# Mechanism of Reduction of the Nitrite Ion by Cu$^{I}$ Complexes

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**Keywords:** Nitrite / Copper(I) / Reduction / NO

The reaction of $Cu^{+}_{aq}$ with $NO_2^-$ was studied. The results are consistent with the mechanism: $Cu^{+}_{aq} + NO_2^- \rightleftarrows (Cu^+NO_2^-)$, $K = 140 \pm 30$ M$^{-1}$; $(Cu^+NO_2^-) + H^+ \rightarrow Cu^{2+} + NO + OH^-$, $k = 4.6 \times 10^5$ M$^{-1}$s$^{-1}$. It is proposed that the copper is bound to the nitrogen in the transient complex $(Cu^+NO_2^-)$. In addition, the reaction was studied with $(Cu^IL^i)^+$ complexes. The results show that the ligands reduce the value of $K$ and that their effect on $k$ depends on the redox potential of the $(Cu^{II/I}L^i)$ couple. This reaction is the simplest model for the CuNIR (NIR = Nitric reductase) enzymes.

## Introduction

The one-electron reduction of nitrite to NO is of major importance in a variety of biological processes[1−3] and in waste water treatment.[4−6] The reducing agent, or the electro-catalytic active agent,[7−9] is usually a low valent transition metal complex, e.g. Fe$^{II}$,[9−13] Co$^{I}$,[14,15] Ru$^{II}$,[8,16] Cu$^{I}$.[17−20] For example, one of the steps in the important nitrogen cycle is the reduction of nitrite to nitric oxide.[21] Dinitrogen is released into the atmosphere by the activity of denitrifying bacteria found in soils and sediments.[22−25] The enzyme nitrite reductase (NIR) is essential for these bacteria.[1,2] Several mechanistic studies on the reduction of $NO_2^-$ by Cu$^{I}$ complexes in organic solvents were performed in order to elucidate the reduction mechanism, and as plausible models for the enzymatic process.[26−35] In the present work, we decided to study the mechanism and kinetics of the reaction between $Cu^{+}_{aq}/(Cu^IL)^+$ complexes and $NO_2^-$. The results indicate that a transient complex, $(Cu^+NO_2^-)$, is formed initially, and that this complex reacts with $H_3O^+$ to yield the final products.

It is of interest to note that the oxidation of NO by Fe$^{III}$L complexes proceeds via the reverse mechanism[35] [see reaction (1)].

$$[Fe^{III}L] + NO \rightleftarrows [Fe^{III}L{\cdot}NO] \xrightarrow{OH^-} [Fe^{II}L] + HNO_2 \qquad (1)$$

## Results and Discussion

When $Cu^{+}_{aq}$ is introduced into a solution containing excess $NaNO_2$ (2.5−100) × $10^{-3}$ M in the pH range 4.15−5.45 (buffered by 0.10 M acetate, the results are independent of the acetate concentration in the range 0.10−0.50 M), a decrease in the absorption at 355 nm due to nitrite is observed. The kinetics of this process, followed using the stopped-flow technique, obey a first order rate law on Cu$^{I}$. The observed rate increases with increasing $[NO_2^-]$. However, the results indicate that a limiting rate is observed at high $[NO_2^-]$ (see Figure 1).

Analysis of the results assuming a mechanism involving the fast formation of an intermediate complex fits well with the experimental data [Figure 2, reaction (2)]. The value of $k_2$ (Table 1) derived from the intercepts of plots analogous to Figure 2 is pH-dependent. A plot of $k_2$ vs. $[H_3O^+]$ (Figure 3) yields a straight line ($k_2 = k_{*2}[H_3O^+]$). From the slope of the line in this figure one obtains $k_{*2} = (4.6 \pm 0.5) \times 10^5$ M$^{-1}$ s$^{-1}$.

