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Homolysis of the Peroxynitrite Anion Detected with Permanganate

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The reaction of peroxynitrite with violet-colored MnO_4^- leads to the formation of green MnO_4^{2-} . The rate constant for the reaction at pH 11.7, 5.5 mM ionic strength, and 25 °C, $0.020 \pm 0.001 \text{ s}^{-1}$, is independent of the MnO_4^- concentration; homolysis of ONOO^- to NO^\bullet and $\text{O}_2^{\bullet-}$ is the rate-determining step. Both NO^\bullet and $\text{O}_2^{\bullet-}$ react with MnO_4^- with rate constants of $(3.5 \pm 0.7) \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ and $(5.7 \pm 0.9) \times 10^5 \text{ M}^{-1}\text{s}^{-1}$, respectively. The activation volume and activation energy for breaking the N–O bond are $12.6 \pm 0.8 \text{ cm}^3\text{mol}^{-1}$ and $102 \pm 2 \text{ kJ mol}^{-1}$, respectively. In combination with the known standard Gibbs energies of formation of NO^\bullet and $\text{O}_2^{\bullet-}$, the rate of the reaction of NO^\bullet and $\text{O}_2^{\bullet-}$, and the pK_a of ONOOH , we find a standard Gibbs energy of formation of ONOO^- of $+68 \pm 1 \text{ kJ mol}^{-1}$, and of ONOOH of $+31 \pm 1 \text{ kJ mol}^{-1}$.

Introduction

Peroxynitrite¹ is formed from the diffusion-controlled reaction of $\text{O}_2^{\bullet-}$ with NO^\bullet , and $k = (1.6 \pm 0.3) \times 10^{10} \text{ M}^{-1}\text{s}^{-1}$.² ONOO^- is fairly stable at pH values above 10. In the protonated form, ONOOH isomerizes to NO_3^- with a rate constant of 1.2 s^{-1} (reaction 1).³ The pK_a of the $\text{ONOOH}/\text{ONOO}^-$ system is 6.5–6.8, depending on the ionic strength.³ Homolysis of ONOO^- is thought to occur with a rate constant of 0.017 s^{-1} (reaction 2).



The first investigation of the kinetics of reaction 2 was made with tetranitromethane, which consumes $\text{O}_2^{\bullet-}$ very rapidly with a rate constant of $2 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$.⁴ When we

reinvestigated the homolysis of ONOO^- with tetranitromethane, we reported a rate constant of $0.005\text{--}0.0185 \text{ s}^{-1}$ that appears to be dependent on NO^\bullet concentration, although the presence of NO^\bullet should have blocked the formation of trinitromethanide.⁵ However, other investigators reported inhibition by NO^\bullet .^{6,7} Lymar and Poskrebyshev used the methyl viologen radical to probe the kinetics of the homolysis reaction and obtained a rate constant of $0.017 \pm 0.001 \text{ s}^{-1}$.⁸ The methyl viologen radical does not react with nitrogen monoxide but reacts with $\text{O}_2^{\bullet-}$ with a rate constant of $(3.5 \pm 0.2) \times 10^9 \text{ M}^{-1}\text{s}^{-1}$. Thus, nitrogen monoxide accumulates, and the rate of superoxide production decreases; this was observed and taken into account.⁸ As we found only a small effect of nitrogen monoxide on the peroxynitrite–tetranitromethane system,⁵ we set out to find a different detection reaction.

In 1929, it was reported that the reaction of peroxynitrite with violet MnO_4^- leads to the formation of green MnO_4^{2-} , (reaction 3)⁹ and, in 1964, this reaction was used for the determination of ONOO^- concentration by potentiometric titration.¹⁰ Although the reaction was first reported nearly

