. Let us remember that I_{412} attains its full development up to 1 min after mixing of the GSNO/HS$^-$ reactants, simultaneously with the onset of NO, supporting a half-life of 6 s for {(H)SNO}.

IR Results. Table 1 reveals some ambiguity on the IR assignments to ν_{NO} in SSNO$^-$. As said above, the initially observed 1515 cm^{-1} → 1568 cm^{-1} conversion is consistent with GSNO → HSNO.[17] We believe that the peak corresponding to SSNO$^-$ might be buried in that broad band, as is predictable from the values for NOSR's.[34] Although the additional peak at 1625 cm^{-1} could also be a candidate,[21] it probably corresponds to NO-containing decomposition intermediates or products,[53] together with that at 1750 cm^{-1}. The FTIR absorptions of {PNP$^+$SSNO$^-$} in an acetone solution display a pattern of bands at much lower frequencies (1270−1330 cm^{-1});[24] we feel that hydrogen-bond interactions in water might induce ν_{NO} to reach higher values, with strengthening of the N−O bond. Some support for these predictions come from our preliminary IR calculations on HSSNO, selected as a model (ν_{NO} 1550(s),1700(w) cm^{-1}),[41] as well as on the corresponding changes in the optimized geometries calculated for *cis*-HSSNO and *cis*-SSNO$^-$ (d_{NO} = 1.159 and 1.234 Å, respectively).[24]

Computational Evidence on the Structural Results. There are several reports on the experimental and computational structures of the SSNO$^-$ anion, and we summarize them in Table 2.[19,23,24,41,54] The isomer stabilities and electronic states of different [S,S,N,O]$^-$ species have been recently published through gas-phase calculations, searching on the identification of signaling pathways during the NO/H_2S crosstalk.[54] A main conclusion was that the NS_2O^- isomer is the most stable species relevant to biochemistry; on the other hand, we found *cis*-SSNO$^-$ to be the most stable aqueous species.[41] We also found that all the isomers are significantly more stable in water than in gas-phase or acetone (by *ca.* 38 kJ mol^{-1}).[41] Related to the structural results, Table 2 shows a good agreement between data from the different groups, dealing either with gas-phase calculations or by using continuum solvent models.[41,54] On the other hand, significant deviations can be appreciated with data reported by employing QM/MM simulations.[19] In addition to the sharp decrease of the $N_1-S_1-S_2$ angle,[19] the lengthening of $d(S_1-S_2)$ and of $d(N_1-S_1)$ were observed in water, due to H-bonds, see next section. A comparatively high value for $d(S_1-S_2)$ (though not so high than in water) occurs also in acetone, which we trace to changes in the Mulliken-charges in both solvents, see Figure 2. We conclude that using continuum models do not account

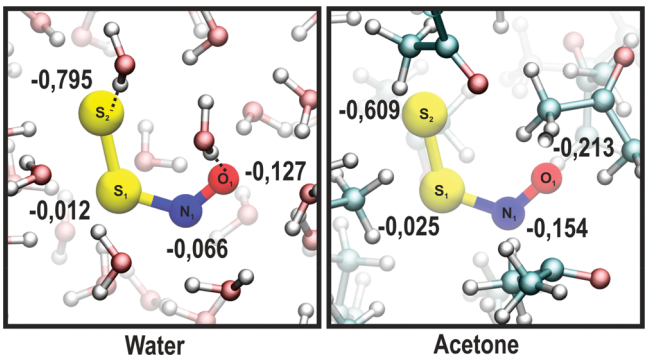


Figure 2. SSNO$^-$−solvent interactions obtained by QM/MM MD simulations. The numbers represent the atomic Mulliken charges, averaged across the simulations. Dotted lines show two selected hydrogen-bond interactions between water molecules and SSNO$^-$. Adapted with permission from ref 19. Copyright 2016 Royal Society of Chemistry.

for the explicit H-bonds for well describing the structures, neither the spectroscopic properties (UV−vis, IR). The H-bonding situation becomes a very important issue for SSNO$^-$, though probably not so much for SNO$^-$. More work is needed to improve the latter theoretical methodologies.[41]

UV−Vis Results and Solvatochromism. We describe in Figure 2 the specific interactions of *cis*-SSNO$^-$ with water and acetone, with the corresponding hydrogen-bond picture and Mulliken charges. The charge distribution has been estimated through the QM/MM MD simulations and might contribute to an understanding of the donor or acceptor character of SSNO$^-$ toward different reactants, as discussed below. Figure 2 highlights how SSNO$^-$ behaves as an electron-pair donor

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Inorg. Chem. XXXX, XXX, XXX−XXX

toward the acceptor solvents, an interaction that stabilizes a higher partial negative charge for S_2 in water than in acetone. Figure 3 shows the calculated UV−vis spectra for $SSNO^-$ in selected protic and aprotic media, with the absorption maxima

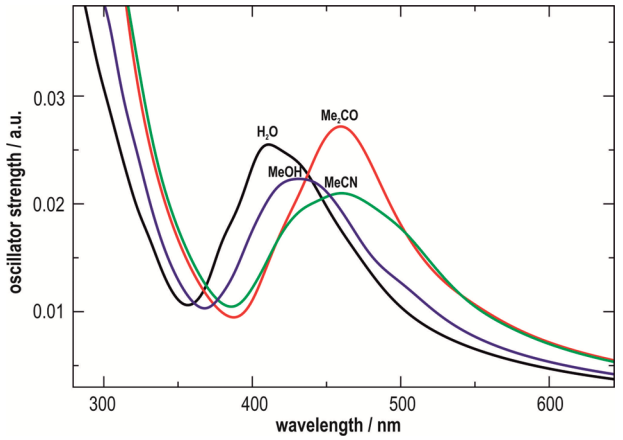

Figure 3. Calculated electronic spectra of $SSNO^-$ in water (black), methanol (blue), acetone (red), and acetonitrile (green) using TD-DFT and QM/MM MD simulations. Adapted with permission from ref 20. Copyright 2017 Elsevier.

shifting toward lower energies upon going from water to alcohols and aprotic solvent solutions. Table 3 contains the

Table 3. TD-DFT Calculations for the Absorption Maxima of N/S Hybrid and Related Species in Water and Aprotic Solvents[a]

compound	solvent	λ_{max}(exp)/nm	λ_{max}(calcd)/nm
$[SSNO]^-$	water	412[22,26]	411
	acetone	448[23,24,27]	458
	acetonitrile	442[23]	458
$[OONO]^-$	water	302[55]	307
	dichloromethane	340[55]	339
EtSNO	water	330[34]	280, 310, 330
ON(SH)S	acetonitrile	358[24]	368
HON(S)S	acetonitrile	358[24]	351
HSNO	water	320,[22] 340[21]	315, 360
SNO^-	acetonitrile	323[23]	315

[a]Experimental values have been slightly modified with respect to ref 19. New values have been added and/or corrected.

experimental and DFT-calculated values of the corresponding absorption maxima for $SSNO^-$ and for diverse relevant small molecules, in water and representative aprotic solvents. It serves as a check on the general validity of the methodology used for getting a reliable spectral discrimination between the involved species.[19,20] We remark on the similar solvatochromism shown by $OONO^-$, a structural analogue of $SSNO^-$.[55] Indeed, the UV−vis spectral results provide additional support for assigning the aqueous I_{412} intermediate to $SSNO^-$, a species that emerges systematically in diverse "crosstalk" reactions afforded in aqueous and nonaqueous media.