$$Cu^{+}_{aq} + NO_2^- \xrightleftharpoons{K_{2app}} [Cu^+NO_2^-] \xrightarrow[H^+]{k_2} Cu^{2+}_{aq} + NO + OH^- \qquad (2)$$

$$-d[NO_2^-]/dt = (k_2K_{2app}[NO_2^-][Cu^{+}_{aq}])/(1 + K_{2app}[NO_2^-])$$

$$1/k_{obsd.} = 1/(k_2K_{2app}[NO_2^-]) + 1/k_2$$

The results indicate that a mechanism in which an unreactive $Cu^I{-}NO_2^-$ complex is formed [reactions (3) and (4)] could yield the same rate law. However, this would require that the reaction according to reaction (4) is a third order process. Thus, this mechanism seems unlikely.

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DOI: 10.1002/ejic.200400255

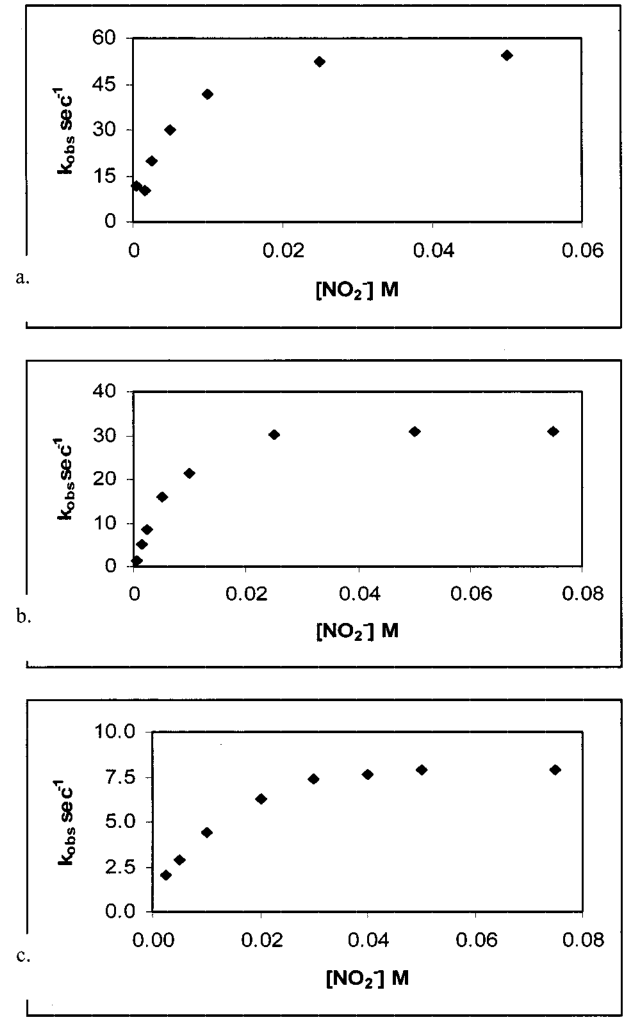

Figure 1. Dependence of $k_{obsd.}$ on the $[NO_2^-]$. The experiments were performed in solutions containing $5.0 \times 10^{-4}$ M $Cu^+_{aq}$, 0.10 M $NaClO_4$, 0.10 M acetic acid, 0.023 M $NH_4^+$, helium saturated at a final pH of: a. 4.3, b. 4.65, c. 5.25.

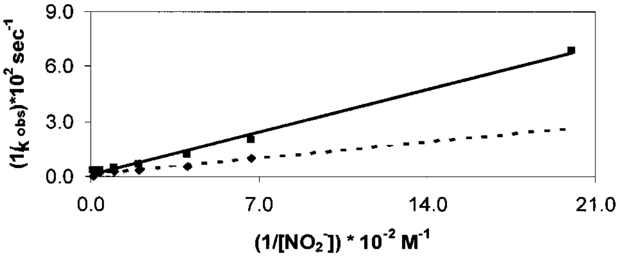

Figure 2. Dependence of $1/k_{obsd.}$ on $1/[NO_2^-]$. The experiments were performed in solutions containing $5.0 \times 10^{-4}$ M $Cu^+_{aq}$, 0.10 M $NaClO_4$, 0.10 M acetic acid, 0.023 M $NH_4^+$, helium saturated, the final pH: 4.3 (-----), 4.65 (—)

