



## Reactivity of iron(II)-bound nitrosyl hydride (HNO, nitroxyl) in aqueous solution

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### ABSTRACT

The reactivity of coordinated nitroxyl (HNO) has been explored with the  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{HNO}]^{3-}$  complex in aqueous medium, pH 6. We discuss essential bio-relevant issues as the thermal and photochemical decompositions, the reactivity toward HNO dissociation, the electrochemical behavior, and the reactions with oxidizing and reducing agents. The spontaneous decomposition in the absence of light yielded a two-electron oxidized species, the nitroprusside anion,  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{NO}]^{2-}$ , and a negligible quantity of  $\text{N}_2\text{O}$ , with  $k_{\text{obs}} \approx 5 \times 10^{-7} \text{ s}^{-1}$ , at 25.0 °C. The value of  $k_{\text{obs}}$  represents an upper limit for HNO release, comparable to values reported for other structurally related L ligands in the  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{L}]^{n-}$  series. These results reveal that the Fe–N bond is strong, suggesting a significant  $\sigma$ – $\pi$  interaction, as already postulated for other HNO-complexes. The  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{HNO}]^{3-}$  ion showed a quasi-reversible oxidation wave at 0.32 V (vs normal hydrogen electrode), corresponding to the  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{HNO}]^{3-}/[\text{Fe}^{\text{II}}(\text{CN})_5\text{NO}]^{3-}, \text{H}^+$  redox couple. Hexacyanoferrate(III), methylviologen and the nitroprusside ion have been selected as potential oxidants. Only the first reactant achieved a complete oxidation process, initiated by a proton-coupled electron transfer reaction at the HNO ligand, with nitroprusside as a final oxidation product. Dithionite acted as a reductant of  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{HNO}]^{3-}$ , in a 4-electron process, giving  $\text{NH}_3$ . The high stability of bound HNO may resemble the properties in related Fe(II) centers of redox active enzymes. The very minor release of  $\text{N}_2\text{O}$  shows that the redox conversions may evolve without disruption of the Fe–N bonds, under competitive conditions with the dissociation of HNO.

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### 1. Introduction

The chemistry of nitrosyl compounds has been under close scrutiny during the recent years, in the context of an explosive interest on the bioinorganic chemistry of NO [1,2]. A specific focus on the one-electron reduction product of NO, the nitroxyl species  $\text{HNO}/\text{NO}^-$  [3,4], was a mandatory consequence of its suspected intermediacy in NO biochemistry. The synthetic aspects, spectroscopic characterization and reactivity of free aqueous HNO have been early considered [5]. Some specific features, like the acid–base and spin-state properties of the  $\text{HNO}/\text{NO}^-$  couple, have been revisited by using theoretical calculations [6], pulse radiolysis and laser flash photolysis techniques [7,8]. Also relevant has been the characterization of adducts formed by nitric oxide and nitroxyl [9,10]. Nitroxyl species have not been directly characterized in biological fluids, though diverse biosynthetic pathways have been well documented [11–15]. Nitroxyl intermediates have been proposed in biological denitrification processes catalyzed by metalloenzymes, thus occupying a strategic place in the natural reversible conversions of nitrates to ammonia [14,16–19]. There is a specific effort trying to discover the role of nitroxyls

in diverse physiological processes, apparently differing from that attributable to NO [20–23].

