# EPR and Spin Trapping Investigations of Nitric Oxide (•NO) from UV Irradiated Nitrite Anions in Alkaline Aqueous Solutions

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The free radicals nitric oxide (•NO) and nitrogen dioxide (•NO$_2$) are common pollutants present in the atmosphere and in aquatic environments. An important source of these radicals is UV light-induced decomposition of the nitrite anion, NO$_2^-$. Based on the results of flash photolysis studies, Treinin and Hayon[1] postulated that the primary chemical event after the absorption of UV light by NO$_2^-$ is the generation of •NO and •OH radicals (eq 1). This is in agreement with the expectation

$$(NO_2^-)^* + H_2O \rightarrow {}^\bullet OH + {}^\bullet NO + HO^- \qquad (1)$$
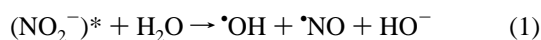
that the photolysis of NO$_2^-$ should proceed in a similar fashion to the photolysis of organic nitrites (RO−NO) which produce nitric oxide and an alkoxyl radical (RO•).[2] In the absence of other solutes •OH reacts with NO$_2^-$ forming NO$_2$• radicals (eq 2).[1]

$$^\bullet OH + NO_2^- \rightarrow {}^\bullet NO_2 + HO^- \qquad (2)$$
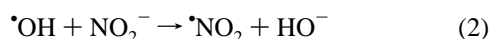
The formation of •OH and NO$_2$• radicals from UV irradiated nitrite anions has been confirmed by EPR and spin trapping;[3] however, neither direct nor indirect EPR evidence was obtained for the second postulated radical photoproduct, •NO, which is, *per se*, the least reactive product of nitrite photolysis. In contrast to •NO$_2$ which dimerizes rapidly to N$_2$O$_4$,[4] dimerization of •NO is negligible in the gas phase and, probably, does not occur in aqueous solutions.[5] And unlike •NO$_2$ whose dimer reacts with water, nitric oxide is stable in aqueous anaerobic solutions. •NO adds slowly to double bonds (including double bonds in spin traps),[3,6] but it reacts very fast with some radicals, such as O$_2$•$^-$,[7] carbon-centered radicals,[8,9] and oxygen.[10] As a result •NO is the most difficult product of nitrite photolysis to detect.

We have recently reported[3] that the *aci* anion of nitromethane, *aci* NM (CH$_2$=NO$_2^-$), is a convenient trapping agent[11] for the NO$_2$• radical photogenerated from nitrite anions (eqs 1 and 2). The radical adds to *aci* NM forming a primary adduct (O$_2$N−

(1) Treinin, A.; Hayon, E. *J. Am. Chem. Soc.* **1970**, *92*, 5821−5828.
(2) Smith, P. A. S. *The Chemistry of Open-Chain Organic Nitrogen Compounds*; W. A. Benjamin, Inc.: New York, 1966; Vol. II, pp 455−513.
(3) Bilski, P.; Chignell, C. F.; Szychlinski, J.; Borkowski, A.; Oleksy, E.; Reszka, K. *J. Am. Chem Soc.* **1992**, *114*, 549−556.
(4) For the reaction 2•NO$_2$ ↔ N$_2$O$_4$ the rate constant and the equilibrium constant have been determined to be 4.5 x 10^8 M^{-1} s^{-1} and 7 × 10^4 M^{-1}, respectively. Schwartz, S. E.; White, W. H. *Adv. Environ. Sci. Technol.* **1983**, *12*, 1−116. Gratzel, M.; Henglein, A.; Lilie, J.; Beck, G. *Ber. Bunsenges. Phys. Chem* **1969**, *73*, 646−653.
(5) Grajower, R.; Jortner, J. *J. Am. Chem. Soc.* **1963**, *85*, 512−516.
(6) Gabr, I. M.; Rai, U. S.; Symons, M. C. R. *J. Chem. Soc., Chem. Commun.* **1993**, 1099−1100.
(7) Huie, R. E.; Padmaja, S. *Free Radical Res. Commun.* **1993**, *18*, 195−199.
(8) Czapski, G.; Holcman, J.; Bilski, B. H. J. *J. Am. Chem. Soc.* **1994**, *116*, 11465−11469.
(9) Mackor, A.; Wajer, Th. A. J.; De Boer, Th. J. *Tetrahedron* **1967**, *24*, 1623−1631.
(10) Pires, M.; Rossi, M. J.; Ross, D. S. *Int. J. Chem. Kinet.* **1994**, *26*, 1207−1227.
(11) Nitromethane can function as a spin trap only when present in the form of an *aci* anion, CH$_2$=NO$_2^-$ (*aci* NM). The transformation of nitromethane to the *aci* form occurs readily in alkaline solutions.[12,13]
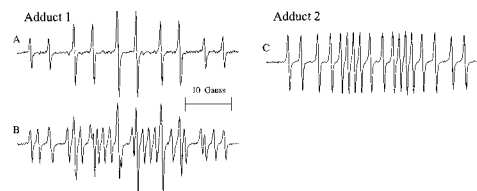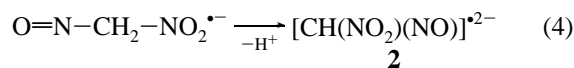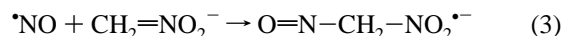(12) Turnbull, D.; Maron, S. H. *J. Am. Chem. Soc.* **1943**, *65*, 212−218.