$$Cu^+_{aq} + NO_2^- \rightarrow (Cu^+NO_2^-) \qquad (3)$$

$$Cu^+_{aq} + NO_2^- \rightarrow P \qquad (4)$$

$$Cu^+_{aq} + NO_2^- + H_3O^+ \rightarrow Cu^{2+}_{aq} + NO + OH^- \qquad (5)$$

Table 1. The effect of $[H_3O^+]$ on $k_2$ and on the observed stability constant of the $(Cu^+NO_2^-)$ complex (the experiments were performed on helium-saturated solutions containing $5.0 \times 10^{-4}$ M $Cu^+_{aq}$, 0.10 M $NaClO_4$, 0.10 M acetic acid, 0.023 M $NH_4^+$)

| $K_{2app}$ ($M^{-1}$) | $k_2$ ($M^{-1}s^{-1}$) | $[H_3O^+]$ (M) | pH |
|---|---|---|---|
| 77 | 2.9 | $3.5 \times 10^{-6}$ | 5.45 |
| 62 | 3.4 | $4.0 \times 10^{-6}$ | 5.40 |
| 62 | 5.0 | $1.3 \times 10^{-5}$ | 4.90 |
| 152 | 8.2 | $2.0 \times 10^{-5}$ | 4.70 |
| 120 | 12 | $2.2 \times 10^{-5}$ | 4.60 |
| 132 | 12.7 | $4.0 \times 10^{-5}$ | 4.40 |
| 110 | 23 | $5.0 \times 10^{-5}$ | 4.30 |
| 154 | 33 | $7.1 \times 10^{-5}$ | 4.15 |

The value of $k_2$ (Table 1), derived from the intercepts of plots analogous to Figure 2, is pH dependent.

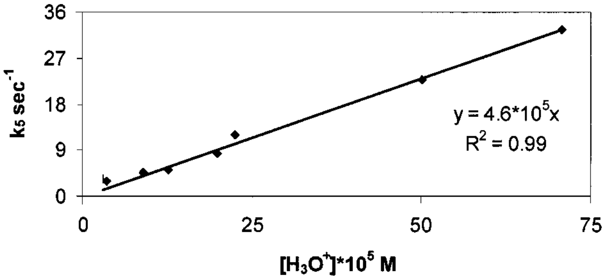

Figure 3. Dependence of $k_5$ on $[H_3O^+]$. The experiments were performed in solutions containing $5.0 \times 10^{-4}$ M $Cu^+_{aq}$, 0.10 M $NaClO_4$, 0.10 M acetic acid, 0.023 M $NH_4^+$, helium saturated.

The apparent values of $K_{2app}$, derived from the slopes of the straight lines, e.g. from Figure 2 (at several pH's in this pH range) are presented in Table 1. The average of $K_{2app}$ in the pH range 4.15−4.70 is 130 ±25 $M^{-1}$ (Table 1). When the pH is increased, pH ≥ 4.7, the formation of CuOH [$K_{sp}(CuOH) = 1 \times 10^{-14}$ $M^{-2}$ [36]] causes an apparent decrease in $K_{2app}$, (Table 1). The contribution of reaction (6)[37] to $K_{2app}$ was examined, $K_2 = K_{2app}(1 + K_a[H^+])$.

$$HNO_2 \rightleftarrows NO_2^- + H^+; pK_a = 3.25 \qquad (6)$$

This analysis indicates that reaction (6) has a minor effect on $K_2$, $K_2 = 140 \pm 30$ $M^{-1}$. From $K_2$, it can be calculated that when $[NO_2^-]$ = 7 mM, the observed rate of reaction is ca. 50% of the maximum rate. Therefore, the binding of $NO_2^-$ to $Cu^+_{aq}$ is ca. 100 times weaker than that to CuNIR, in which $K_M$ = 0.074 mM.[38]