Strikingly, the latter comprehensive results and interpretations have been briefly rejected by questioning the UV−vis spectral assignments, based on a "complete absence of regularity" detected in a plot of the *wavelength* maxima (in nanometers) versus dielectric constants of the solvents (cf. Figure 1b in ref 27).[1,27] The criticism apparently implies that a linear correlation must hold in such a plot if a dielectric continuum model is operative. This is not the case, as analyzed above with the computational results, because these models are not appropriate when *specific, donor−acceptor interactions* are established (i.e., weak covalent bonds). We provide arguments based on Gutmann's approach to solute−solvent donor−acceptor interactions.[56] Figure 4 shows a plot of the *energies* of

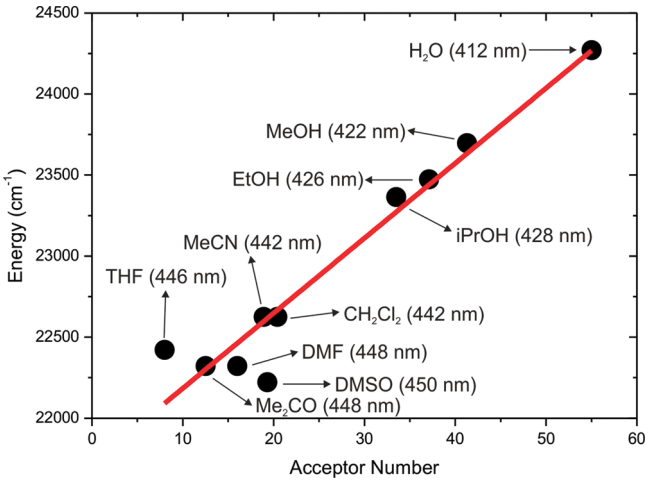

Figure 4. Energy of the absorption maxima of $SSNO^-$ (in cm^{-1}) against the ANs of different solvents. All values of the energy maxima have been extracted from ref 27 (Figure 1b, in a plot of the wavelength maxima versus dielectric constants), except for *N*,*N*-dimethylformamide (DMF)[32] and THF.[35] The ANs correspond to the values published in ref 56.

the electronic absorption maxima for $SSNO^-$ measured in water, alcohols, and diverse aprotic solvents, with values reported in ref 27, against the "acceptor number" (AN) of the solvents. The ANs have been defined by making ^{31}P NMR measurements of triethylphosphine oxide dissolved in the respective solvents[56] and can be expressed by a linear combination of the solvent's polarity/polarizability (π^*) properties and by the solvent's hydrogen-bonding ability (α).[57] Thus, the solvent effects produced by aprotic solvents are determined essentially by the π^* values; on the other hand, the hydrogen-bonding abilities become important for hydroxylic solvents such as water and alcohols. Because the solvents may contain either electrophilic or nucleophilic sites, the donor numbers of the solvents have also been defined and can be used when the corresponding solute is the acceptor.[56] The plot in Figure 4 shows a linear correlation, with only dimethyl sulfoxide (DMSO) and THF as outliers, probably because of their capability of behaving as donors toward the sulfane atom (S_1) in $SSNO^-$. These correlations have been widely used, particularly with coordination compounds containing cyano (as the donor) or ammine (as the acceptor) ligands toward the acceptor or donor solvents, respectively.[58,59] Other properties have also been used in the plots [metal-to-ligand charge transfer (MLCT) band energies, redox potentials, and IR frequencies]. For the IR case, large changes in the ν_{NO} stretching frequencies have been observed by dissolving nitroprusside, $[Fe(CN)_5(NO)]^{2-}$, in different acceptor solvents.[60] Finally, Gutmann's approach has been useful for dealing with solvent mixtures (viz., hydroxylic/aprotic) in a wide relative concentration range, in which a preferential solvation of $SSNO^-$ by water has been established under dilute

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Inorg. Chem. XXXX, XXX, XXX−XXX

conditions with respect to an aprotic solvent.[57,61] We will come back to this property when discussing the nucleophilic reactivities of diverse reagents toward $SSNO^-$.

As reported recently,[19] the main absorption band of $SSNO^-$ can be assigned to a $p(S_2) \rightarrow \pi^*(S_1NO)$ transition. Thus, the observed hypsochromic shift in an aqueous medium can be easily interpreted as due to stabilization of the ground state of $SSNO^-$ through hydrogen bonding between water and the lone pairs at the terminal S_2 atom. Consequently, we might expect significant changes, not only as described by the structures (Table 2) but also in the electronic distribution in $SSNO^-$ when changing the solvents (as suggested by the Mulliken charges), thus influencing the *chemical reactivity*. $SSNO^-$ was shown to be resistant in water to the attack of nucleophiles such as HS^-, CN^-, RSH, and DTT, decaying in hours;[26] we infer that hydrogen bonding to S_2 stabilizes it in water, making heterolytic cleavage of the S_1-S_2 bond not favorable for S^0 transfer to nucleophiles. On the other hand, faster reactions in the minute time scale have been observed in aprotic solvents for the same nucleophiles.[24,37] The differences can be related to the more positively charged S_2 atoms in an acetone (or other aprotic) solution compared to water. We expand below on these nucleophilic reactivity issues.

It has been shown that $SSNO^-$ decomposes in water under anaerobic conditions, in about half an hour, with the rates increasing with the concentration of O_2.[31] In contrast, it is stable for many hours in acetone or other aprotic solvents, with intermediate situations for alcohols. This decomposition mode might be associated with cleavage of the N_1-S_1 bond. Table 2 shows that the latter bond enlarges by 0.1 Å in water, making decomposition much easier than that in acetone. Consistently, $SSNO^-$ decomposes much faster under acidic conditions. Protonation at S_2 is also expected to shift the electron density, making the N_1-S_1 bond weaker. Whether decomposition of $SSNO^-$ ensues through heterolytic and/or homolytic cleavage needs further clarification, given that polysulfides and N_2O[24] (or NO)[26] have been detected as products.

MECHANISM FOR THE CONVERSION OF {(H)SNO} TO $SSNO^-$

We have already analyzed the evidence on {(H)SNO} formation, in either the direct or transnitrosation reaction. Consider some alternative decay modes of {(H)SNO}. In agreement with the authors,[1,21] we already considered reaction (4).

$$\{(H)SNO\} + HS^- \rightleftarrows HNO + HS_2^- \quad (4)$$

Supported by the similar reactivity of *S*-nitrosothiols with nucleophilic RSH/RS^-, the feasibility of reaction (4) has been discussed.[1,20] Despite being endergonic (ΔG_4° = +32 kJ mol^{-1}),[62] it might be plausible under conditions of excess HS^- and/or fast product removal. Evidence of HNO intermediacy and very minor amounts of N_2O was reported at low values of $[HS^-]/[GSNO]$, along with the increased production of N_2O, NH_2OH, and polysulfides under excess sulfide.[21]