A key point of nitroxyl biochemistry deals with the coordination chemistry related to bio-relevant transition metal ions [24]. Well characterized complexes with HNO and  $\text{NO}^-$  as ligands have been prepared in the last decade, noticeably with  $d^6$  low-spin metals such as  $\text{Ru}^{\text{II}}$  [25–28],  $\text{Os}^{\text{II}}$  [27,29–31],  $\text{Re}^{\text{I}}$  [31–33],  $\text{Ir}^{\text{III}}$  [29] (for HNO), and with  $\text{Co}^{\text{III}}$  [34–38],  $\text{Cr}^{\text{III}}$  [39–41],  $\text{Pt}^{\text{IV}}$  [42],  $\text{Ru}^{\text{II}}$  [27], and  $\text{Fe}^{\text{II}}$  [43–47] (for  $\text{NO}^-$ ), generally by using chelating macrocyclic coligands (viz., with porphyrins, aza-macrocycles, and thioether binding species) [24]. In general, a main drawback relates to the low solubility in water and the very limited presence of iron complexes. Among the latter,  $[\text{Fe}(\text{cyclam-ac})\text{NO}]$  [46], may be distinguished as capable of being 1-electron oxidized or reduced, affording the three redox-interconvertible forms of nitrosyl, formally  $\text{NO}^+$ ,  $\text{NO}$  or  $\text{NO}^-$ . Remarkably and most relevant to biochemistry, a complex with myoglobin ( $\text{Mb}^{\text{II}}\text{HNO}$ ) has been prepared in aqueous medium [48–51], and fully characterized by diverse spectroscopic tools. This was followed by the successful preparation of the first non-heme complex,  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{HNO}]^{3-}$  (**I**), by a 2-electron reduction of the nitroprusside ion  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{NO}]^{2-}$  (NP) with dithionite [52].

In the present study, we advance on the poorly cultivated grounds of bound HNO reactivity, and wish to report on some preliminary key issues, namely the thermal (spontaneous) and photochemically induced reactivity of (**I**), as well as the electrochemical properties, as

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revealed by a CV study. We report on the reactions with oxidants that have been previously assayed with free HNO: hexacyanoferrate(III) and methyl viologen [53]. The NP ion has been also selected as a potential oxidant, given the possible reaction between bound NO<sup>+</sup> and HNO, yielding the comproportionation product [Fe<sup>II</sup>(CN)<sub>5</sub>NO]<sup>3-</sup> [52]. By considering our previous work on the estimated pK<sub>a</sub> of (I), 7.7, we select pH 6.0 as representative of dominant speciation of the [Fe<sup>II</sup>(CN)<sub>5</sub>HNO]<sup>3-</sup> ion. Given that an excess of dithionite led to the decay of the characteristic absorbance of (I) at 445 nm, we also present a kinetic study of its reduction reaction.

## 2. Experimental

**Materials:** Sodium nitroprusside (SNP, Na<sub>2</sub>[Fe(CN)<sub>5</sub>NO]·2H<sub>2</sub>O), sodium cyanide (NaCN), ethylenediaminetetraacetic acid (EDTA, C<sub>10</sub>H<sub>16</sub>N<sub>2</sub>O<sub>8</sub>), sodium chloride (NaCl), potassium hexacyanoferrate(III) (K<sub>3</sub>[Fe(CN)<sub>6</sub>]), methyl viologen (C<sub>12</sub>H<sub>14</sub>Cl<sub>2</sub>N<sub>2</sub>), dimethylsulfoxide (CH<sub>3</sub>)<sub>2</sub>SO, potassium chloride (KCl), sodium hydroxide (NaOH), hydrochloric acid (HCl), mercaptosuccinic acid (C<sub>4</sub>H<sub>6</sub>O<sub>4</sub>S) and pyrazinamide (C<sub>5</sub>H<sub>5</sub>N<sub>3</sub>O) were of analytical grade (Sigma) and used as received. Sodium dithionite solutions (86% Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>, λ = 315 nm, ε = 6900 M<sup>-1</sup> cm<sup>-1</sup>) were prepared in 0.1 M NaOH, and standardized with [Fe<sup>III</sup>(CN)<sub>6</sub>]<sup>3-</sup> (λ = 418 nm, ε = 1012 M<sup>-1</sup> cm<sup>-1</sup>), under Ar-atmosphere [54,55]. All the solutions were prepared with distilled and purified water using a Mili-Q water purification system. The pH was adjusted to 6 with hydrochloric acid.