Solutions of $NaNO_2$ were mixed with $Cu^+_{aq}$ at a final pH of 2.20 (at this pH the nitrite is present mostly as $HNO_2$). Figure 4 describes $k_{obsd.}$ as a function of $[NaNO_2]$ at this acidic pH. It is apparent that there is no limiting rate (at higher pH's and the same $NO_2^-$ concentrations a deviation from linearity is observed, as shown in Figure 1). Though

$HNO_2$ is a stronger oxidant than $NO_2^-$,[37] there is no reason to suggest that reaction (7) occurs.

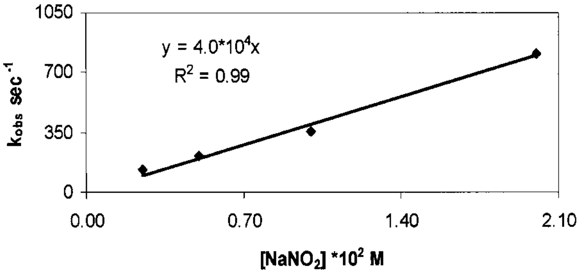

Figure 4. Dependence of $k_{obsd.}$ on $[NaNO_2]$. The experiments were performed in solutions containing $5.0 \times 10^{-4}$ M $Cu^+_{aq}$, 0.10 M $NaClO_4$, 0.023 M $NH_4^+$, the final pH was 2.20, helium saturated

$$Cu^+_{aq} + HNO_2 \rightleftarrows (Cu^+HNO_2)^+ \tag{7}$$

Moreover, if $(Cu^+HNO_2)^+$ were formed its $pK_a$ is expected to be lower than the $pK_a$ of $HNO_2$. Therefore, when the pH ≥ 4.15, $(Cu^INO_2^-)$ is clearly the complex formed. At pH values lower than the $pK_a$ of $HNO_2$, the $NO_2^-$ concentration is low so that the apparent value for $k_{*5}K_5$ is expected to be lower at pH 2.20 than that observed at pH 4.15−5.45. The slope of the line in Figure 4 equals the apparent value of $k_{*2}K_2[H_3O^+] = 4.0 \times 10^4$, i.e. $k_{*2}K_2$ (at pH 2.20) = $5.5 \times 10^6$ M$^{-2}$sec$^{-1}$ as compared to $k_{*2}K_2 = 4.6 \times 10^5 \times 140 = 6.4 \times 10^7$ M$^{-2}$sec$^{-1}$ at pH = 4.15−4.70. Thus, at pH 2.20 the apparent value of $k_{*2}K_2$ equals ca. 10% of that observed at pH 4.15−4.80. The nitrite concentration at pH 2.20 is ca. 10% of its concentration at pH ≥ 4.15. This result is in agreement with the suggested mechanism: It should be pointed out that if $HNO_2$ were the oxidant, a lower apparent rate is expected also at pH 2.20, as the concentration of $HNO_2$ is not proportional to $[H_3O^+]$ below the $pK_a$ of $HNO_2$. However, the nonlinear dependence of the observed rate at pH ≥ 4.1 and the linear dependence at pH 2.20 on $[NO_2^-] + [HNO_2]$, proves that the active oxidant is $NO_2^-$.

The yield of NO from the reaction was measured by two techniques: 1. EPR, by measuring the effect of the addition of NO to 4,4,5,5-tetramethyl-2-(1-pyryl)-2-imidazoline-1-oxyl 3-oxide (PN) on the EPR spectrum. 2. Mass spectrometry. Both techniques revealed that [NO] ≥ 75% $[Cu^+_{aq}]$. This result demonstrates that the yield of NO is quantitative as some dioxygen always enters the samples during the handling of the solutions.