Another meaningful proposal for HSNO consumption is reaction (9), implying homolysis of the N–S bond.[21]

$$\{(H)SNO\} \rightleftarrows NO^\bullet + HS^\bullet \quad (9)$$

A value of k_9 = 0.12 s^{-1} ($t_{1/2}$ = 6 s) for NO production has been measured with a specific NO electrode,[21] in agreement with the results using chemiluminiscent methods.[22] Reaction (9) evolves faster than that in the homolytic decompositions of RSNOs, consistent with the S–N bond dissociation energy being smaller in HSNO versus RSNO by 12 kJ mol^{-1},[62] as well as by the longer N–S distance in HSNO (1.834 Å) compared to RSNO's (1.75 Å).[17] Reaction (9) can proceed through the fast removal of sulfur products, as shown in reactions (10) and (11).[63]

$$HS^\bullet \rightleftarrows S^{\bullet-} + H^+ \qquad pK_{10} = 3.4 \quad (10)$$

$$S^{\bullet-} + HS^- \rightleftarrows HS_2^{2\bullet-} \qquad K_{11} = 9 \times 10^3\ M^{-1} \quad (11)$$

Because the radical anion produced in reaction (11) is a strong reductant (E = −1.13 V),[63] a third decaying mode for {(H)SNO} can be proposed, reaction (12).

$$\{(H)SNO\} + HS_2^{2\bullet-} \rightarrow NO^\bullet + HS_2^- + HS^- \quad (12)$$

Further fast reduction of NO to HNO by $HS_2^{2\bullet-}$ can also be envisaged, reaction (13) (as well as successive one-electron reductions of HNO to NH_2OH/NH_3). This proposal is supported by the previously discussed reactivity of NO, giving HNO by using strong biologically relevant reductants.[38]

$$NO^\bullet + HS_2^{2\bullet-} + H^+ \rightarrow HNO + HS_2^- \quad (13)$$

Reactions (4), (9), (12), and (13) allow for the colocalized formation of NO/HNO, thus promoting irreversible N_2O release.[1] The mechanism can be related to the generation and further reactivity of hyponitrite radicals $HN_2O_2^\bullet$, which give $N_3O_3^-$ as an intermediate that slowly produces N_2O and NO_2^-.[53]

Disulfides are proposed to be produced through reactions (4), (12), and (13), along with the onset of both the transnitrosation and direct reaction processes. The tendency of sulfur to catenate extends from disulfides to a series of polysulfide dianions $[S_n]^{2-}$ (n = 2–9) and related radical monoanions $[S_n]^{\bullet-}$.[12] In addition to organic polysulfanes R–S_n–R, partially substituted hydropolysulfides R–S_n–H have been described. We focus here on aqueous polysulfides $[HS_n]^-$ under pH 7 conditions, containing partially oxidized soluble sulfur compounds, with a sulfide species bound to chains of sulfanes. $[HS_n]^-$ are pH-dependent unstable species and usually contain equilibrium mixtures of components with different numbers of S atoms, formed through disproportionation. The sulfur chains are thermodynamically stable toward homolytic S–S dissociation in water and interconvert rapidly between different HS_n^-. At pH values lower than 7–8, these reactions can irreversibly lead to insoluble cyclic S_8, with the slow onset of colloidal solids. Reaction (14) describes this complex process in a simplified way:[13]

$$HS^- + nS^\circ \rightleftarrows HS_2^- \rightleftarrows HS_3^- \ldots HS_9^- \rightarrow HS^- + S_8 \quad (14)$$

HS_2^- is reported to be the dominant species in diluted aqueous solutions in the pH 5–9.5 range.[12] Importantly, HS_2^- (as well as other HS_n^-) can survive enough at pH 7.4 to engage in specific reactions with other substrates through S^0 transfer. We propose reaction (15) as one of the possible steps for buildup of $SSNO^-$. It can be described as a transnitrosopersulfidation reaction.[6] Other feasible production steps for $SSNO^-$ could be the direct recombination of NO with equilibrated $S_2^{\bullet-}$[26] or reaction of the NO_2^- product with di(poly)sulfides, namely, a nitrosation process with the NO^+ intermediate.

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Inorg. Chem. XXXX, XXX, XXX–XXX

$$\{(H)SNO\} + HS_2^- \rightleftarrows SSNO^- + HS^- + H^+ \quad (15)$$

In a recent work,[19] we performed QM/MM MD simulations in aqueous solution to determine the free energy profile of the reaction HSNO + HS_2^- ⇄ HSSNO + HS^-, which differs from reaction (15) in proton transfer from the weak acid HS_2NO to the medium. The latter reaction proceeds with $\Delta G^{\ddagger}$ = 32 kJ mol^{-1}, ΔG_r° = −8 kJ mol^{-1}, with $K \sim 25$ and can be described as a fast equilibrium process. By assuming $pK_a(HS_2NO) = 5$, we estimated K_{15} to fall in the range 10^2−10^3 at pH 7, consistent with the relative values of the forward/reverse specific rate constants for HS_2^-/HS^- reactivity.

Knowing the exact speciation of polysulfides inside the cells is a currently investigated problem. To identify each polysulfide in solution, alkylation techniques have been used in both organic and aqueous models.[11−13,64] Caution must be exercised for interpreting the results when the alkylation rates are not faster than the onset dynamic equilibrium of HS_n^-. In those cases, the electrophilic labeling is said to be under Curtin−Hammett control, meaning that the final alkylated mixture may not represent the speciation that prevailed before alkylation. In this context, reaction (15) could imply any other HS_n^- other than disulfide.

With reactions (1)−(15), we can explain the observed conversion of GSNO into I_{412}, up to 1 min after mixing,[21] consistent with $SSNO^-$ reaching steady-state conditions by way of the removal of HSNO through reaction (9). This agrees with the early generation of NO,[21,22] and most revealing is the *simultaneous* buildup of NO and I_{412} ($t_{1/2}$ = 6 s), which means that reaction (15) is reached in a fast way. Not unexpectedly, HS_2^- behaves as a stronger nucleophile than HS^-, in agreement with its greater polarizability, a result also found when the reactivities of persulfides RSS^- and RS^- were compared.[65] For the same reasons, reaction (4) becomes less favorable than reaction (15), unless we employ excess sulfide conditions. The competitive decay routes for {(H)SNO}, reactions (4) and (15), necessarily lead to smaller yields for $SSNO^-$ production with respect to the initial oxidant concentration, given N_2O formation. Finally, the observation of a second delayed process of NO generation[22,26] indicates that $SSNO^-$ could be the source of long-lasting NO release promoting the sustained activation of guanylate cyclase (sGC).[66] The decomposition mechanisms of aqueous $SSNO^-$, reported to generate NO and $S_2^{\bullet-}$,[26] might be open for further analysis.

Additional evidence for the mechanistic discussion comes from the results on O_2 consumption during the initial instances of the reaction, as well as from the onset of induction times for the initial reaction advancements.[26] Closely related early thiolate-radical consumptions in oxygenated media have been reported for the reactions of nitroprusside with thiols.[67] The O_2 decay occurs in both anaerobic and aerobic media. It has been suggested that O_2 can attack the intermediate sulfide radicals formed in reactions (10)−(12). A mechanistic proposal on the reaction of the inert one-electron oxidant $[IrCl_6]^{2-}$ with H_2S provides solid evidence on the participation of sulfide radicals for the production of $[IrCl_6]^{3-}$ and polysulfides.[68] Again, the onset of polysulfides was found to begin after the (nearly) complete consumption of the oxidant.

CAN $SSNO^-$ SURVIVE IN AQUEOUS MEDIA? CAN IT HAVE A SUSTAINED BIOACTIVITY?