Figure 1. EPR spectrum generated by UV irradiation (>300 nm) of NaNO$_2$ (143 mM) in NaOH (ca. 0.5 N) in the presence of NM (19 mM): (A) during exposure; (B) in the dark, 16 min after irradiation (the sample was purged with N$_2$ for 5 min prior to irradiation); and (C) spectrum of adduct **2** produced using gaseous •NO. Instrumental settings: micowave power 10 mW, modulation amplitude 0.165 G, gain 10 × 10^3 (A), 2 × 10^4 (B), and 1.6 × 10^4 (C), time constant 0.25 s, scan rate 8 min.

CH$_2$−NO$_2^-$•) which at high pH undergoes ionization yielding the dianion radical $^-$O$_2$N−•CH−NO$_2^-$, adduct **1**.[3,13] It has also been found[14] that at high pH gaseous •NO reacts with *aci* NM affording a nitro−nitroso dianion radical, [CH(NO$_2$)(NO)]•$^{2-}$, adduct **2**. The EPR spectrum of this species consists of 18-lines (3 × 3 × 2), originating from interaction of the unpaired electron with one hydrogen and two non-equivalent nitrogen atoms. Radical **2** is very persistent because its EPR signal could be observed for several hours. It was therefore expected that *aci* NM could be a useful trapping agent for •NO radicals generated during NO$_2^-$ photolysis. We report here the first EPR spectroscopic evidence for the formation of •NO from photo-irradiated nitrite anions.

A. Photolysis of NO$_2^-$. UV irradiation ($\lambda > 300$ nm) of NO$_2^-$ in the presence of *aci* NM in N$_2$-saturated NaOH generates a strong EPR spectrum of adduct **1** (Figure 1A). No other signals were observed even upon prolonged exposures (*ca.* 30 min). However, when irradiation was stopped and the sample was allowed to stay in the dark for several minutes, a new signal emerged superimposed on that of **1**. Figure 1B shows the EPR spectrum observed ca. 16 min after irradiation had ceased. This newly formed species has been identified as [CH(NO$_2$)(NO)] •$^{2-}$, adduct **2,** because its hfsc's (Table 1) are identical to those of the radical formed by reacting gaseous •NO with *aci* NM (Figure 1C).[14]

The probable mechanism of the formation of adduct **2** is *via* the addition of •NO to CH$_2$=NO$_2^-$ to give a primary spin adduct O=N−CH$_2$−NO$_2$•$^-$ (eq 3), which at high pH undergoes ionization forming a dianion radical (eq 4).

$$^\bullet NO + CH_2{=}NO_2^- \rightarrow O{=}N{-}CH_2{-}NO_2{}^{\bullet-} \qquad (3)$$

$$O{=}N{-}CH_2{-}NO_2{}^{\bullet-} \xrightarrow{-H^+} [CH(NO_2)(NO)]^{\bullet 2-} \qquad (4)$$
$$\mathbf{2}$$

The slow increase of the EPR signal intensity of adduct **2** in the dark can be contrasted with an almost immediate formation of a strong signal of adduct **2** in a sample which was purged with N$_2$ after the photolysis. Purging with N$_2$ merely removes volatile products accumulated during the nitrite photolysis, mostly •NO. Therefore it seems that •NO, which is not trapped by *aci* NM, reacted with adduct **2** either yielding a non-radical product or causing EPR line broadening, which renders the species undetectable. This effect is similar to the observation that broad, unresolved, low intensity EPR spectra generated by •NO oxidation of methyl- or *tert*-butyl-substituted phenols could

(13) Behar, D.; Fessenden, R. W. *J. Phys. Chem.* **1972**, *76*, 1710−1721.
(14) Reszka, K. J.; Bilski, P.; Chignell, C. F. *J. Am. Chem. Soc.* **1994**, *116*, 4119−4120.