**Preparation of [Fe<sup>II</sup>(CN)<sub>5</sub>HNO]<sup>3-</sup> (I), in solution:** A freshly prepared solution of 0.19 M Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> in degassed 0.1 M NaOH was added to a 3 × 10<sup>-4</sup> M solution of SNP, at pH 10, 1 × 10<sup>-2</sup> M phosphate buffer, 5 × 10<sup>-4</sup> M EDTA, 6 × 10<sup>-3</sup> M NaCN and I = 0.1 M (NaCl), in a 10 mm quartz cuvette, under nitrogen atmosphere and with continuous stirring. The sequential addition of two equivalents of reductant was spectroscopically monitored, and yielded a product with λ<sub>max</sub> = 445 nm, ε = 4000 M<sup>-1</sup> cm<sup>-1</sup>. Then the pH was adjusted to 6 with 1.2 M HCl, after the addition of 5% excess of Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> in order to prevent the back-oxidation reaction at pH 10 [52].

**Decomposition of [Fe<sup>II</sup>(CN)<sub>5</sub>HNO]<sup>3-</sup>:** The absorbance decay of a solution of (I) (λ<sub>max</sub> = 445 nm, ε = 4000 M<sup>-1</sup> cm<sup>-1</sup>, pH 6) in the absence of light was determined by the integral method, applied to isolated measurements.

**Determination of products:** For [Fe<sup>II</sup>(CN)<sub>5</sub>NO]<sup>2-</sup>: standard solutions of 1–5 × 10<sup>-4</sup> M of SNP were prepared at pH 10 in 10<sup>-3</sup> M phosphate buffer, saturated with KCl, and 0.26 M mercaptosuccinic acid was added in a 100-fold excess. The absorbance of the red, stable adduct formed between [Fe<sup>II</sup>(CN)<sub>5</sub>NO]<sup>2-</sup> and mercaptosuccinate (λ<sub>max</sub> = 526 nm, ε = 5600 M<sup>-1</sup> cm<sup>-1</sup>) was used to produce a calibration curve [56,57]. All the solutions were deoxygenated before mixing. For [Fe<sup>II</sup>(CN)<sub>5</sub>H<sub>2</sub>O]<sup>3-</sup>: titration with pyrazinamide led to the well characterized [Fe<sup>II</sup>(CN)<sub>5</sub>(pyrazinamide)]<sup>3-</sup> complex, λ<sub>max</sub> = 494 nm, ε<sub>max</sub> = 4590 M<sup>-1</sup> cm<sup>-1</sup> [58]. Quantification of ammonia was performed both with the indophenol colorimetric test [59], and with an ammonia combination electrode (Hach), calibrated separately against a standard commercial solution, and [Fe<sup>II</sup>(CN)<sub>5</sub>NH<sub>3</sub>]<sup>3-</sup> at pH 11. Potential H<sub>2</sub> formation during the spontaneous decay of [Fe<sup>II</sup>(CN)<sub>5</sub>HNO]<sup>3-</sup> was explored by gas chromatography–mass spectrometry.

**Kinetic measurements:** UV–visible (UV–vis) spectra were recorded in a Hewlett-Packard 8453 diode array spectrophotometer (200–900 nm), at 25.0 ± 0.1 °C; by using a Lauda E103 thermostat. In all the kinetic experiments, the pseudo-first order constants k<sub>obs</sub> (s<sup>-1</sup>) were obtained by plotting ln [A<sub>t</sub> - A<sub>∞</sub>] vs time, where A<sub>t</sub> was the absorbance measurement at time t, and A<sub>∞</sub> was the absorbance at infinite time. The values of k<sub>obs</sub> were determined from at least three half-lives, and are the result of at least two independent measurements, reproducible with an accuracy of ca. 2%. The second order rate constants were obtained from the slopes of the plots of k<sub>obs</sub> against the concentration of the excess reactant.