In 2015, Wedmann and co-workers tried to demonstrate why $SSNO^-$ was unable to account for sustained bioactivity of NO,[24] in response to previous reports,[22,26] and focused on the putative role of $SSNO^-$ in cell signaling. An exhaustive characterization of the anion was accomplished with the solid salt (PNP^+SSNO^-}, in aprotic or aprotic/water solvent mixtures, by employing UV−vis, FTIR, ^{15}N NMR, and computational methodologies.[24] The X-ray structure showed no new information compared to that originally reported by Seel and Krebs.[23] Other spectroscopic properties in acetone showed notorious differences compared to water. Most importantly, the studies on the reactivity of $SSNO^-$ in acetone toward diverse nucleophiles—H_2S, glutathione, and cyanide—revealed a fast reaction with $SSNO^-$,[24,27] in sharp contrast with the reported unreactivity in an aqueous medium.[22,26] The latter differences were attributed to the unknown composition of the "NO mix" solutions used by Cortese-Krott and co-workers,[24,27] in contrast with the authors employing "pure" $SSNO^-$ derived from the solid salt. The experiments in aprotic media might provide useful information on the chemistry expected at hydrophobic biological compartments, and we describe here how important changes in the reactivities can be onset with respect to aqueous solutions because of strong solvatochromism. These results add an important caveat upon analysis of the putative reactivity of $SSNO^-$ in specific cell compartments, in addition to the uncertainties in the real, steady-state concentrations of the reactants, pH, O_2 availability, etc., eventually determined by the complex enzyme-generating machineries that might operate inside a given local environment.

With the previous considerations in mind, we afford the first question of the title by considering the *most relevant and significant* material in ref 24, namely, Figure 7, describing experiments on the transfer of "pure" $SSNO^-$, either as a solid or dissolved in acetone, by mixing with pure or mixed solutions. The main conclusion by the authors is that a band at ca. 420 nm, appearing in nearly all experimental situations, cannot be $SSNO^-$; instead, it corresponds to polysulfides. In contrast, we highlight our interpretation, which is just the opposite. We anticipate the equilibrium reaction (15′), written in an inverted way to reaction (15), in order to better illustrate how $SSNO^-$ might evolve to a quasi-equilibrium situation by mixing with HS^-, and subsequently tracked to the final products through subsequent decay of {(H)SNO} and HS_2^-.

$$SSNO^- + HS^- + H^+ \rightleftarrows \{(H)SNO\} + HS_2^- \quad (15')$$

(1) The broad bands in Figure 7A,B in ref 24, centered at 420 nm (I_{420}), build up immediately after mixing, emerging over the tail of intense absorptions in the far-UV region from the polysulfide/sulfur solids, revealing the onset of suspensions. In parts A (final 50% H_2O/acetone mixtures) and B (10% H_2O/90% acetone) of Figure 7 in ref 24, both under excess sulfide, I_{420} should be assigned to $SSNO^-$, consistent with solvatochromism. Hydroxylic/aprotic mixed solutions frequently show "positive deviations" from ideal behavior (i.e., a nonlinear dependence of a given property on the composition of the solution); thus, water occupies preferentially the solvent shell of $SSNO^-$, over the aprotic component; this phenomenon is best appreciated in the solutions more diluted in water. On the other hand, for more enriched water

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Inorg. Chem. XXXX, XXX, XXX−XXX

solutions, the formation of hydrogen-bonded water polymers makes the occupancy of the $SSNO^-$ sites by the aprotic component easier. These results have been described as a preferential solvation of either component in the environment of the solute[57,61] and explain why the maxima locate at 420 nm, not at 412 nm, as found for a pure aqueous solution (considering that the maxima for $SSNO^-$ lie around 450 nm for pure aprotic solvents). There are other factors probably influencing the actual composition of the solvation shell, viz., the presence of countercations, sulfides, and soluble/colloidal polysulfides and dispersion effects, although the preferential solvation issue seems to be the determinant.

(2) In Figure 7C in ref 24, concentrated PNP^+SSNO^- was mixed with excess sulfide (1:10), filtered, and diluted further. After 90 min, I_{420} disappears completely and a well-defined band appears at 345 nm, a signature for SNO^- that could also include NO_2^-. We propose that reaction 15′ onsets after mixing; I_{345} evolves under steady-state conditions with further decomposition through reaction (9); similarly, HS_2^- leads to sulfur solids, reaction (14). We consider that aqueous $SSNO^-$ gives SNO^- through a S^0 transfer to sulfide; the rate is significantly *slower* than that observed in nonaqueous media.[24,27]

(3) In Figure 7D in ref 24, no traces of I_{420} show up after a direct transfer of the solid $\{PNP^+SSNO^-\}$ to the aqueous buffered solution at pH 7.4; instead, again a very intense absorption in the far-UV region appears. This has been interpreted as due to a fast decomposition of $SSNO^-$, giving polysulfide/sulfur solids and N_2O.[24] However, such a transfer procedure could have immediately precipitated all the solid salt. Therefore, the assertion on that extreme instability of aqueous $SSNO^-$ at pH 7.4 is at least doubtful for us, although it is generally accepted that decomposition through S−N bond cleavage ensues under more acidic conditions. Besides, the inset in Figure 7D in ref 24 showing that I_{448} appears in acetone for a similar concentration of PNP^+SSNO^- is perfectly normal, as $SSNO^-$ is known to be stable for hours in organic media under deaerated conditions.[35]

(4) For Figure 7E in ref 24, in the presence of excess sulfide (1:10), I_{420} decays, forming I_{345} with a half-life of ca. 7 min. Because the elapsed time is smaller than that in Figure 7C in ref 24, I_{420} is still present under *equilibrium* conditions [reaction (15′)].

(5) Figure 7G in ref 24 shows the spectral NMR outputs generated with solvent mixtures and confirms the $SSNO^-$ identity in them. By mixing Na_2S and $PNP^+SS^{15}NO^-$ in 10% water/acetone, a peak emerged at 314 ppm,[24,27] moderately displaced from the value for $SSNO^-$ in pure acetone at 354 ppm.[35] The value at 314 ppm is consistent with the differential solvation properties of water toward $SSNO^-$ (see above).

(6) In Figure 7I,J in ref 24, successive UV−vis spectra show the decay of I_{448} or I_{420} when PNP^+SSNO^- is dissolved either in pure acetone or in a 10% H_2O/acetone mixture, after the addition of a 10-fold excess of dithiothreitol (DTT). For pure acetone, I_{448} decays with a half-life of 7 min until complete consumption of $SSNO^-$. On the other hand, the decay of I_{420} is slower with the mixture, although still in the minute time scale. We remark that DTT was shown to be unreactive toward I_{412} formed as a product of aqueous transnitrosation.[26]