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(1) Systematic names and *trivial* names in italics: ONOO^- , oxidoperoxynitrite(1-), *peroxynitrite*; ONOOH , (hydridodioxido)oxidonitrogen, *peroxynitrous acid*; NO^\bullet , oxidonitrogen(•) or nitrogen monoxide, *nitric oxide*; $\text{O}_2^{\bullet-}$, dioxide(•1-), *superoxide*; O_2 , dioxygen; $\text{CO}_2^{\bullet-}$, dioxidocarbonate(•1-), *carbonate radical*; MnO_4^- , tetraoxido-manganate(1-), *permanganate*; MnO_4^{2-} , tetraoxidomanganate(2-), *manganate*; Connelly, N. G.; Damhus, T.; Hartshorn, R. M.; Hutton, A. T. *Nomenclature of Inorganic Chemistry. IUPAC Recommendations 2005*; Royal Society of Chemistry: Cambridge, U.K., 2005.

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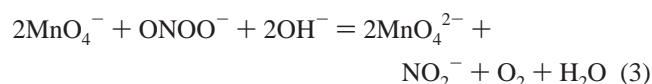
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eight decades ago, no data regarding the kinetics or a mechanism have been published. We report here that ONOO^- reduces MnO_4^- in a reaction that is zero-order in permanganate.



Materials and Methods

Chemicals. ONOO^- was synthesized from NO^\bullet and $(\text{Me}_4\text{N})\text{O}_2$ according to the method of Bohle et al.¹¹ NO^\bullet and argon were obtained from PanGas. All of the other chemicals were purchased at the highest grade available. Deionized water was purified with a Millipore Milli-Q unit. ONOO^- solutions in 10 mM KOH were freshly prepared for each experiment and stored in an ice bath. All of the stock solutions were protected from light.

Instrumentation. Kinetics experiments were carried out with OLIS RSM 1000 and Applied Photophysics SX 17MV stopped-flow spectrophotometers operating in the symmetric mixing mode at ambient pressure and 25 °C. For each experiment, the mixed solution from the stop syringe was collected, and the pH was measured with a Metrohm glass electrode.

An Applied Photophysics SX 18MV stopped-flow spectrophotometer was used for the determination of the activation energy. The temperature range was 15–55 °C at ambient pressure (ca. 950 mbar). The decay of MnO_4^- was followed at 524 nm, and the formation of MnO_4^{2-} was monitored at 420 and 610 nm.

High-pressure stopped-flow experiments were carried out with a Hi-Tech HPSF-56 equipped with a diode-array spectrophotometer, which has been described previously.^{12,13} The pressure range was 5–175 MPa. Absorbance changes at 370–750 nm were followed at 25 °C and pH 11.7. Kinetics data collected at 420, 524, and 610 nm were analyzed, and reaction rates and constants were extracted with *KaleidaGraph* software.

Pulse radiolysis experiments were carried out by irradiation of the samples with a Febetron 705 accelerator as described earlier.¹⁴ All of the solutions were saturated with O_2 (1.16 mM, corrected for pressure and ionic strength)¹⁵ and then irradiated directly after mixing in the symmetric mode.

Errors are reported as t^*/\sqrt{n} at the 95% probability level, where n is the number of samples, s/\sqrt{n} is the standard deviation, and t^* is the distribution over $n - 1$ degrees of freedom, unless otherwise indicated.

Results

The decay of MnO_4^- (50–500 μM) observed at 524 nm and the formation of MnO_4^{2-} observed at 420 and 610 nm upon mixing with ONOO^- (20 μM in 5 mM KOH) in aqueous solution at 25 °C over a time scale of 200 s at pH 11.7 are shown in Figure 1. Furthermore, the figure shows that the observed rates of both decay and formation are