The mechanism observed here is in agreement with that observed for several other $Cu^I$ complexes in methanol/acetonitrile.[26,28,29] However, the rate constant is considerably higher for $Cu^+_{aq}$ than for those complexes. This observation is attributed to the stabilization of $Cu^I$ by the ligands used in those studies, caused by an anodic shift of the redox potential of the $Cu^{II/I}$ couple. Therefore, it seemed of interest to study the mechanism and kinetics of reduction of $NO_2^-$ by $(Cu^IL)^+$ complexes in aqueous solutions. Two complexes were studied:

1. A copper(I) complex which is a stronger reducing agent than $Cu^+_{aq}$. For this purpose, $(Cu^IL^1)^+$ ($L^1$ = 2,5,8,11-tetramethyl-2,5,8,11-tetraazadodecane, Figure 5) was chosen, $E^0(Cu^{II}L/Cu^IL^1) = 0.116$ V (vs. NHE),[39] at pH 5.0. At this pH $k[NO_2^- + (Cu^IL^1)^+] = 35 \pm 10$ M$^{-1}$s$^{-1}$ is obtained. The observed rate is nearly independent of $[H_3O^+]$ in the pH range 5.0−6.0. This rate is considerably higher than that obtained for $Cu^+_{aq}$ over this pH range. The lack of dependence on $[H_3O^+]$ suggests that, in this case, $H_2O$ acts as the proton donor, i.e. the general mechanism for the decomposition of the complex $[(Cu^IL^i)^+NO_2^-]$ is according to reaction (8).

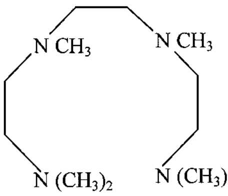

Figure 5. $L^1$ = 2,5,8,11-tetramethyl-2,5,8,11-tetraazadodecane

$$[(Cu^IL^i)^+NO_2^-] + H_3O^+/H_2O \rightarrow (Cu^{II}L^i)^{2+} + NO + (OH^- + H_2O)/2OH^- \tag{8}$$

From the results, which indicate that the observed rate increases linearly with $[NO_2^-]$, it can be estimated that $K = [(Cu^IL^1)^+NO_2^-)]/([(Cu^IL^1)^+][NO_2^-]) \leq 7$ M$^{-1}$, i.e. the ligation of $NO_2^-$ to the complex is considerably weaker than to $Cu^+_{aq}$. This is probably due to steric hindrance caused by the ligand $L^1$, which is a very bulky ligand, or to the competition for a ligation site between a terminal tertiary amine group of $L^1$ and $NO_2^-$.

2. Copper(I) complexes which are weaker reducing agents[40,41] than $Cu^+_{aq}$. For this purpose, the complexes $[Cu^I(CH_3CN)_n]^+$ were chosen. These solutions contain a mixture of several species: $[Cu^+_{aq}$, $Cu^I(CH_3CN)]^+$, $[Cu^I(CH_3CN)_2]^+$, $[Cu^I(CH_3CN)_3]^+$, and the detailed composition of each solution can be calculated from the known stability constants of these complexes.[40,41] Solutions of $[Cu^I(CH_3CN)_n]^+$ $5.0 \times 10^{-4}$ M were mixed with $NO_2^-$ [(2.5−100) $\times 10^{-3}$ M) at different pH's (4.0−4.8) and different $CH_3CN$ concentrations (0.048−0.96) M. The results demonstrate that:

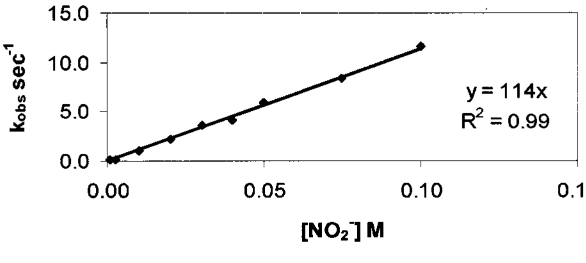

Figure 6. Dependence of $k_{obsd.}$ on $[NO_2^-]$. The experiments were performed in solutions containing $5.0 \times 10^{-4}$ M $Cu^+_{aq}$, 0.10 M $NaClO_4$, 0.10 M acetic acid, 0.048 M $CH_3CN$, the final pH was 4.40, helium saturated.