(7) In a more recent work,[27] Wedmann and colleagues revisited the reactions of $SSNO^-$ toward various nucleophiles: sulfide, cyanide, and glutathione. For HS^-, reaction (15′) has been proposed to occur, written as an irreversible process. ^{15}N NMR was used for identifying products by using either 10% water/acetone solutions or pure aprotic solvents (THF). For 10% water mixtures, the reaction was shown to be slow; a sample taken 1 h after mixing showed a peak at 314 ppm, which the authors assigned to SNO^-. The NMR region has not been informed up to 600 ppm, in order to check what we analyze in Table 1 and subsequent text; besides, it is unclear for us whether the signal at 354 ppm for pure acetone conditions is still present after 1 h, in order to confirm that the equilibrium reaction reached completion. On the other hand, the reaction evolved much faster for the pure aprotic solutions, as revealed by the ESI-time-of-flight (TOF)-MS experiments taken 1−4 min after mixing, which allowed the detection of both products, SNO^- and HS_2^-. Finally, the authors studied a similar reaction with CN^-, leading to thiocyanate. The reactions in pure acetone showed again a fast evolution, with complete loss of the 448 nm band in 200 s. On the other hand, the measurements with 5:2 acetone/methanol solutions were much slower; when recorded 4 h after mixing, the NMR results showed the ^{15}N peak at 314 ppm, along with evidence of thiocyanate generation. Finally, the reactions with glutathione were shown to behave similarly. In conclusion, all of the experiments and interpretations confirm that the S-transfer reaction from $SSNO^-$ to the nucleophiles behaves quite differently according to the nature of the solutions used (protic, aprotic, and mixed), as explained above. Besides, our interpretations here agree with the previous conclusions on the identity of $SSNO^-$ (as demonstrated by the well-disclosed UV−vis and ^{15}N NMR properties with respect to SNO^-) and the structural explanations accounting for the different behaviors of $SSNO^-$ in pure aprotic solutions compared to that in hydroxylic media.

Turning back to the UV−vis evidence, we highlight a recent report by Jacob and co-workers on the role of polysulfanes (RS_xR', x = 1−4) in the *aqueous-phase* decompositions of GSNO, giving NO in the presence or absence of thiol reducing agents: cysteine and glutathione.[69] The observed enhancement of NO production in their presence allowed one to propose that the intermediate H_2S generation could explain the *observed* I_{412}, consequently assigned to $SSNO^-$. Not unexpectedly, it was found that *no influence* of the addition of DTT exists on the decay of $SSNO^-$, in contrast with the results of Figure 7I,J in ref 24 and the complementary results more recently reported in acetone.[27]

Ivanovic-Burmazovic and Filipovic have made comments[1] on the recent results dealing with the NO/H_2S crosstalk by Pluth and colleagues.[35] Because the issue is most relevant to the content of this section, we make precisions. In that work, the authors did not "readdress the $SSNO^-$ formation from NO and H_2S", thus being unable to find "any proof of its existence" (this was stated by them in a previous article; it is a generally accepted conclusion). Instead, as the title indicates, the studied reaction dealt with the reaction of *nitrite* with organic hydropersulfides RSSH in THF solutions, forming a mixture of polysulfide intermediates containing the trisulfide radical anion $S_3^{\bullet-}$ and organic polysulfanes RS_nR. The polysulfide mixture reacted further with nitrite, and $SSNO^-$ was identified through its maximum absorbance value at 446 nm as a new intermediate, I_{446}, which subsequently decayed to NO and polysulfides, although it could be stabilized for hours under the absence of O_2 or light.[35] The confirmed formation of I_{446} assignable to $SSNO^-$ was also achieved by reacting nitrite with S_8 in THF and, most importantly, by using labeled $SS^{15}NO^-$ to

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Inorg. Chem. XXXX, XXX, XXX−XXX

obtain a signal at 332 ppm (Table 1). Finally, by arguing specifically on the controversial issue of stability in water, they quoted that $SSNO^-$ was *stable upon the addition of water* to the THF solutions under dry anaerobic conditions and that the absorbance maxima *shifted toward lower wavelengths* (from 445 to 422 nm) with increasing water concentrations up to 1:1 H_2O/THF, supporting its *stability in aqueous environments* (Figure SI8 in ref 35).

As a conclusion on the claimed *extreme reactivity* of aqueous $SSNO^-$,[1,24] wrong interpretations of their own results (viz., I_{420} is a polysulfide; the NMR signal at 322 ppm pertains to SNO^-)[21,24,27] led the authors to miss the main points related to $SSNO^-$ identity. It is demonstrated again that I_{420} is $SSNO^-$. Most important is the recognition that the reactivity of $SSNO^-$ differs dramatically in water and aprotic solvents.

Related to the second question in the section title, we consider $SSNO^-$ accounting for the sustained bioactivity of NO, as focused on in ref 24. We appreciate that this could be possible, as reported elsewhere,[22,26,66] given that aqueous $SSNO^-$ behaves as a late source of NO. Another signaling pathway associated with the expression of heme oxygenase has been proposed for $SSNO^-$.[70] Filipovic and Ivanovic-Burmazovic concluded that only HNO, HSNO, and RSSH could have biological relevance on the basis of reactions (2)−(7) proposed for the direct reaction;[1] they argued on the putative decay of the intermediate radical $HSNO^{\bullet-}$ through reaction with NO and with the most probable physiologically available electron acceptors O_2 and protein thiols RSH. However, we showed that HSNO survives enough ($t_{1/2}$ = 6 s) for reaction with abundant HS_2^-/HS_n^- [reaction (15)], as well as with HS^- [reaction (4)]. Besides, no other role is assumed for HS_2^- other than it being very unstable and irreversibly reactive toward disproportionation. However, HS_2^- is a member of the reversibly and rapidly interconverting colloidal polysulfides, which might survive enough to give $SSNO^-$ through S^0 transfer. Indeed, both the HSNO and HS_2^- intermediates can be generated under similar space-timing (colocalized) conditions, favoring reaction (15).

The impossibility of $SSNO^-$ being generated in cell compartments has also been raised by Koppenol and Bounds,[62] by performing calculations based on the thermochemical and kinetic data, for both transnitrosation and direct reactions, by assuming concentrations of H_2S/NO in the micromolar/nanomolar range, respectively. We commented above on the difficulties of getting trustable evidence of real reactivity situations for different biological local sites, as revealed by the current discussions on how we need to account for the speciation of RSS inside the cells.[64] Besides, the authors used tentative rate laws, omitted mentioning reaction (15), and neglected the role of polysulfides, all crucial important factors for $SSNO^-$ generation, as demonstrated above. Therefore, we prefer to leave these calculations and consequent interpretations as an open issue.

REACTIONS BETWEEN $SSNO^-$ AND HEMEPROTEINS

In 2016, Bolden and co-workers showed biorelevant evidence on the chemical reactivity of $SSNO^-$ toward ferrous and ferric iron-containing proteins.[31] The transnitrosation reaction of GSNO with H_2S was employed as a source of I_{412}, with 2:1 $[HS^-]/[GSNO]$, under conditions similar to those used before[21] and with closely similar absorbance−time spectral features. A bimolecular rate constant for I_{412} formation was estimated, $k = 640 \pm 200\ M^{-1}\ s^{-1}$. By using deoxyhemoglobin as a target, I_{412} reacted through the formation of $Hb^{II}NO$, as indicated by the electron paramagnetic resonance evidence. Although mechanisms have not been defined, the authors proposed the cogeneration of $S_2^{\bullet-}$, resembling the reported decomposition of $SSNO^-$ through homolytic N−S cleavage.[26] Interestingly, $Hb^{II}NO$ was not formed when HbCO or oxyhemoglobin was used, showing that a vacant heme site is needed; a previous coordination event for $SSNO^-$ into Hb^{II} could not be demonstrated however. The reaction of I_{412} with metMb also led to $Mb^{II}NO$ through a direct reaction, with proposed cogeneration of elemental S, implying cleavage of the N−S bond in $SSNO^-$.

