Transient Reaction Intermediates

Solving the 170-Year-Old Mystery About Red-Violet and Blue Transient Intermediates in the Gmelin Reaction

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Abstract: The Gmelin reaction between nitroprusside and sulfides in aqueous solution is known to produce two transient intermediates with distinct colors: an initial red-violet intermediate that subsequently converts into a blue intermediate. In this work, we use a combination of multinuclear ($^{17}$O, $^{15}$N, $^{13}$C) NMR, UV/Vis, IR spectroscopic techniques and quantum chemical computation to show unequivocally that the red-violet intermediate is [Fe(CN)$_5$(NO)SS]$^{4-}$ and the blue intermediate is [Fe(CN)$_5$(N(O)SS)]$^{4-}$. While the formation of [Fe(CN)$_5$(N(O)S)]$^{4-}$ has long been postulated in the literature, this study provides the most direct proof of its structure. In contrast, [Fe(CN)$_5$(N(O)SS)]$^{4-}$ represents the first example of any metal coordination complex containing a perthionitrite ligand. The new reaction pathways found in this study not only provide clues for the mode of action of nitroprusside for its pharmacological activity, but also have broader implications to the biological role of H$_2$S, potential reactions between H$_2$S and nitric oxide donor compounds, and the possible biological function of polysulfides.

In 1843, Scottish chemist Lyon Playfair described the transient formation of “the most magnificent purple colored solution” when nitroprusside (NP, [Fe(CN)$_5$NO]$^{2-}$) was treated with alkaline sulfides. Since he credited the first observation of this phenomenon to Leopold Gmelin, this reaction is thus often referred to as the Gmelin reaction in the modern chemical literature. The red-violet coloration of the Gmelin reaction is so intense that it can be easily detected by human eyes as soon as the color changes when the concentration of Na$_2$S(aq) is as low as 20 μM (about 2 μg mL$^{-1}$). Historically, the Gmelin reaction is among the most sensitive color reactions studied primarily relying on UV/Vis, IR, kinetic measurement, and chemical analysis of the decomposed products, direct structural information on the transient intermediates is lacking. For this reason, we decided to employ a combined multinuclear ($^{17}$O, $^{15}$N, $^{13}$C) NMR and computational approach to elucidate the structures of these famous transient intermediates.

Figure 1 shows the red-violet solution formed immediately upon reacting NP with Na$_2$S$_2$(aq) and its subsequent conversion into a blue color, which then fades over time. If the reaction is carried out in a phosphate buffer (pH 7.4), it is difficult to capture the red-violet color, because the solution immediately turns to blue ($\lambda_{\text{max}} = 577$ nm), as shown in Figure 1b. Under the high pH conditions (e.g. pH 11-12), the UV/Vis absorption band displays a bathochromic shift over time, as seen in Figure 1c, which is due to a gradual conversion of the initial red-violet intermediate ($\lambda_{\text{max}} = 542$ nm, $t_{1/2} = 1.5$ min) to the blue intermediate ($\lambda_{\text{max}} \approx 570$ nm, $t_{1/2} = 90$ min); see Figure 1d. Following Rock and Swinehart,[2] we will also refer these two intermediates as A and B.

To gain direct information about the molecular structures of these unstable intermediates, we first recorded time-dependent $^{17}$O ($I = 5/2$) NMR spectra for a freshly prepared reaction solution (see Figure 1e and 1f). The initial product of the reaction exhibits a $^{17}$O NMR signal at 1028 ppm, which is then quickly converted to a new signal at 938 ppm. The close correlation between the time-resolved $^{17}$O NMR and UV/Vis data strongly suggests that the two $^{17}$O NMR signals correspond to the red-violet (A) and blue (B) intermediates, respectively. Recently, Feelisch and co-workers[20] reported that S-nitrosothiols (R–S=NO) react with excess HS$^-$ to produce [SNO]$^-$ (thionitrite) which is then converted into [SSNOCN]$^-$ (perthionitrite). We hypothesized that a similar reaction occurs between NP and HS$^-$.

Therefore, the observed $^{17}$O NMR signals at 1028 ppm (intermediate A) and 938 ppm (intermediate B) should arise from the following two Fe$^+$ complexes: A, [Fe(CN)$_5$(N(O)S)]$^{4-}$; B, [Fe(CN)$_5$(N(O)SS)]$^{4-}$.