a) The observed rate depends linearly on $[NO_2^-]$ at all the $CH_3CN$ concentrations studied (for example, see Figure 6). From these results, it can be calculated that $K_{10} = [(Cu^I(CH_3CN)_nNO_2^-)]/([Cu^I(CH_3CN)_n]^+[NO_2^-]) \leq 30$ M$^{-1}$ [reaction (9)].

$$[Cu^I(CH_3CN)_n]^+ + NO_2^- \overset{K_9}{\rightleftharpoons} [Cu^I(CH_3CN)_nNO_2^-] \xrightarrow{k_9} \text{products} \quad (9)$$

The observation that $K_9 < K_2$ is probably due to one or more of the following facts:

I. Competition between one of the $CH_3CN$ ligands and $NO_2^-$ for the same ligation site. II. A change of hybridization from sp to sp$^3$ is required for the binding of $NO_2^-$ to $[Cu^I(CH_3CN)_2]^+$, which is the major complex present in the solution.

b) The observed rate increases linearly with $[H_3O^+]$ at all the $CH_3CN$ concentrations studied, e.g. Figure 7. The slope of the graph equals $k_9K_9 = 4.0 \times 10^5$ M$^{-2}$sec$^{-1}$. $k^*{}_2K_2$ for the $Cu^+_{aq}$ system equals $140 \times 4.6 \times 10^5 = 6.4 \times 10^7$ M$^{-2}$sec$^{-1}$, i.e. at this $CH_3CN$ concentration, acetonitrile reduces the rate of reaction by a factor of 150.

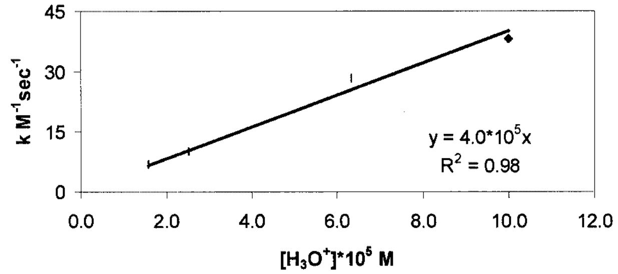

Figure 7. Dependence of $k$ on $[H_3O^+]$. The experiments were performed in solutions containing $5.0 \times 10^{-4}$ M $[Cu^I(CH_3CN)_n]^+$, 0.10 M $NaClO_4$, 0.10 M acetic acid, 0.96 M $CH_3CN$, helium saturated.

c) The observed rate at constant pH and $[NO_2^-]$ decreases with $[CH_3CN]$, (e.g. Figure 8).

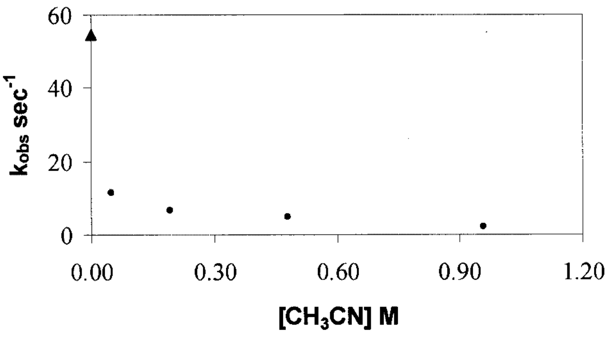

Figure 8. Dependence of $k$ on $[CH_3CN]$. The experiments were performed in solutions containing $5.0 \times 10^{-4}$ M $[Cu^I(CH_3CN)_n]^+$, 0.10 M $NaClO_4$, 0.10 M acetic acid, 0.10 M $NO_2^-$, helium saturated. solid triangle: result in the absence of $CH_3CN$, solid circles: results in presence of $CH_3CN$.