All of the latter experiments seem conclusive on I_{412} behaving as a NO-containing species, namely, $SSNO^-$, excluding its assignment to a polysulfide.[21] Related to the objections of using solutions in which $SSNO^-$ appears as mixed with unknown species,[1] the authors used $SSNO^-$ stocks generated anaerobically within 10 or 30 min after mixing GSNO with H_2S, subsequently degassed with argon, and used immediately for reaction with the heme proteins. Therefore, considering that {(H)SNO} had already been consumed during the 10−30 min incubation period, we find no other possible NO-generating species other than $SSNO^-$ that could lead to $Mb^{II}NO$ or $Hb^{II}NO$ under the conditions used.

REACTION OF $ONOO^-$ (PEROXYNITRITE) WITH H_2S

Remarkably, a very similar UV−vis spectral display was reported for the reaction of H_2S with peroxynitrite at pH 7.4 (in fact, a mixture of $ONOO^-$ and ONOOH). Figure 5 shows

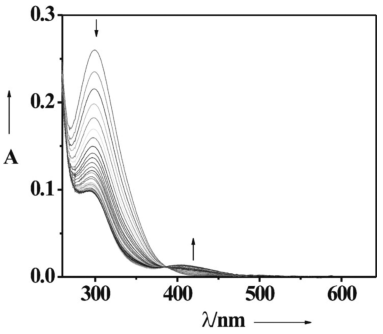

Figure 5. Successive UV−vis spectra for the reaction of 140 μM $ONOO^-$ with 1.25 mM H_2S at 37 °C. Adapted from ref 71. Copyright 2012 Portland Press Ltd.

the decay of the main band of $ONOO^-$, centered at 302 nm, along with the onset of a "yellow reaction product", with a maximum at 408 nm (I_{408}). I_{408} was stable under anaerobic conditions, although it decayed in 30 min under air bubbling, with the formation of a new broad peak centered at 350 nm.[71]

In a subsequent study a pH-independent rate constant was reported, $k = 5.6 \times 10^4\ M^{-1}\ s^{-1}$, along with reformulation of the previously proposed mechanism.[72] We point to some significant issues: (i) Figure 6 shows a slow absorbance *increase* at 302 nm, starting *after the nearly complete consumption* of $ONOO^-$ concomitant with the onset of I_{408}.[72] The timing features suggest positive evidence for polysulfides *delayed* generation and negative evidence on I_{408} being a polysulfide. (ii) A short induction time can be observed for the buildup of I_{408}, seemingly related to the delayed production of HS_2^-. (iii)

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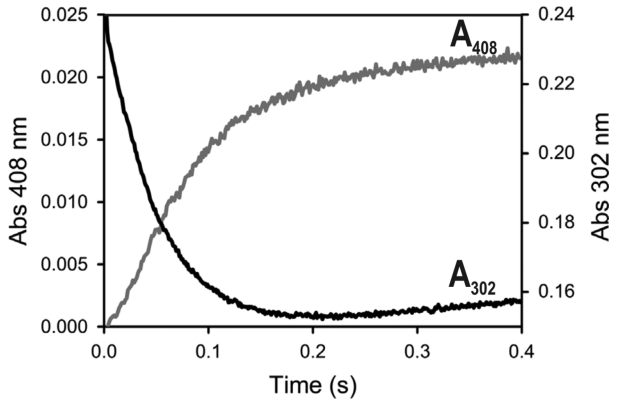


Figure 6. Absorbance traces at 302 nm (decay) and 408 nm (onset) for the reaction of 0.2 mM $ONOO^-$ with 2.1 mM Na_2S at pH 7.3 and 37 °C. Adapted with permission from ref 72. Copyright 2015 Elsevier.

Early consumption of O_2 has been detected, which probably relates to an attack on the intermediate sulfur radicals.[71] (iv) The value of the rate constant for the reaction of $ONOO^-$ with cysteine was similar to that with HS^-, although *without* the appearance of I_{408}, consistent with the formation of unreactive or poorly reactive disulfides R_2S_2.[72,73]

The authors proposed a nucleophilic addition of HS^- to ONOOH, with formation of the sulfenic derivative HSOH + NO_2^- in the first step, followed by a faster attack of HS^- at HSOH, giving HS_2^-, which finally reacted with ONOOH, leading to the yellow product.[72] They concluded, aided by theoretical calculations, that a mixture of isomers was formed, namely, protonated thionitrate $HSNO_2$ or sulfinyl nitrite HS(O)NO. We guess that HS_2^- might also react as a nucleophile toward ONOOH, giving $SSNO^-$ + H_2O_2, as well as sulfur solids in a longer time scale. Finally, and closely related to the latter guess, we remark on a reported mass signal (ESI-ion-trap MS equipment; positive ionization mode)[71] at m/z 96, which shifted to m/z 97 upon ^{15}N enrichment ($HSSNO + H^+$?). This was never discussed further.

REACTION OF NO_2^- WITH H_2S

The direct outer-sphere reaction seems to be thermodynamically impossible based on the one-electron redox potentials of the reactants, imposing an activation barrier of +70 kJ mol^{-1}, which agrees with observations that mixed solutions of NO_2^- and HS^- at pH 7.4 remain stable for at least 1 h. Previous results indicate, however, that nitrite-reduction products are formed, even under neutral anaerobic conditions and free of metal ions. Cortese-Krott and co-workers have reviewed this subject,[74] inspired by the recognition that nitrite and nitrates could serve as alternative endogenous sources of NO in an oxygen-dependent manner, through the onset of enzymatic and nonenzymatic pathways. They concluded that formation of the final products NO, HNO/N_2O, and S_8 could be explained through the crucial intermediacy of HSNO.

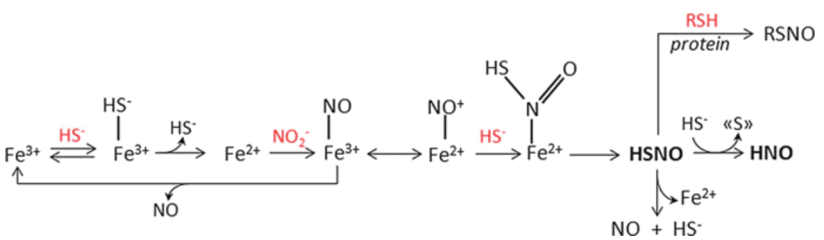
By analogy with the recent mechanistic routes proposed for the endergonic first step in diverse reactions of HS^-, RS^-, and ROH with NO, we suggested an associative route leading to a PCNA from HS^- to the antibonding vacant orbital in NO_2^-, leading to $S^{\bullet-}$ and NO.[7] As discussed above, by reaction with HS^-, $S^{\bullet-}$ is a precursor of the stable and strongly reducing $HS_2^{\bullet 2-}$ radical, allowing for a fast formation of NO, HNO/N_2O, and HS_2^-. Thus, $SSNO^-$ could be formed in a manner similar to that proposed for the direct NO/H_2S reaction [cf. reaction (15)]. Finally, HS_2^- reacts competitively through disproportionation, leading to polysulfides and sulfur solids. We conclude that NO_2^- can very slowly produce NO under in vitro conditions. In the vasculature, high [HS^-] and anoxic conditions could favor the reaction, given that O_2 is an effective trapping agent for $S^{\bullet-}$, much faster than that for NO.[75] In short, here again, association could provide a favorable route, along with the coupling of subsequent exergonic steps.