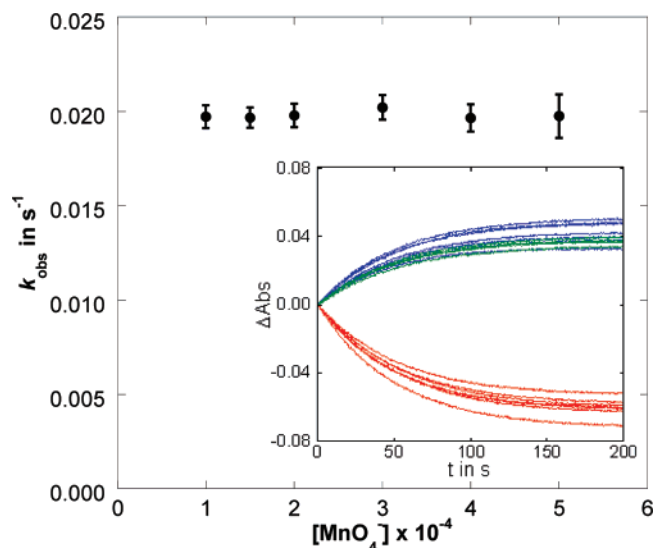


Figure 1. Observed rate constants for the decay of MnO_4^- with 20 μM ONOO^- as a function of MnO_4^- concentration at pH 11.7 and 25 °C. Inset: Decay of MnO_4^- (50–500 μM) with 20 μM ONOO^- at 524 nm (red) and formation of MnO_4^{2-} at 420 nm (green) and 610 nm (blue) at pH 11.7 and 25 °C. Rates of decay of MnO_4^- and of formation of MnO_4^{2-} are independent of the MnO_4^- concentration.

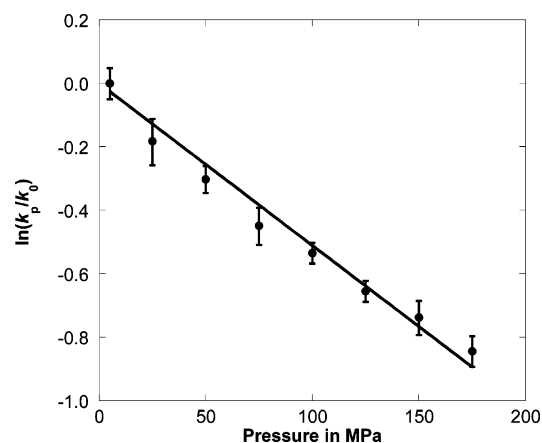


Figure 2. Dependence of the rate constants for the homolysis of peroxynitrite on pressure at 40 μM ONOO^- , 400 μM MnO_4^- , pH 11.7, and 25 °C.

independent of MnO_4^- concentration, and a first-order rate constant of $0.020 \pm 0.001 \text{ s}^{-1}$ at an ionic strength of 5.5 mM and 25 °C was obtained. All of the concentrations are those after mixing. In control experiments, we mixed NaNO_2 (10 μM) with MnO_4^- and followed at 300 and 524 nm whether a reaction took place; no absorbance changes were observed during the 200 s analysis period. We also verified that MnO_4^- is not decomposed by hydroxide alone at pH 11.7.

If the bond scission of N–O were the rate-determining step, the reaction rate would be inversely proportional to pressure. Figure 2 shows the rate of reaction as a function of pressure up to 175 MPa; the volume of activation, $\Delta V^\ddagger = 12.6 \pm 0.8 \text{ cm}^3\text{mol}^{-1}$, was calculated from the plot of $\ln(k/k_0)$ versus pressure.

The rate of reaction of MnO_4^- with the homolysis products NO^\bullet and $\text{O}_2^{\bullet-}$ were determined separately. Figure 3 shows the rate of disappearance of NO^\bullet (20 μM) as a function of MnO_4^- concentration (50–250 μM) under mostly pseudo-

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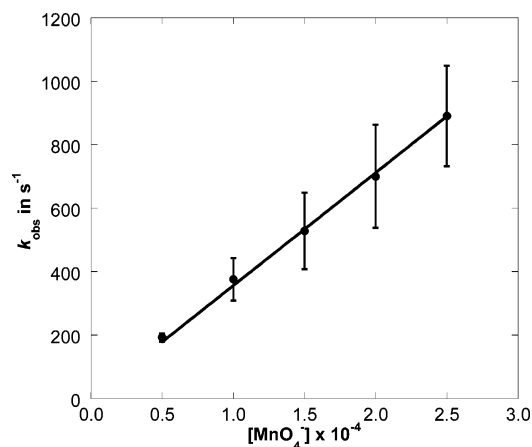


Figure 3. Rate constants for the decay of MnO_4^{2-} as a function of MnO_4^{2-} concentration at $20 \mu\text{M NO}^\bullet$, pH 11.7, and 25°C .