To confirm the identity of B, we investigated the reaction between NP and Na$_2$S$_2$. Because HSSH has $pK_a < 7$ and $pK_{a1} > 10$,[24] Na$_2$S$_2$ produces predominantly HSS$^-$ under the conditions used in this study. Like HS$^-$, HSS$^-$ would undergo nucleophilic attack to NP to form [Fe(CN)$_5$(N(O)SSH)]$^{4-}$.[25] Its subsequent deprotonation would produce B. Indeed, upon mixing NP and Na$_2$S$_2$, the solution immediately turns blue with an absorption band at $\lambda_{\text{max}} = 575$ nm (see the Supporting Information). This blue solution also gives rise to a $^{17}$O NMR signal at 938 ppm, as shown in Figure 2b, whose position is invariant over the pH range between 7 and 12. Over time the intensity of this
ONMR signal and the blue coloration of the solution decrease simultaneously. These observations strongly support our hypothesis that B is indeed $[^{15}N](^{15}N)(^{15}N)(^{15}N)(^{15}N)(^{15}N)(^{15}N)$/CO. We further obtained a complete set of $^{15}N$ and $^{13}C$ NMR data for both A and B. As shown in Figure 2, the A and B intermediates exhibit $^{15}N$ chemical shifts of 700 and 632 ppm, respectively. The blue product from the reaction between NP and Na$_2$S$_2$ exhibits only one $^{15}N$ NMR signal at 632 ppm. As a result of the Fe$^{II}$ coordination, the $^{15}N$ and $^{17}O$ chemical shifts for A and B are more shielded than those reported for free S- and C-nitroso compounds, approximately $\delta(^{15}N) = 900–700$ ppm$^{[25]}$ and $\delta(^{17}O) = 1200–1300$ ppm.$^{[26]}$ We also obtained the NMR signals for $[^{15}N](^{15}N)(^{15}N)(^{15}N)(^{15}N)(^{15}N)(^{15}N)$/CO, which is the closest analog of A. Interestingly, $[^{15}N](^{15}N)(^{15}N)(^{15}N)(^{15}N)(^{15}N)(^{15}N)$/CO exhibits $\delta(^{17}O) = 690$ ppm and $\delta(^{15}N) = 555$ ppm, both values being considerably further shielded compared to those found for A. This discrepancy is clearly due to the increased N=O double bond character in $[^{15}N](^{15}N)(^{15}N)(^{15}N)(^{15}N)(^{15}N)(^{15}N)$/CO as compared with $[^{15}N](^{15}N)(^{15}N)(^{15}N)(^{15}N)(^{15}N)(^{15}N)$/CO. We found that $[^{15}N](^{15}N)(^{15}N)(^{15}N)(^{15}N)(^{15}N)(^{15}N)$/CO and $[^{15}N](^{15}N)(^{15}N)(^{15}N)(^{15}N)(^{15}N)(^{15}N)$/CO have very similar $\delta(^{13}C_{eq})$ values, suggesting that [NO$_2$] (nitro), [NO]$^{[27]}$ (nitro) and [NO]$^{[27]}$ (perthionitro) ligands have similar p-acceptor capabilities. In contrast, NP shows a completely different set of NMR parameters: $\delta(^{17}O) = 419$, $\delta(^{15}N) = 376$, $\delta(^{13}C_{eq}) = 134.5$ and $\delta(^{13}C_{ax}) = 132.3$ ppm.$^{[27]}$

To further link the experimental NMR chemical shifts to molecular structure, we performed quantum chemical calculations, because no crystal structure has yet been reported in the literature for any metal coordination complexes containing thionitrite and perthionitrite ligands. Figure 3b shows the optimized
structures of \([\text{Fe(CN)}_5\text{N(O)S}]^{4-}\) and \([\text{Fe(CN)}_5\text{N(O)SS}]^{4-}\). Here we followed the procedure suggested by Olabe and co-workers\(^{14}\) to add three water molecules to each of the models in order to mimic the solvent environment. The overall geometries of the Fe\(^{II}\)-bound thionitrite and perthionitrite anions\(^{28}\) and \([\text{Fe(CN)}_5\text{N(O)S}]^{4-}\) and \([\text{Fe(CN)}_5\text{N(O)SS}]^{4-}\), fully optimized at the B3LYP/6-311G + + (3df,3pd) level of theory with the IEFPCM solvent model. c) Correlation between computed and observed NMR chemical shifts for closely related compounds. The dash line is along the diagonal. Computational details are given in the Supporting Information.