Table 1. Hyperfine Splitting Constants (G) of the *aci* Anion Nitromethane Spin Adducts Produced during UV Photolysis of NO_2^- in NaOH Solutions

spin adduct	$^{\bullet}NO$ source/solvent	$a_N^{NO_2}$	a_N^{NO}	a_H
$^-O_2N^{\bullet}CHNO_2^-$	$NaNO_2/NaOH/NM/UV$	9.62	9.62 (NO_2)	4.13
$[CH(NO_2)NO]^{\bullet 2-}$	$^{\bullet}NO$ gas/NM/NaOH	11.50	6.50	2.80
	$NaNO_2/NaOH/NM/UV$	11.50	6.50	2.80
	$KNO_2/t\text{-}BuOH/UV^a$	11.46	6.42	2.81
	acetaldehyde/$NaNO_2$/ascorbic acid/base^b	11.9	6.0	2.6
$[CH(^{14}NO_2)(^{15}NO)]^{\bullet 2-}$	$Na^{15}NO_2/NaOH/NM/UV$	11.50	9.10 (^{15}NO)	2.80

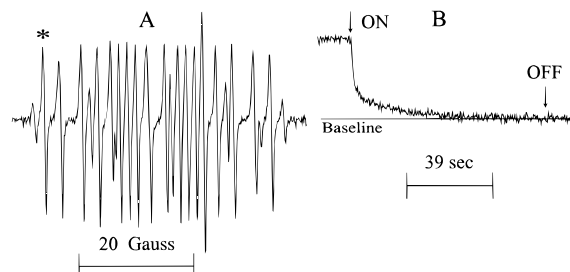^{a} From ref 19a. ^{b} From ref 19b.

Figure 2. (A) EPR spectrum of adducts **1** and **2** observed in the dark following irradiation (>300 nm) of NO_2^- (300 mM) in the presence of *aci* NM (20 mM) in NaOH (0.5 N). The sample was purged with N_2 prior to and after exposure. (B) Photoinduced ($\lambda_{irr} > 300$ nm) decay of adduct **2** recorded at the peak position of the first low-field component of the adduct (marked with an asterisk). Modulation amplitude 0.66 G and other recording parameters same as in Figure 1.

be transformed into intense, well-resolved spectra by purging the samples with nitrogen gas.[15]

Figure 2A shows a composite EPR spectrum of adducts **1** (minor) and **2** (dominating) from a sample containing NO_2^- and *aci* NM which was irradiated outside the EPR cavity for 5 min and then bubbled again with N_2 for 1 min to remove unreacted $^{\bullet}NO$. Kinetic measurements showed that adduct **2** disappears rapidly upon UV irradiation (Figure 2B).[16] In contrast, irradiation of a sample free of nitrite anions but containing only adduct **2** (produced chemically by reacting gaseous $^{\bullet}NO$ with *aci* NM under nitrogen) did not cause such a decrease (not shown). These observations suggest that adduct **2** undergoes a nitrite-photosensitized destruction to a non-radical product(s), which may explain why adduct **2** cannot be observed during the nitrite photolysis.

B. Photolysis of $^{15}NO_2^-$. To examine whether all detected $^{\bullet}NO$ molecules originate from nitrite anions we used ^{15}N-labeled nitrite, $^{15}NO_2^-$. It was expected that if nitrite is the only source of $^{\bullet}NO$, then only the $[CH(^{14}NO_2)(^{15}NO)]^{\bullet 2-}$ radical should be observed. If photodegradation of *aci* NM also produces $^{\bullet}NO$, then the EPR spectrum of adduct **2** should contain contribution from $[CH(^{14}NO_2)(^{15}NO)]^{\bullet 2-}$ and $[CH(^{14}NO_2)(^{14}NO)]^{\bullet 2-}$ radicals.

The EPR spectrum observed during anaerobic irradiation of $^{15}NO_2^-$ and *aci* NM (^{14}N) in NaOH contains adducts of both the $^{14}NO_2^{\bullet}$ and $^{15}NO_2^{\bullet}$ radicals, in agreement with our previous report.[3] When the photolyzed solution was briefly purged with N_2 (to speed up the appearance of adduct **2**) the EPR spectrum shown in Figure 3A was observed in the dark. This multicomponent spectrum contains signals from *aci* NM adducts with $^{14}NO^{\bullet}$, $^{15}NO^{\bullet}$, $^{14}NO_2^{\bullet}$, and $^{15}NO_2^{\bullet}$, in relative concentrations of ca. 43%, 42%, 5% and 10%, respectively. Simulated spectra of the individual species $[CH(^{14}NO_2)(^{14}NO)]^{\bullet 2-}$, $[CH(^{14}NO_2)(^{15}NO)]^{\bullet 2-}$, $[CH(^{14}NO_2)_2]^{\bullet 2-}$, and $[CH(^{15}NO_2)(^{14}NO_2)]^{\bullet 2-}$ are
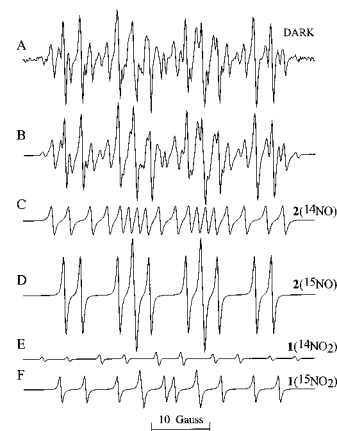