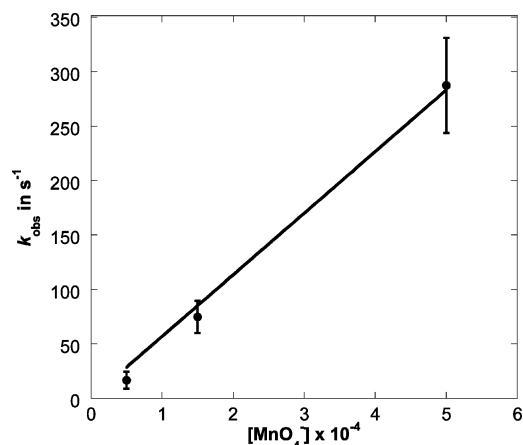


Figure 4. Rate constants for the decay of MnO_4^{2-} as a function of MnO_4^{2-} concentration at $30 \mu\text{M O}_2^{\bullet-}$, pH 11.7, and 25°C . The error bars are $2s$.

first-order conditions; a rate constant of $(3.5 \pm 0.7) \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ was obtained. In Figure 4, the rate constant for the reaction of $\text{O}_2^{\bullet-}$ ($30 \mu\text{M}$) is plotted as a function of MnO_4^{2-} concentration ($50\text{--}500 \mu\text{M}$). This reaction was investigated by pulse radiolysis with HCOO^- ($5\text{--}50 \text{ mM}$) irradiated at a dose of 50 Gy to yield $\text{O}_2^{\bullet-}$. The calculated rate constant is $(5.7 \pm 0.9) \times 10^5 \text{ M}^{-1}\text{s}^{-1}$; because the lowest concentration of MnO_4^{2-} does not meet pseudo-first-order conditions, it is not included in the fit.

The Arrhenius plot in Figure 5 shows the temperature dependence of the rate constant for homolysis; an activation energy of $102 \pm 2 \text{ kJ mol}^{-1}$ was derived, and the frequency factor $A = (1.2 \pm 0.5) \times 10^{16} \text{ s}^{-1}$ was obtained by extrapolation.

The rate constants determined here were used to calculate an equilibrium constant for the homolysis of ONOO^- , which could then be combined with values from the literature to generate thermodynamic parameters for ONOO^- and ONOOH . These parameters, together with those for nitrate for comparison, are collected in Table 1.

Discussion

We studied the reactions of MnO_4^{2-} with ONOO^- , NO^\bullet , and $\text{O}_2^{\bullet-}$. The observed rate constant of $0.020 \pm 0.001 \text{ s}^{-1}$

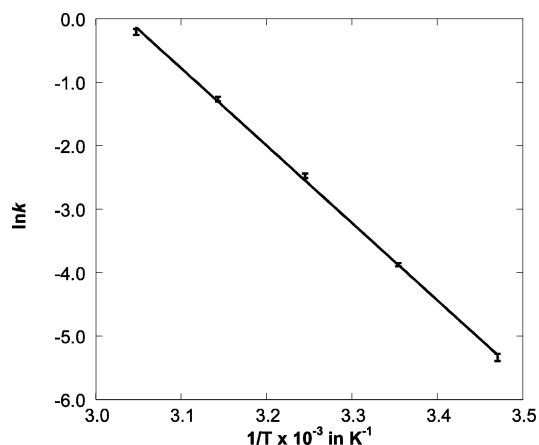


Figure 5. Arrhenius plot for the homolysis of ONOO^- over the temperature range $15\text{--}55^\circ\text{C}$ at $20 \mu\text{M ONOO}^-$, $400 \mu\text{M MnO}_4^{2-}$, and pH 11.7.

