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Preventing Nitrite Contamination in Tetramethylammonium Peroxynitrite Solutions

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Peroxynitrite prepared from superoxide and nitric oxide in liquid ammonia does not contain detectable levels of nitrite. However, the dissolution of nitrite salts can lead to variable levels of peroxynitrite depending on the conditions used to disolve the salt. Low levels of nitrite result when frozen peroxynitrite solutions are first brought to +1 °C and then to room temperature. These undergo only 2–3% decomposition after 1 h, in contrast with the findings of a recent report (Lymar, S. V.; Khairutdinov, R. F.; Hurst, J. K. *Inorg. Chem.* **2003**, *42*, 5259–5266), where high levels of nitrite (∼20%) result from rapid thawing of these solutions to room temperature. Warming the frozen peroxynitrite solution directly to room temperature in 30 min leads to a nitrite level of 28%.

The mechanism of peroxynitrite [systematic name: oxoperoxonitrate(1−)] decomposition has been intensively studied and vigorously debated in the past decade. Among the critical mechanistic issues are the reaction products, which include nitrite and dioxygen when the initial concentration of peroxynitrite is high.

$$\mathrm{ONOOH} + \mathrm{ONOO^-} \rightarrow 2\mathrm{NO_2^-} + \mathrm{O_2} + \mathrm{H^+} \qquad (1)$$

In a recent paper on the thermodynamics of peroxynitrous acid, Hurst and co-workers[1] criticized our model to describe the formation of nitrite and dioxygen and claimed that their high-pressure studies support homolysis. Furthermore, these authors mention that the peroxynitrite supplied by us contained 20% nitrite. Whereas the first two points have already been addressed,[2,3] the origin of the nitrite contamination needs to be scrutinized carefully.

Peroxynitrite decomposition yields nitrite and dioxygen in a 2:1 ratio,[4] and the yields of these products, relative to peroxynitrite, can provide information about the mechanism of decomposition. Generally, frozen stock solutions are melted and warmed to 0,[5] 25,[6,7] or 37 °C[4] and then mixed with varying concentrations of phosphoric acid at the appropriate temperature to obtain different pH values at 25 or 37 °C, followed by a nitrite determination. If nitrite is already generated by the warming process and if the concentration of nitrite is a function of the time that the stock solution is kept at a particular temperature, then this would complicate the analysis, even if a determination is made at low pH some time after the stock solution has melted. This issue of nitrite generation in the alkaline solution needs to be resolved for progress to be made on the mechanistic front.

Herein, we demonstrate how, through warming above 0 °C, alkaline (0.01 M potassium hydroxide) stock solutions can become heavily contaminated with nitrite, and we describe the steps that should be followed to avoid this problem.

The tetramethylammonium peroxynitrite used in these studies was prepared by treating tetramethylammonium superoxide with nitrogen monoxide at −77 °C in liquid ammonia, followed by isolation as a crystalline solid via removal of the ammonia. From combustion analysis, this solid corresponds to $(\mathrm{NMe_4})(\mathrm{O_3N})$; contamination with as little as 6.6% $(\mathrm{NMe_4})(\mathrm{NO_2})$ would significantly alter these figures.[8−10] Clearly, the C, H, and N analyses alone are not consistent with 20% contamination with nitrite. Greater precision might be possible when oxygen analysis is included

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in this determination. Furthermore, given the absence of the strong $\nu(\mathrm{NO})_{\mathrm{sym}}$ band at 1040 cm^{-1} in the Raman spectrum, the salt is not contaminated with nitrate. This band and other characteristic signals for nitrite and nitrate are also not observed by IR spectrum. Finally, the ^{15}N NMR spectra of these salts in both liquid ammonia at −40 °C and D_2O at pH 14 are consistent with the presence of tetramethylammonium and peroxynitrite nitrogens alone. Because peroxynitrite and nitrite both have geometries bent at the nitrogen atom, we expect similar relaxation times for these species. The ^{15}N NMR results are consistent with less than 1% contamination with nitrite. Given that nitrate exhibits D_{3h} geometry and longer relaxation times, we also used longer relaxation delays in measurements in an attempt to detect its presence in these experiments as well. None was observed, and the clear conclusion from all of these experiments is that nitrite is not formed during synthesis.[8−10] Thus, if it is present, it must result from either the dissolution or the subsequent treatment. Finally, we note that only one conformation of peroxynitrite is observed in these spectroscopic methods, and this is consistent with the cis geometry determined by X-ray diffraction.[11]

To prepare aqueous solutions of peroxynitrite, it is best to dissolve the tetramethylammonium salt in cold (0−2 °C) 0.01 M potassium hydroxide that has been freshly prepared to avoid carbonate contamination. Either this alkaline peroxynitrite solution can be used immediately, or it can be stored, e.g., as 1-mL aliquots, ca. 20 mM in peroxynitrite, in small Eppendorf tubes and frozen by dropping the tubes into liquid dinitrogen. Peroxynitrite so prepared was stored at −80 °C. The sample of peroxynitrite used by Prof. Hurst and co-workers was prepared in this way and was sent on dry ice by courier.