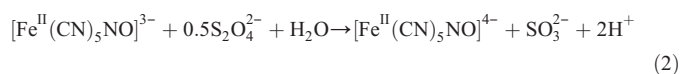
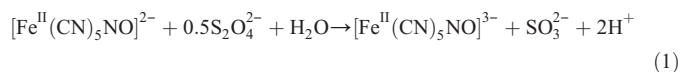
**Irradiation of [Fe<sup>II</sup>(CN)<sub>5</sub>HNO]<sup>3-</sup>:** UV–vis spectra corresponding to the decomposition of (I) under visible light exposure (λ = 450 nm) were recorded every 90 s. [Fe<sup>II</sup>(CN)<sub>5</sub>HNO]<sup>3-</sup> initial = 2 × 10<sup>-4</sup> M; [CN<sup>-</sup>]<sub>initial</sub> = 10<sup>-3</sup> M; HPO<sub>4</sub><sup>2-</sup>/H<sub>2</sub>PO<sub>4</sub><sup>-</sup> buffer 10<sup>-3</sup> M, pH 6; EDTA = 10<sup>-3</sup> M; I = 0.1 M (NaCl). T = 25.0 °C.

The voltammetric measurement system comprised a Princeton Applied Research (PAR) 273A instrument and a PAR303A mercury drop electrode (area, 0.024 cm<sup>2</sup>), a Ag/AgCl reference electrode (3.5 M KCl), and a platinum wire counter electrode. The complex [Fe<sup>II</sup>(CN)<sub>5</sub>HNO]<sup>3-</sup> (I) (c = 2.5 × 10<sup>-3</sup> M) was prepared in a phosphate buffer medium (3.4 × 10<sup>-3</sup> M, pH 10) containing 10<sup>-4</sup> M EDTA, 0.06 M [CN<sup>-</sup>], with a final I = 0.1 M (KNO<sub>3</sub>), at T = 25.0 °C. Then the pH was immediately adjusted to pH 6 with 2.4 M acetic acid. The samples were introduced in 10 ml PAR voltammetric cell cups, and were deoxygenated with nitrogen. Blanks of the reagents present in the synthesis of the complex were also measured.

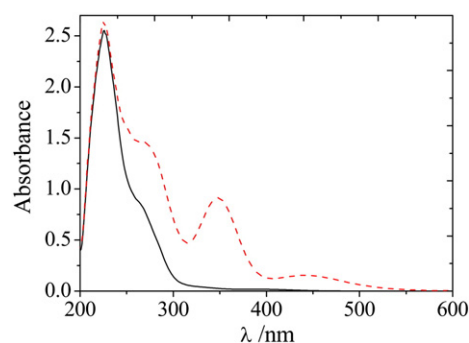
## 3. Results and discussion

### 3.1. Synthesis of the [Fe<sup>II</sup>(CN)<sub>5</sub>HNO]<sup>3-</sup> (I) complex

The preparation of (I) in aqueous solution has been achieved by starting with the nitroprusside ion and using dithionite as a reductant, in the presence of excess cyanide and under anaerobic conditions. By working at pH 10, a 2-electron reduction process can be observed by adding successively one, and later another equivalent of dithionite. Fig. 1 shows the formation of the 1-electron reduced product, [Fe<sup>II</sup>(CN)<sub>5</sub>NO]<sup>2-</sup>, which has been well characterized in the literature (λ<sub>max</sub> at 345 nm, ε<sub>345</sub> = 3500 M<sup>-1</sup> cm<sup>-1</sup>, and at 430 nm, ε<sub>430</sub> = 550 M<sup>-1</sup> cm<sup>-1</sup>) [60]. Fig. 2 shows the complete decay of [Fe<sup>II</sup>(CN)<sub>5</sub>NO]<sup>2-</sup>, with the formation of a new species absorbing at 445 nm. The overall process can be described by reactions 1 and 2:



The nitroxyl complex produced in reaction (2) showed to be unstable, with reversal to [Fe<sup>II</sup>(CN)<sub>5</sub>NO]<sup>3-</sup>. This slow decomposition (t<sub>1/2</sub> ~ 50 min) could be greatly diminished through a rapid pH-shift to 6, thus reaching a very stable species with similar absorption properties than at pH 10, with ε = 4000 M<sup>-1</sup> cm<sup>-1</sup> [52]. This species was identified as the [Fe<sup>II</sup>(CN)<sub>5</sub>HNO]<sup>3-</sup> ion (I) by performing <sup>1</sup>H NMR experiments, which also allowed establishing a value of pK<sub>a</sub> = 7.4 for its conversion to [Fe<sup>II</sup>(CN)<sub>5</sub>NO]<sup>4-</sup>.



**Fig. 1.** One-electron reduction of [Fe<sup>II</sup>(CN)<sub>5</sub>NO]<sup>2-</sup> (solid line) with dithionite, forming [Fe<sup>II</sup>(CN)<sub>5</sub>NO]<sup>3-</sup> (λ<sub>max</sub> at 345 and 430 nm, ε = 3500 and 550 M<sup>-1</sup> cm<sup>-1</sup>, respectively, dashed line).

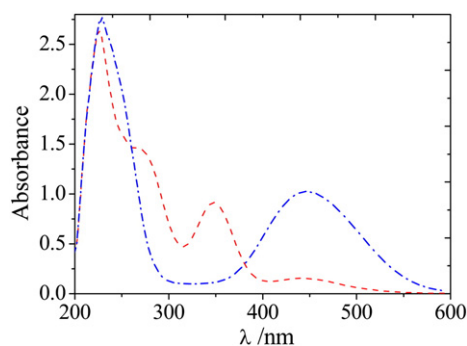
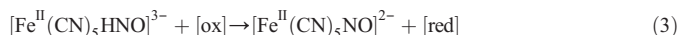


Fig. 2. Formation of  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{NO}]^{4-}$  ( $\lambda_{\text{max}}$  at 445 nm,  $\epsilon = 4000 \text{ M}^{-1} \text{ cm}^{-1}$ , dot-dashed line) after the addition of one equivalent of dithionite to  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{NO}]^{3-}$  (dashed line).

### 3.2. Thermal reactivity of $[\text{Fe}^{\text{II}}(\text{CN})_5\text{HNO}]^{3-}$ (**I**)

Complex (**I**) showed to be very robust, though a very slow decay of the band at 445 nm could be observed. A value of  $k = 1.4 \times 10^{-5} \text{ s}^{-1}$  was formerly reported for this spontaneous decomposition process, at pH 6. We found later that the decay profile showed a strong dependence on the number of spectra collected per unit of time, accounting for a photodegradative mechanism. Besides, though we also found  $\text{N}_2\text{O}$  as a marginal product of this experiment, detected by IR spectroscopy ( $2230 \text{ cm}^{-1}$ ), we must correct our previous contention that the decomposition should be related to the release of the HNO ligand and further dimerization/dehydration giving  $\text{N}_2\text{O}$  [52]. A significantly slower, complete absorbance decay of the 445 nm band, as well as of the 265 nm band characteristic of (**I**), could be obtained in the absence of light, rendering  $k_{\text{obs}} \approx 5 \times 10^{-7} \text{ s}^{-1}$ , at 25.0 °C. The main product was nitroprusside, with a 90% yield. We propose Eq. (3) as representative of the stoichiometry:



Reaction (3) implies a formal 2-electron conversion in the bound-HNO  $\rightarrow$  NO<sup>+</sup> system. The nature of the oxidant cannot be defined unambiguously at the moment. The formation of  $\text{H}_2$  has been proposed in an analogous decomposition process of the  $[\text{Ru}(\text{py}^{\text{bu}}\text{S}_4)\text{HNO}]$  complex [25]. As we did not find  $\text{H}_2$  by gas chromatography–mass spectrometry of the head space of the reaction vessel, we speculate that an oxidative action of sulfite is operative, giving sulfide (cf. reaction (2)). A spurious  $\text{O}_2$ -leakage cannot be discarded either. The spontaneous redox decomposition can be understood on the basis of the electron-rich character of the NO-group in complex (**I**), which can be also prepared by electrochemical means through the reduction of SNP at a very negative potential,  $-1.1 \text{ V}$  (vs normal hydrogen electrode, NHE) [61]. The slow decomposition may be traced to the stabilizing influence of the proton in HNO. We are still able to assign the  $k_{\text{obs}}$  value as an upper limit to  $k_{-\text{HNO}}$ , the specific dissociation rate constant of HNO from complex (**I**). By looking at Table 1, we can appreciate that HNO should be released with a comparable rate as other structurally related L ligands in the  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{L}]^{n-}$  series, like nitrosobenzene [62]. Other nitroso-derivatives bound to  $[\text{Fe}^{\text{II}}(\text{CN})_5]^{3-}$  also behave as robust complexes [60,63]. An important conclusion is that HNO binds to Fe(II) through a strong  $\sigma$ - $\pi$  interaction, as already postulated for  $\text{Mb}^{\text{II}}\text{HNO}$  [48–51], and other HNO complexes, and recently discussed by means of theoretical calculations [64].

### 3.3. Photochemical reactivity

By illuminating complex (**I**) with 450 nm laser light, the complete decay of the band at 445 nm was achieved in  $\approx 10 \text{ min}$ . The products were SNP (ca. 50%) and  $[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}$ , as detected by a new band at 320 nm [65,66]. As the 445 nm band may be reasonably assigned to

Table 1  
Specific rate constants (25.0 °C) for the dissociation of L in  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{L}]^{n-}$  complexes.

| Ligand          | $k_{-L} \text{ (s}^{-1}\text{)}$ | Reference |
|-----------------|----------------------------------|-----------|
| NO <sup>+</sup> | Not detected                     | –         |
| CO              | $< 10^{-8}$                      | [83]      |
| CN <sup>−</sup> | $\approx 4 \times 10^{-7a}$      | [84]      |
| HNO             | $< 4.7 \times 10^{-7}$           | This work |
| NOBz            | $1.6 \times 10^{-6}$             | [62]      |
| NO•             | $1.6 \times 10^{-5}$             | [85]      |
| dmsO            | $7.5 \times 10^{-5}$             | [86]      |
| pz              | $4.2 \times 10^{-4}$             | [87]      |
| py              | $1.1 \times 10^{-3}$             | [87]      |
| NH <sub>3</sub> | $1.8 \times 10^{-2}$             | [78]      |

<sup>a</sup> Extrapolated to 25 °C from reported data at higher T.

a metal to ligand charge transfer process (MLCT), and by analogy with a recent study on the photolysis of  $\text{Mb}^{\text{II}}\text{HNO}$  [67], we propose that the primary excitation step could be related to the formation of the aminoxyl radical, NHOH, together with Fe(III). Secondary oxidation processes should expectedly lead to NP, as previously observed [68]. The  $[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}$  ion has been also reported as a main product of photolysis of the  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{L}]^{n-}$  ions [69]. A detailed photochemical investigation is in order.