If $Cu^+_{aq}$ were the only reacting species in solutions containing $CH_3CN$, i.e. if the rate of reaction of $[Cu^I(CH_3CN)_n]^+$ were negligible, then the expected observed rate in solutions containing 0.96 mol·dm$^{-3}$ $CH_3CN$ at pH 4.4 would be $k_{*2}K_2 \times [H_3O^+] \times ([Cu^+_{aq}]/[Cu^I]_T) = 140 \times 4.6 \times 10^5 \times 4 \times 10^{-5} \times (2.1 \times 10^{-9}/5.0 \times 10^{-4}) = 0.011$ M$^{-1}$s$^{-1}$. ($Cu^+_{aq}$ concentration in this equation was calculated using the stability constants[40,41] of the $[Cu^I(CH_3CN)_n]^+$ complexes). However, $k_{obsd.} = 24$ M$^{-1}$s$^{-1}$ is obtained under these conditions. These results clearly demonstrate that the $[Cu^I(CH_3CN)_n]^+$ complexes react with $NO_2^-$ and contribute to the observed rates of reaction. The results are not accurate enough to evaluate the rate constant of the reaction of each of the $[Cu^I(CH_3CN)_n]^+$ species accurately. However, the results (Figure 8) clearly demonstrate that the rate constants decrease with an increase in $n$.

## Conclusions

The value of $K_2 = [(Cu^+NO_2)]/([Cu^+_{aq}][NO_2^-]) = 140 \pm 25$ M$^{-1}$ is similar to values reported in the literature for $Cu^I$ complexes with other ligands in organic solvents,[28,29] but higher than the values measured in this work for $[(Cu^IL^1)^+NO_2^-]$ $K \leq 7$ M$^{-1}$ and for $[(Cu^I(CH_3CN)_n)^+NO_2^-]$, $K \leq 30$ M$^{-1}$. The stability constant of the $(Cu^+NO_2^-)$ complex suggests that nitrite binds to the copper via the nitrogen atom, as the stability constants of $Cu^I$ complexes with oxo-anions are expected to be considerably lower.[42,43]

## Experimental Section

**Materials and Methods:** $NaNO_2$, $CuSO_4$, $Cu^0$, $NH_3$, $L^1$ ($L^1$ = 2,5,8,11-tetramethyl-2,5,8,11-tetraazadodecane), acetonitrile, acetic acid, $NaClO_4$, and $HClO_4$ were purchased from Aldrich. PN [4,4,5,5-tetramethyl-2-(1-pyryl)-2-imidazoline-1-oxyl 3-oxide] was prepared according to modified procedures, along with small amounts of 4,4,5,5-tetramethyl-2-(1-pyryl)-2-imidazoline.[44] All solutions were prepared using A.R. grade chemicals and from distilled water further purified by passing through a Milli Q Millipore setup, final resistivity >10 mΩ/cm. The experiments were carried out at $[NaClO_4]$ = 0.1 mol·dm$^{-3}$, at room temperature, 22±2 °C.

Solutions of $[Cu^I(NH_3)_2]^+$ and $(Cu^IL^i)^+$ were prepared via the conproportionation process, reaction (10). Solid copper metal was added to deaerated solutions containing $CuSO_4$ and an excess of $NH_3$, or $L^i$ at the appropriate pH values.

$$Cu^0_s + (Cu^{II}L_n)^{2+}_{aq} + (2m - n)L \leftrightarrows 2(Cu^IL_m)^+_{aq} \quad (10)$$

$Cu^+_{aq}$ was obtained by injecting $[Cu^I(NH_3)_2]^+$ into an acetate buffer solution (acidic solution), or into an $HClO_4$ solution containing the nitrite. Under these conditions the following reactions (11) and (12) occur.

$$[Cu^I(NH_3)_2]^+ \overset{\text{fast}}{\rightleftharpoons} Cu^+_{aq} + 2NH_3 \quad (11)$$

$$NH_3 + H^+ \underset{}{\overset{\text{fast}}{\rightleftarrows}} NH_4^+ \qquad (12)$$

Thus, at pHs considerably lower than the p$K_a$ of $NH_4^+/NH_3$ all the $[Cu^I(NH_3)_2]^+$ is transformed into $Cu_{aq}^+$ (The ligand exchange of Cu$^I$ is fast[45]).