Table 1. Thermodynamic Parameters for Nitrate, Peroxynitrite, and Peroxynitrous Acid

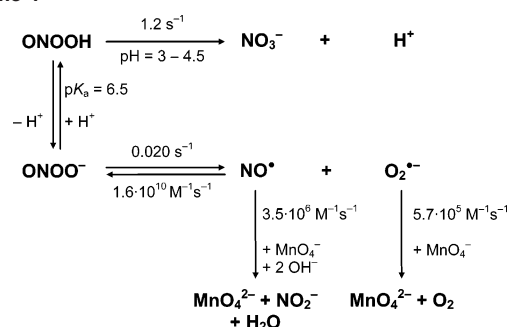
	$\Delta_r G^\circ, \text{ kJ mol}^{-1}$	$\Delta_r H^\circ, \text{ kJ mol}^{-1}$	$S^\circ, \text{ J K}^{-1} \text{ mol}^{-1}$
NO_3^-	-111.3^a	-207.4^a	146.4^a
ONOO^-	68^b	-43^c	89^b
	67^d		
	69^e	-42^e	96^e
	69^f		
ONOOH	31^b	-61^b	153^b
	30^d		
	31^f		
	32^g		

^a Ref 23. ^b This work. ^c Ref 37. ^d Ref 36. ^e Ref 6. ^f Ref 8. ^g Ref 4.

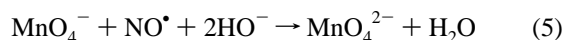
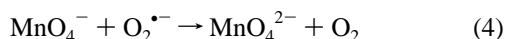
for the reaction of ONOO^- with MnO_4^{2-} is quite close to that ($k = 0.017 \text{ s}^{-1}$) reported for the reaction of ONOO^- with tetranitromethane⁴ and with the methyl viologen radical.⁸ In the reaction of ONOO^- with MnO_4^{2-} , both homolysis products, NO^\bullet and $\text{O}_2^{\bullet-}$, are rapidly consumed by the detection reagent. The rate constant we obtained for the reaction of NO^\bullet with MnO_4^{2-} , $(3.5 \pm 0.7) \times 10^6 \text{ M}^{-1}\text{s}^{-1}$, is in excellent agreement with previously reported values of 3.93×10^6 ,¹⁶ 1.18×10^6 ,¹⁷ and $5.60 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$.¹⁸ For the reaction of $\text{O}_2^{\bullet-}$ with MnO_4^{2-} , we determined a rate constant of $(5.7 \pm 0.9) \times 10^5 \text{ M}^{-1}\text{s}^{-1}$ at pH 11.7, which is lower than the earlier published value of $(9.5 \pm 0.8) \times 10^5 \text{ M}^{-1}\text{s}^{-1}$ at pH 9.4,¹⁹ but the discrepancy may be ascribed to the difference in pH. The results are summarized in Scheme 1. Rate constants ranging from 3.8×10^9 to $1.9 \times 10^{10} \text{ M}^{-1}\text{s}^{-1}$ have been reported for reaction 2, the formation of peroxynitrite from superoxide and nitrogen monoxide.^{2,3,7,20–22} Three mechanistically different flash photolysis experiments led to the value of $(1.6 \pm 0.3) \times 10^{10} \text{ M}^{-1}\text{s}^{-1}$ used here;

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Scheme 1



additionally, a pulse radiolysis experiment indicated a rate constant larger than $1 \times 10^{10} \text{ M}^{-1}\text{s}^{-1}$.² Given the rate constant of $(1.6 \pm 0.3) \times 10^{10} \text{ M}^{-1}\text{s}^{-1}$ and the concentrations used in this study, NO[•] and O₂^{•-} are consumed about 2.5 to 25 times faster than ONOO⁻ is formed. Both reactions are favorable: from $E^\circ(\text{NO}_2^-, \text{H}_2\text{O}/\text{NO}^\bullet, 2\text{OH}^-) = -0.46 \text{ V}$,²³ $E^\circ(\text{O}_2/\text{O}_2^{\bullet-}) = -0.35 \text{ V}$,²⁴ and $E^\circ(\text{MnO}_4^-/\text{MnO}_4^{2-}) = +0.56 \text{ V}$,²³ we calculate Gibbs energy changes for reactions 4 and 5 of -88 and -98 kJ/mol , respectively.