CROSSTALK INCLUDING IRON BINDING. NITRITE REDUCTION BY H_2S CATALYZED BY AN IRON PORPHYRIN

We reviewed recently the activation of NO and H_2S on heme platforms, as well as the role of heme compounds in the NO/H_2S crosstalk.[7] On the basis of the seminal work of Kim-Shapiro and Gladwin,[76] the role of NO_2^- in NO homeostasis under hypoxic conditions led to wide interest in the enzymatic systems for conversion of NO_2^- to NO mediated by a diverse set of nitrite reductases.[77] The molecular basis of the mechanism of heme-based reduction of NO_2^- was studied by Heinecke and co-workers by using a water-soluble heme compound, Fe^{III}(TPPS).[78] The results proved the catalytic reduction of NO_2^- to NO and to HNO at pH 5.8. A detailed reaction pathway implied an O-atom-transfer mechanism from NO_2^- to the acceptor.

The assay of H_2S as a possible regulator of these nitrite reductase heme model systems, with clear significance as a putative crosstalk mechanism, was soon performed by Miljkovic and co-workers by selecting an octaanionic water-soluble ferric porphyrin to perform catalysis.[79] Under excess NO_2^-, at pH 7.4 and strict anaerobiosis, the concomitant addition of NO_2^- and HS^- was proposed to yield the reductive nitrosylation product $Fe^{II}NO^{\bullet}$ and, supposedly, the HSNO species as the outcome of sulfide oxidation. Scheme 2 describes a situation with HS^- excess over NO_2^-.[79] Although the concentrations of H_2S in whole tissues are orders of magnitude

Scheme 2. Catalytic Reactivity of NO_2^- with an Excess of HS^- in an Iron Porphyrin Environment in Anaerobic Conditions (Extracted from Reference 7. Copyright 2016 Elsevier BV)



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lower than the previously accepted values,[80] excess sulfide over nitrite could exist in an intracellular microenvironment in order to activate a local signaling mechanism, as suggested in Scheme 2. Detection of the ferrous porphyrin revealed an alternative reaction pathway, where the low-spin $Fe^{III}(SH^-)$ adduct and the crosstalk product $Fe^{II}(HSNO)$ could be detected by high-resolution cryospray ESI-TOF-MS in the negative mode at −20 °C.

In light of the presently analyzed HSNO reactivity issues, a putative $SSNO^-$ onset could be in order under specific conditions.

REACTIONS OF NONHEME METALLONITROSYLS WITH H_2S. IRON-BOUND HSNO/SNO^- *AND* $SSNO^-$?

We deal with the old "Gmelin" reaction, for which basic mechanistic considerations are still under dispute. The stoichiometry of the $[Fe(CN)_5NO]^{2-}/H_2S$ reaction led to S_8 and NH_3/N_2O (with pH-dependent yields) and to Prussian Blue-type precipitates as final products. We dealt with a comprehensive mechanistic revision by working under anaerobic conditions in the pH 10−13 range.[81] The first observed intermediate, I_{535}, and a second emerging species, I_{575}, were tentatively traced to $[Fe(CN)_5(SNO)]^{4-}$ and $[Fe(CN)_5(HSNO)]^{3-}$, respectively. The latter assignments were reasonably rejected, on the basis that the bound $SNO^- \rightarrow$ HSNO conversion, apparently occurring in seconds, could not be compatible with an expected very fast rate for protonation/deprotonation reactions on the ligands.[82] A follow-up mechanistic proposal was raised by extending the studied pH range to more biorelevant conditions.[83] Although new significant evidence has recently been published (I_{575} was assigned to $[Fe(CN)_5(SSNO)]^{4-}$),[84] the authors currently confirmed their mechanistic proposal, which implies excluding $[Fe(CN)_5(SSNO)]^{4-}$ as a possible intermediate.[1,28,29] In our hands, the chemistry of the Gmelin process shows notorious differences from that of the analogous situation comprising the reactions of $[Fe(CN)_5NO]^{2-}$ with thiolates.[85] The identities and yields of intermediates and products appear to be dependent on the pH and O_2 availability, as well as on the initial relative concentrations of the reagents. Our main guidelines are now closely related to the new results and interpretations on the chemistry of free {(H)SNO}, SNO^-, and $SSNO^-$, as detailed in this Viewpoint. In fact, $[Fe(CN)_5(SNO)]^{4-}$ (I_{535}) appears to be a moderately reactive species toward N−S homolysis, while $[Fe(CN)_5(HSNO)]^{3-}$ still eludes clear identification, suggesting a high reactivity. The intermediacy of HNO (free and/or bound) is still a matter of further analysis. We advanced some new information recently on the feasibility of I_{575} being assigned to bound $SSNO^-$,[20] and we hope to provide a new comprehensive mechanistic proposal in due course. Indeed, reports on related systems comprising other metallonitrosyls reacting with H_2S are highly desirable.[1]

CONCLUSION AND FUTURE PERSPECTIVES

We conclude that $SSNO^-$ can *survive* transiently in aqueous solutions for minutes/hours, depending on $[O_2]$ and light, in order to react with nonbiologically or eventually biologically relevant targets. Thus, $SSNO^-$ is more stable and less reactive than its precursor {(H)SNO} at pH 7.4. The decay routes established for aqueous $SSNO^-$ may imply either S^0 transfer to substrates or cleavage of the N−S bond, leading to NO and/or N_2O together with polysulfides, HS_n^-. Although $SSNO^-$ decomposes in water faster tan in nonaqueous solvents through N−S bond cleavage, the hydrogen bonding interaction determines a notorious decrease in the S^0-transfer aqueous reactivity of $SSNO^-$ toward biorelevant nucleophiles (viz., HS^-, RS^-, DTT, CN^-) compared to the same reactions in aprotic solvents. Studies on the formation/decomposition mechanisms of $SSNO^-$/{(H)SNO} still merit further investigation. In the latter context, we are cautious to suggest specific signaling abilities that could be onset in biorelevant media other than those related to NO/NO^-/HNO production and subsequent vasodilatory activation through mechanistic routes that have currently been addressed elsewhere. Recently, a new path consistent with $SSNO^-$ signaling has been identified in studies on the expression of heme oxygenase.[70] Furthermore, for soluble sGC activation, the detailed mechanistic features might be complex: in addition to NO, NO^- could be a very effective player by acting as a strong σ-donor ligand, thus reinforcing the ability of the iron-based enzyme to best promote the release of trans ligands and subsequent triggering of cGMP production;[86,87] on the other hand, HNO has been demonstrated to be a negligible trans-ligand activator.[77] To the types of well-recognized biorelevant post-translational processes (persulfidations, sulfenylations, nitrosations, and transnitrosations),[7] we suggest adding the putative involvement of $SSNO^-$ in transnitrosopersulfidation reactions affording S^0-transfer mechanistic features, useful for protein modifications, which are reported to occur with several enzymes.[4] Finally, the current studies on the N/S crosstalk allow one to obtain more clear insight into the role of polysulfides, which are well-recognized candidates for signaling; besides the complex nature of the aqueous equilibrium mixtures of HS_n^-, we showed that they can exert dual roles, comprising the formation of $SSNO^-$, as well as being a decomposition product through homolysis. Evidently, aqueous $SSNO^-$ and HS_n^- provide new elements to enrich the NO/HS^- story focused in biological signaling features.