**Kinetic Measurements:** Stopped-flow experiments were carried out using an SX.18 Applied PhotoPhysics stopped-flow instrument that enables kinetic measurements of reactions with lifetimes $\geq 2$ msec. A 05−109 Spectra Kinetics Monochromator, which enables measurements in the range of 200−700 nm, was used. The optical path was 10 mm. All the kinetic runs are the average of at least a triplicate. The error limit for the rate constants and for the equilibria constants is ±10%.

The time-dependence of the absorbances at 355 nm was observed . This wavelength is the maximum of the absorption band of $NO_2^-$ for the reactions of $Cu_{aq}^+$ and $Cu^I(CH_3CN)_n{}^+$. Alternatively, the absorbance of the band at 635 nm, the maximum of the absorption band of $(Cu^{II}L^1)^{2+}$ for the reaction of $(Cu^IL^1)^+$, was observed.

Electron Paramagnetic Resonance (EPR) spectra were recorded using a Bruker EMX-220 digital X-band (ν = 9.4 GHz) spectrometer equipped with a Bruker EP 4241VT temperature control system. All spectra were recorded at room temperature ($T = 297$ K) with the following parameters: 20.12 mW nonsaturating microwave power, 100 kHz field modulation of 0.1 mT amplitude. EPR spectra were processed using the Bruker WIN-EPR and Microcalc™ Origin™ software.

Solutions of $(Cu^IL)^+$, nitrite and PN were mixed producing 4,4,5,5-tetramethyl-2-(1-pyryl)-2-imidazoline (PPN), which has a completely different EPR spectrum (Figure 9) from PN (PN reacts only with NO to produce PPN).
Mass Spectra: The mass spectrometer used in this study was a Balzers Quadrupole model QMG-421 instrument, with an SEM (secondary electron multiplier) detector.

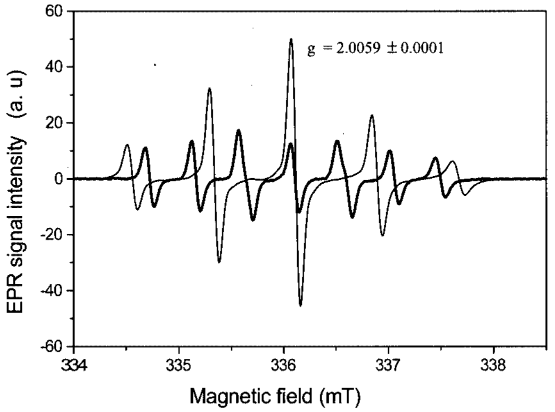

Figure 9. The EPR spectrum of PPN (bold line), formed by mixing 1:1 $1.0 \times 10^{-3}$ M $[Cu^I(NH_3)_2]^+$ with $5.0 \times 10^{-2}$ M $NaNO_2$; this solution was mixed 4:1 with $1.55 \times 10^{-3}$ M PN. The experiment was carried out in solutions containing also 0.08 M $NaClO_4$ and 0.08 M acetic acid. The EPR spectrum of PN (thin line) which does not change after mixing 1:1 $2.0 \times 10^{-4}$ M PN with 0.03 M $NaNO_2$, 0.10 M $NaClO_4$, 0.20 M acetic acid. The final pH was 4.7, the solutions were helium-saturated, ν = 9.424 GHz.

## Acknowledgments

This study was supported in part by a grant from the Budgeting and Planning Committee of The Council of Higher Education. D. M. wishes to express his thanks to Mrs. Irene Evens for her ongoing interest and support.

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Received March 30, 2004
Early View Article
Published Online July 15, 2004