We analyze stored peroxynitrite by ion chromatography as previously described[5] from time to time for nitrite, and values of ca. 1% or less are always found. The claim that the material contains 25% nitrite is, thus, misleading. That analyses by Hurst and co-workers of peroxynitrite decomposition products[1,6] generally show higher nitrite values and more scatter than ours[2;57] under comparable conditions does not account for the difference between 1% and 20%. Also, the addition of hydroxide[1] is not expected to increase the nitrite level.

We suggest that the nitrite might have originated through decomposition (reaction 1), which is much enhanced at higher concentrations of peroxynitrite.[5] To investigate this possibility, we took several frozen 1-mL samples, warmed them in three different ways, and analyzed them for nitrite. One sample was brought directly from −80 °C to room temperature (heat exchange by air), and others were allowed to melt in an ice-bath at or near 0 °C first or to remain at that temperature. None of the samples were stirred. One sample was warmed by hand until the frozen pellet fell into ice-cold potassium hydroxide. The results of the nitrite analyses, by way of the Griess method and anion chromatography, are shown in Figure 1. The temperature−time profiles were obtained with frozen 0.01 M potassium hydroxide samples and are shown in Figure 2. The samples "warmed up" in ice water reached 0 °C within 100 s; those in air took ca. 250 s and stayed near 0 °C for ca. 12 min, after which their temperature increased again.

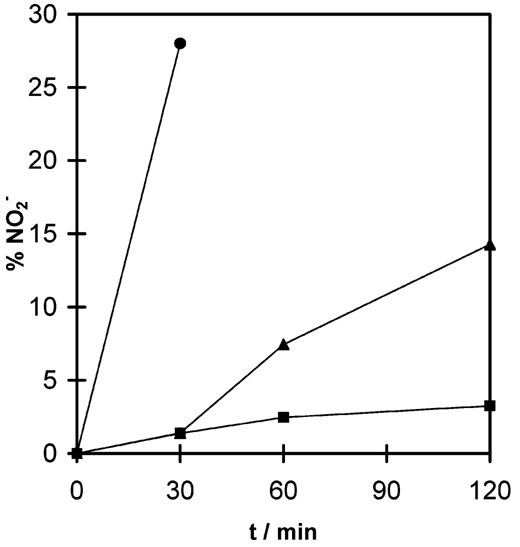

Figure 1. Decomposition of $[(CH_3)_4N]^+[ONOO]^-$ as a function of temperature and time. One-milliliter samples of 20.1 mM $[(CH_3)_4N]^+$-$[ONOO]^-$, pH = 12, were thawed and warmed as indicated: ●, from −80 °C directly to room temperature; ▲, from −80 °C to +1 °C in ice-cold water and then to room temperature; and ■, from −80 °C to +1 °C in ice-cold water and kept at that temperature. The samples were immediately analyzed for nitrite by the Griess method and by ion chromatography. No significant differences were found between these two analytical methods.

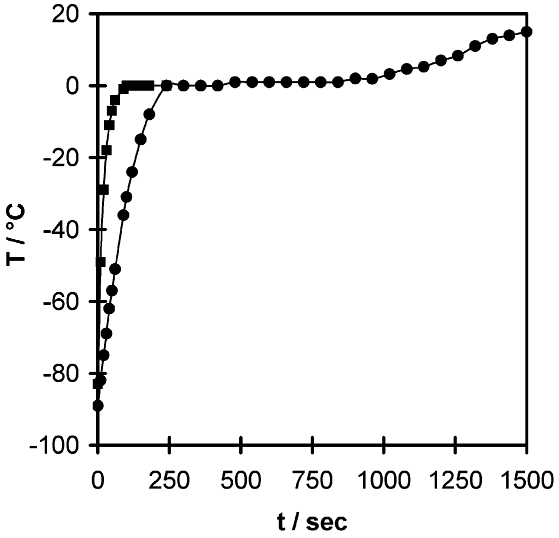

Figure 2. Temperature vs time profiles of the samples during warming. Symbols as described in the caption of Figure 1.

The sample allowed to warm to room temperature directly had a nitrite level comparable to that reported by Hurst and co-workers in less than 30 min.[1] The other samples had nitrite levels near 1% after 1 h that increased only slowly with time. This applied also to the sample that was diluted while melting. We are of the opinion that the 1% nitrite is also caused by decomposition. Clearly, temperatures above 0 °C

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lead to a significant conversion of peroxynitrite to nitrite and dioxygen, in agreement with earlier observations.[5]

We conclude that handling and storage procedures are critical for the quality of tetramethylammonium peroxynitrite solutions, and we speculate that accelerated decomposition might be due to high heats of dissolution and solvation. It is quite plausible that the high levels of nitrite contamination reported[1] were caused by keeping the peroxynitrite solution at room temperature. Alternatively, it is possible that, during shipment or storage, a temperature of at least −20 °C was not maintained. In either case, the high nitrite levels reported by these workers could result.

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