![Diagram](https://example.com/diagram.png)

**Figure 3.** Selected bond lengths [Å] and angles [°] for a) [S–N=O]– and [S–S–N=O]– anions\(^{28}\) and b) \([\text{Fe(CN)}_5\text{N(O)S}]^{4-}\) and \([\text{Fe(CN)}_5\text{N(O)SS}]^{4-}\), fully optimized at the B3LYP/6-311G + + (3df,3pd) level of theory with the IEFPCM solvent model. c) Correlation between computed and observed NMR chemical shifts for closely related compounds. The dash line is along the diagonal. Computational details are given in the Supporting Information.

have pK\(_a\) values of 2.9 and 1.3, respectively; see the Supporting Information. We also performed TD-DFT calculations for UV/Vis spectra; see the Supporting Information. The calculations properly reproduced the observed 30 nm bathochromic shift on going from \([\text{Fe(CN)}_4\text{N(O)}]^2-\) to \([\text{Fe(CN)}_4\text{N(O)}]^3-\). Furthermore, the calculations reveal that the origin of the absorption band in both compounds is primarily the ligand based \(\pi\) to \(\pi^*\) transition.

To complete the spectroscopic characterization, we obtained FTIR data for \([\text{Fe(CN)}_5\text{NO}]^3-\), \([\text{Fe(CN)}_5\text{N(O)}]^2-\) and \([\text{Fe(CN)}_5\text{N(O)}]^3-\) in aqueous solution; see the Supporting Information. The observed \(\nu_{\text{NO}}\) bands are essentially the same for the nitro, thionitro and perthionitro complexes, suggesting that the three ligands have very similar \(\pi\)-acceptor capabilities. This observation is thus in full agreement with the \(^{13}\)C NMR results discussed earlier. More interestingly, we observed the \(\nu_{\text{NO}}\) stretching bands at 1254.3 and 1358.3 cm\(^{-1}\) for \([\text{Fe(CN)}_5\text{N(O)}]^2-\) and \([\text{Fe(CN)}_5\text{N(O)}]^3-\), respectively. These IR bands have never been reported before. The slightly higher \(\nu_{\text{NO}}\) in \([\text{Fe(CN)}_5\text{N(O)}]^3-\) is consistent with the slightly shorter N–O bond in the optimized molecular structure shown in Figure 3b. Quite remarkably, the \(\nu_{\text{CN}}\) and \(\nu_{\text{NO}}\) values observed for \([\text{Fe(CN)}_5\text{N(O)}]^2-\) and \([\text{Fe(CN)}_5\text{N(O)}]^3-\) are lower than the corresponding values observed for the so-called “red product” from the reaction between NP and ETSH, \([\text{Fe(CN)}_5\text{N(O)}^{\text{SET}}]^3-\), \(\nu_{\text{CN}} = 2072\) and \(\nu_{\text{NO}} = 1380\) cm\(^{-1}\).\(^{29}\) If this latter complex can be considered as a model for the protonated form of the ligand, the IR data of \([\text{Fe(CN)}_5\text{N(O)}]^3-\) and \([\text{Fe(CN)}_5\text{N(O)}]^3-\) once again confirm that thionitro and perthionitro ligands are indeed in the deprotonated states. For \([\text{Fe(CN)}_5\text{N(O)}^{\text{SET}}]^3-\), we were also able to detect the \(\nu_{\text{NO}}\) stretch band at 805.6 cm\(^{-1}\). The \(\nu_{\text{NO}}\) bands for free S-nitrosothiols typically appear around 650–670 cm\(^{-1}\) with the S–N bond lengths in the range of 1.792–1.763 Å.\(^{100}\) The S–N bond length in the optimized structure of \([\text{Fe(CN)}_5\text{N(O)}]^3-\) is 1.749 Å (shown in Figure 3b), which is consistent with the experimental \(\nu_{\text{NO}}\) stretch frequency. In contrast, the S–N bond in \([\text{Fe(CN)}_5\text{N(O)}^{\text{SET}}]^3-\) is considerably longer, and we were unable to detect its \(\nu_{\text{NO}}\) band.