The current studies highlighted a broad mechanistic picture (viz., one-electron/two-electron redox changes) available by studying the chemistry of the three redox states of the nitrosyl group (NO^+, $NO^\bullet$, and NO^-), enlarged by the sulfur triad (S^{2-}, $S^{\bullet-}$, and S^0) with the crosstalk reactions, leading to HSNO, $SSNO^-$, or others. A higher complexity level arises by introducing the oxygen triad (O_2, $O_2^{\bullet-}$, and O_2^{2-}), thus comprising the S/O crosstalk features on the generation of sulfites, sulfates, etc., in the mitochondria, which have been avoided in this Viewpoint. Finally, by introducing new dimensions such as light or the coordination of most of these species into the redox-active transition-metal centers, we drive the complexities to a fascinating research scenario.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: doctorovich@qi.fcen.uba.ar.
*E-mail: olabe@qi.fcen.uba.ar.

ORCID

Juan P. Marcolongo: 0000-0001-9711-4095
Willian R. Rocha: 0000-0002-0025-2158
Fabio Doctorovich: 0000-0003-1088-2089

Notes

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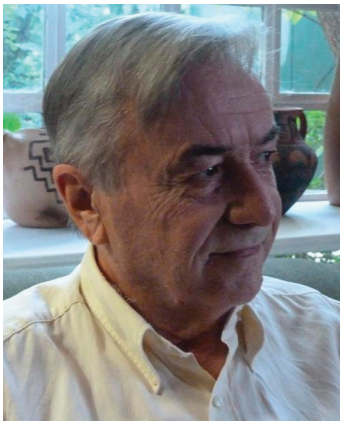
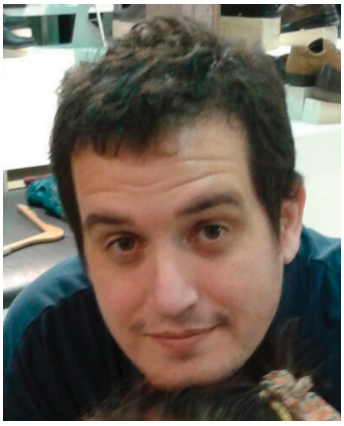
Biographies

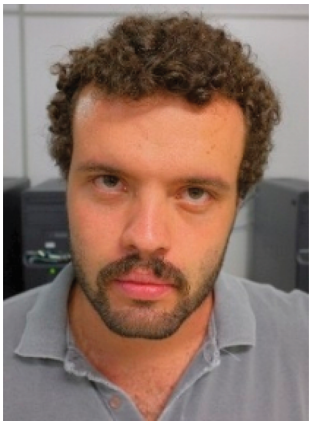


José A. Olabe was born in San Sebastian, Spain, in 1941. He carried out basic studies in Argentina and obtained his Ph.D. at the University of La Plata in 1968, working in the electrochemistry field with Prof. A. J. Arvia. He undertook postdoctoral studies at the same university and was a Visiting Professor at the State University of New York (Stony Brook), working with Prof. A. Haim in 1989. Since 1986, he has been Professor of Inorganic and Physical Chemistry at the Universities of Lujan, Mar del Plata, and Buenos Aires, and a member of the staff of CONICET, the National Research Council in Argentina and was elected vice-Dean at the Facultad de Ciencias Exactas y Naturales, being a member of diverse academic-leading committees. His scientific interest relies in transition-metal coordination chemistry, comprising structural and kinetic/mechanistic issues mostly focused in studies with small molecules: nitric oxide, nitrogen hydrides, hydrogen sulfide, and redox derivatives. He has engaged in sustained collaborative work with Professors W. Kaim (Stuttgart) and R. van Eldik (Erlangen, Krakow), as well as with other Argentinian colleagues. He authored over 100 articles and reviews in qualified journals and is currently Emeritus Professor at University of Buenos Aires.

Fabio Doctorovich is a Full Professor at the University of Buenos Aires (UBA) and a Top-Class Researcher at CONICET. He earned his Ph.D. in Organic Chemistry from UBA in 1990. He was a postdoctoral fellow at the Georgia Institute of Technology, working with Prof. E. C. Ashby and K. Barefield, first on single-electron transfer and afterward on chemical reactions taking place in nuclear waste tanks. Back in Argentina, he started to work on nitric oxide (NO), including organic nitroso compounds, inorganic iron, rhodium, ruthenium, and iridium nitrosyl complexes, and the reactivity of metalloporphyrins toward HNO. He also worked on CO complexes, catalytic reactions, and other topics. Nowadays, his research focus is on reactions involving HNO, as well as the catalytic production of hydrogen from water. Prof. Doctorovich has published over 100 works in international journals such as *Accounts of Chemical Research*, *Journal of the American Chemical Society*, *Inorganic Chemistry*, and *Nature Communications*, as well as the book *The Chemistry and Biology of Nitroxyl (HNO)* at Elsevier in 2016. He has supervised more than 10 Ph.D. students. In 2011 he received the Guggenheim Fellowship and in 2016 the Innovar Prize.



Juan P. Marcolongo earned his Ph.D. at University of Buenos Aires, working in the synthesis, characterization, thermal, and photochemical reactivity of coordination compounds under the supervision of Prof. Leonardo D. Slep in 2015. From 2016 to 2018, he was a postdoctoral fellow in the laboratory of Prof. Damián Scherlis at the Molecular Modelling Group at INQUIMAE, researching on real-time TD-DFT, focusing on the prediction of electronic spectra of inorganic species, and combining real-time TDDFT with QM/MM MD simulations. He stands as Head Teaching Assistant at the Department of Inorganic, Analytical and Physical Chemistry in the School of Exact and Natural Sciences, University of Buenos Aires. He has been a member of the National Research Council (CONICET) since 2019, and his research interests involve both experimental and theoretical aspects related to photochemical processes in coordination chemistry.



Mateus F. Venâncio received his bachelor's degree in Chemistry in 2011 from Federal University of Minas Gerais. In 2013, he earned a master's degree in Physical Chemistry for the development of a new approach to obtain thermodynamic properties on the internal rotation of substituted ethanes, also at Federal University of Minas Gerais. In 2017, he obtained his title of Doctor in Sciences: Chemistry for studies on nitric oxide behavior in aqueous solution, redox potentials, and excited-state ab initio MD at the same university. After two postdocs at Federal University of Juiz de Fora and Federal University of Minas Gerais, he is now assuming a position as Adjunct Professor at Federal University of Bahia. His interests are related to the investigation and understanding of mechanisms of reactions of

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Inorg. Chem. XXXX, XXX, XXX−XXX

bioinorganic relevance and multiconfigurational problems involving transition-metal complexes, as well as metal nitrosyl complexes.

Willian R. Rocha received his B.S. in Chemistry from Federal University of Minas Gerais (1994) in Brazil and his Doctor of Science: Chemistry from the same university in 2000 under the guidance of Prof. Wagner B. De Almeida, working on the theoretical studies of homogeneous transition-metal-catalysed reactions and solvent effects on chemical reactions. He was Adjunct Professor at Federal University of Pernambuco from 2002 to 2006 and is currently Professor of Chemistry at the Chemistry Department of Federal University of Minas Gerais in Brazil. His research interests include the use of theoretical and computational chemistry for the study of chemical reactions in gas and condensed phases, particularly those related to transition-metal catalysis and bioinorganic processes, and molecular spectroscopy.

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