Figure 3. (A) EPR spectrum observed in the dark following UV irradiation (>300 nm) of ^{15}N-nitrite anion (100 mM) in the presence of *aci* NM (20 mM) in N_2-saturated NaOH (0.5 N). (B) Simulated spectrum using hfsc's for the ^{14}N and ^{15}N $^{\bullet}NO$ and $^{\bullet}NO_2$ adducts (from Table 1). (C–F) Simulated individual spectra of adduct **2** containing ^{14}NO, $[CH(NO_2)(^{14}NO)]^{\bullet 2-}$ (C) and ^{15}NO, $[CH(NO_2)(^{15}NO)]^{\bullet 2-}$ (D) and of adduct **1** containing $^{14}NO_2$, $[CH(^{14}NO_2)_2]^{\bullet 2-}$ (E) and $^{15}NO_2$, $[CH(^{15}NO_2)(^{14}NO_2)]^{\bullet 2-}$ (F) of relative contributions of ca. 43, 42, 5, and 10%, respectively.

shown in Figures 3C, 3D, 3E, and 3F, respectively. Hyperfine splitting constants used in these simulations are summerized in Table 1.

Detection of the $[CH(^{14}NO_2)(^{15}NO)]^{\bullet 2-}$ radical confirms the formation of $^{\bullet}NO$ from nitrite.[17] The spectrum in Figure 3A also shows the presence of unlabeled adduct **2**. Because the nitroso moiety of this adduct must be derived from *aci* NM, it appears that the spin trap may undergo nitrite-sensitized photodegradation and release $^{\bullet}NO$ and $NO_2^{\bullet}$ radicals.[18]

In conclusion, this study has provided unambiguous EPR spectroscopic evidence showing that UV irradiation (>300 nm) of NO_2^- in aqueous solutions generates the nitric oxide radical.[19a,b]

(15) Janzen E. G.; Wilcox, A. L.; Manoharan, V. *J. Org. Chem.* **1993**, *58*, 3597−3599.

(16) The decay of adduct **2** does not seem to be related to the formation of $^{\bullet}OH$ or $NO_2^{\bullet}$ radicals. Addition of $^{\bullet}OH$ scavengers, MeOH or formate, did not accelerate the appearance of adduct **2**. Radicals derived from these additives are also known to react with $^{\bullet}NO$ (see ref 9), and $NO_2^{\bullet}$ is efficiently trapped by *aci* NM.[3,13]

(17) Previously $^{\bullet}NO$ from nitrite photolysis has been detected fluorometrically (Zafiriou, O. C.; McFarland, M. *Anal. Chem.* **1980**, *52*, 1662−67) which does not provide any "molecular fingerprint" to indicate the source of the $^{\bullet}NO$. In contrast, the EPR method employed in this work allowed us to assign the $^{\bullet}NO$ radical unambiguously to the nitrite by the use of ^{15}N-labeled NO_2^-, and detection of the corresponding ^{15}N-labeled *aci* NM/^{15}NO adduct.

(18) Under our conditions photodegradation of *aci* NM is responsible for ca. 50% of adduct **2**. Irradiation of $^{15}NO_2^-$ in the presence of $CH_2=^{14}NO_2^-$ also yields a small amount of adduct **1** containing two $^{14}NO_2$ moieties, $[CH(^{14}NO_2)_2]^{\bullet 2-}$. These observations show that caution has to be exercised when using *aci* NM for quantitative analysis of $^{\bullet}NO$ and $NO_2^{\bullet}$ radicals from UV photolyzed nitrite solutions.

(19) (a) There are at least two other routes to adduct **2**. Zeldes and Livingston in their study of free radicals produced by UV irradiation of KNO_2 in a *tert*-butyl alcohol−water mixture observed an EPR spectrum with hyperfine parameters identical to that of our adduct **2** (Table 1). However at this time the species responsible for this spectrum has not been identified (Zeldes, H.; Livingston, R. *J. Am. Chem. Soc.* **1968**, *90*, 4540−4544). (b) Recently, it has been found that the same species is produced by reacting sodium nitrite with acetaldehyde in the presence of ascorbate, followed by alkalization (Lagercrantz, C. *J. Chem. Soc., Perkin Trans. 2* **1995**, 101−105).