Our observation of an activation volume of $12.6 \pm 0.8 \text{ cm}^3\text{mol}^{-1}$ also supports that bond cleavage in ONOO⁻ is homolytic. The significantly positive volume can be ascribed to desolvation²⁵ and compares well with other known volumes of activation for bond scission in peroxides.²⁶

The Arrhenius plot yields an activation energy of $102 \pm 2 \text{ kJ mol}^{-1}$ for the decomposition of ONOO⁻ to NO[•] and O₂^{•-}. From an Eyring plot (not shown), we derived an activation enthalpy of $99 \pm 2 \text{ kJ mol}^{-1}$ and an activation entropy of $54 \pm 4 \text{ J mol}^{-1} \text{ K}^{-1}$. These parameters were used to calculate a Gibbs activation energy of $83 \pm 3 \text{ kJ mol}^{-1}$ at 25 °C. According to ab initio calculations, the activation energy for cleavage of the N–O bond lies in the range 90–110 kJ mol⁻¹.²⁷ The activation energy for the homolysis of

peroxynitrite obtained in this work, although somewhat higher, is comparable to the $93 \pm 7 \text{ kJ mol}^{-1}$ for the N–O scission of O₂NOOH to form NO₂[•] and HOO[•],²⁸ and for the decay of ONOO⁻ under basic conditions (91 kJ mol^{-1}).^{29,30} The frequency factor A , $(1.2 \pm 0.5) \times 10^{16} \text{ s}^{-1}$, also indicates homolysis, values for which are typically in the range 10^{15} – 10^{17} s^{-1} .³¹

We obtained an equilibrium constant K of $(1.25 \pm 0.2) \times 10^{-12} \text{ M}$ for the homolysis of ONOO⁻. From this K , we calculate a standard Gibbs energy of $67.9 \pm 0.4 \text{ kJ mol}^{-1}$ for reaction 2, which is significantly different from our earlier estimates.^{5,32,33} $\Delta_f G^\circ(\text{O}_2^{\bullet-})$ is $33.8 \pm 1.1 \text{ kJ mol}^{-1}$ based on $E^\circ(\text{O}_2/\text{O}_2^{\bullet-}) = 350 \pm 11 \text{ mV}$ ²⁴ and $\Delta_f G^\circ(\text{NO}^\bullet)\text{aq} = 102.0 \pm 0.2 \text{ kJ/mol}$, calculated from $\Delta_f G^\circ(\text{NO}^\bullet)\text{g} = +86.57 \text{ kJ/mol}$ ³⁴ and a Henry constant of $1.92 \times 10^{-3} \text{ M/0.100MPa}$ ³⁵ at 25 °C, from which follows a $\Delta_f G^\circ(\text{ONOO}^-)$ of $68 \pm 1 \text{ kJ mol}^{-1}$, very close to earlier reported values of 69 and 67 kJ mol⁻¹ (Table 1).^{4,8,36} On the basis of the pK_a for ONOOH, 6.5 ± 0.1 ,³ we calculate that $\Delta_f G^\circ(\text{ONOOH}) = 30.8 \pm 1.2 \text{ kJ mol}^{-1}$. Given the agreement, we recommend $\Delta_f G^\circ(\text{ONOO}^-) = 68 \pm 1 \text{ kJ mol}^{-1}$ and $\Delta_f G^\circ(\text{ONOOH}) = 31 \pm 1 \text{ kJ mol}^{-1}$ for further usage.

In conclusion, we determined a rate constant of 0.020 s^{-1} for the homolysis of ONOO⁻ to NO[•] and O₂^{•-} with MnO₄⁻. The detection with MnO₄⁻ is facilitated because the reaction is driven to completion by the simultaneous consumption of both homolysis products.

Acknowledgment. We thank Dr. P. L. Bounds for helpful discussions and linguistic advice. These studies were supported by the ETH Zurich and the Swiss National Foundation.

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