After having firmly established the identities of the A and B intermediates, we now propose a mechanism to explain how they are produced during the reaction. In this study, we focus only on the generation of A and its subsequent conversion to B. As shown in Figure 4, upon nucleophilic attack of HS\(^-\) to NP, \([\text{Fe(CN)}_5\text{N(O)}^{\text{SH}}]^3-\) is formed which spontaneously deprotonates under the basic or neutral conditions. At the same time, the S–N bond in \([\text{Fe(CN)}_5\text{N(O)}^{\text{SH}}]^3-\) can homolytically cleave producing HS\(^-\) and \([\text{Fe(CN)}_5\text{NO}]^3-\). It is well known that, under the aerobic condition, \([\text{Fe(CN)}_5\text{NO}]^3-\) can be oxidized back to NP. In principle, \([\text{Fe(CN)}_5\text{NO}]^3-\) can also decompose into \([\text{Fe(CN)}_5\text{NO}]^2- + \text{CN}^-\).\(^{31}\) However, under the high pH conditions employed in this study, we did not observe any \([\text{Fe(CN)}_5\text{NO}]^2-\), \((\nu_{\text{CN}} = 615\) nm) during the early stage of the reaction (i.e., when A and B are produced). Once HS\(^-\) is produced from recombination of two HS\(^-\) radicals, there are two possible ways that \([\text{Fe(CN)}_5\text{N(O)}^{\text{SH}}]^3-\) can be generated. One is the transnitration reaction between HSS\(^-\) and \([\text{Fe(CN)}_5\text{N(O)}]^3-\). The other is the direct nucleophilic attack of HSS\(^-\) on NP (if NP is still
available in solution). It is interesting to note that, the generation of a Fe-"perthionitriite complex is unique to the reaction of HS\textsuperscript{-}, as other thiolates, RS\textsuperscript{-}, would form the unreactive RSSR as one of the final products.\textsuperscript{[12]} It is important to point out that our experimental results, together with those from other workers,\textsuperscript{[23,33,34]} clearly point to a parallelism between S-nitrosothiols and NP in their reactions with H\textsubscript{2}S. It can be further speculated that all NO donor compounds may share the same reaction pathway when interacting with H\textsubscript{2}S. While the mechanisms of pathway when interacting with H\textsubscript{2}S. While the mechanisms of formation of perthionitrite anion and its Fe\textsuperscript{II} coordination complex under physiological conditions should be considered to be a critical step in a chain of reactions contributing to a variety of biological functions. The formation of [Fe(CN)\textsubscript{5}N(O)SS\textsuperscript{4}] can also be related to the mode of hypotensive action of NP at the cellular level.

In summary, we have utilized multinuclear (\textsuperscript{17}O, \textsuperscript{15}N, \textsuperscript{13}C) NMR, UV/Vis, FTIR spectroscopic measurement and quantum mechanical computation to establish unequivocally the molecular structures of the red-violet and blue intermediates in the Gmelin reaction. The time-dependent \textsuperscript{17}O NMR data provided the key clues, leading to the final structural elucidation of these unstable intermediates. [Fe(CN)\textsubscript{5}N(O)SS\textsuperscript{4}] represents the first metal coordination complex containing a perthionitro ligand. The proposed reaction pathways between NP and HS\textsuperscript{-} have important implications in such areas as the chemical biology of H\textsubscript{2}S, NO donor biochemistry, and the biological role of polysulfides, all of which are under intense investigation presently in several disciplines.

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**Keywords:** NMR spectroscopy · hydrogen sulfide · nitroprusside · perthionitrite · thionitrite

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\[ \text{O}_2 \]

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\begin{align*}
\text{[Fe(CN)\textsubscript{5}N(O)SS\textsuperscript{4}]} + \text{HS}^\text{-} & \rightarrow \text{[Fe(CN)\textsubscript{5}N(O)SSH\textsuperscript{3}]} + \text{H}^+ \\
\text{pK}_5 < 3 & \quad \text{transnitrosation} \\
\text{[Fe(CN)\textsubscript{5}N(O)SS\textsuperscript{4}]} + \text{H}^+ & \rightarrow \text{[Fe(CN)\textsubscript{5}N(O)SS\textsuperscript{4}]} + \text{H}_2\text{O}
\end{align*}
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Figure 4. Proposed reaction pathways for the generation of [Fe(CN)\textsubscript{5}N(O)SS\textsuperscript{4}] and [Fe(CN)\textsubscript{5}N(O)SS\textsuperscript{4}] in the Gmelin reaction.

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170 years later: The molecular structures of the red-violet and blue transient intermediates in the Gmelin reaction have finally been elucidated with a combination of multinuclear (\(^{17}\)O, \(^{15}\)N, \(^{13}\)C) NMR spectroscopy and quantum chemical computation (see scheme).

Old Mystery Solved

For 170 years the true identities of the red-violet and blue transient intermediates of the Gmelin reaction between nitroprusside and hydrogen sulfide have remained unknown and often disputed in the literature. In their Communication on page \(\text{ff.}^\text{ff.}\), Wu et al. report the structural elucidation of these colorful but rather unstable compounds in this historically important chemical reaction.