### 3.4. Electrochemical reactivity of (**I**)

The electrochemistry of solutions of SNP has been studied at the hanging Hg-electrode showing the successive reductions of bound NO<sup>+</sup> [61]. The first reduction wave at  $-0.35 \text{ V}$  is pH-independent, leading to bound NO. A second pH-dependent reduction wave at ca.  $-0.6 \text{ V}$  has been associated to the reduction of  $[\text{Fe}(\text{CN})_4\text{NO}]^{2-}$ , formed by cyanide labilization of  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{NO}]^{3-}$ . A third reduction at more negative potentials leads presumably to  $\text{NH}_2\text{OH}/\text{NH}_3$ . Fig. 3 shows the CV results with a solution of complex (**I**) at pH 6. By running in the oxidative sense, a wave with  $E_{1/2} = +0.32 \text{ V}$  (vs NHE) appears, with a quasi-reversible behavior, as shown in the inset by the independence of  $E_{1/2}$  on the scan rates ( $\nu$ ), as well as by the linear relation between the current and  $\nu^{1/2}$ . A very good reproducibility of this wave has been achieved along 4–5 successive scans [70]. Given that the difference between the peak potential and  $E_{p/2}$  is  $\approx 68 \text{ mV}$ , we estimate that one electron is transferred in the process, and we ascribe the measured potential to

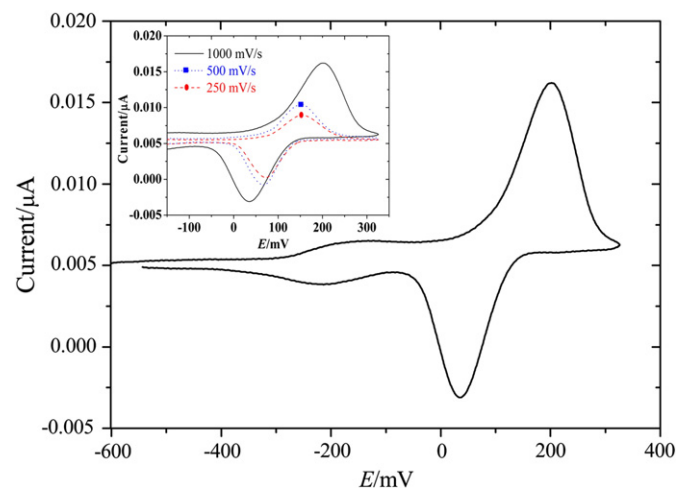


Fig. 3. Cyclic voltammogram of  $2.5 \times 10^{-3} \text{ M}$   $[\text{Fe}^{\text{II}}(\text{CN})_5\text{HNO}]^{3-}$  (mercury drop electrode vs Ag/AgCl, 3.5 M), pH 6. Inset: Runs at different scan rates (1000, 500 and 250 mV/s in solid, dotted and dashed lines, respectively).

the bound  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{HNO}]^{3-}/[\text{Fe}^{\text{II}}(\text{CN})_5\text{NO}]^{3-}, \text{H}^+$  redox couple. We rule out the onset of a potential related to the electron exchange at the iron center, because this potential should expectedly appear at more positive values, in the range  $\geq 0.4$  V [71].

### 3.5. Reactivity of (I) toward oxidants: hexacyanoferrate(III)

Fig. 4a,b shows the successive spectra upon addition of  $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$  to complex (I). In Fig. 4a, the addition of 1 equivalent leads to the consumption of half of the initial reactant (I). Besides, by adding another equivalent, a full decay of the 445 nm band is observed. In Fig. 4b, a continuous decrease of absorbance indicates the full consumption of complex (I) through the initial addition of 2 equivalents of the oxidant in a single step. The insets show the corresponding exponential traces, measured at 490 nm, to avoid the band's overlap at 445 nm.

The stoichiometry of the title reaction can be described by reaction (4), as supported by the quantitative generation of the 2-electron oxidized product nitroprusside (1:1 with respect to I), and by the

signatures in the final spectra of Fig. 4, which correspond to  $[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}$  [65].<sup>1</sup>

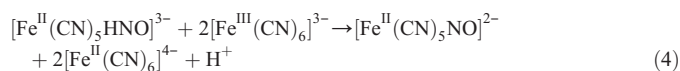
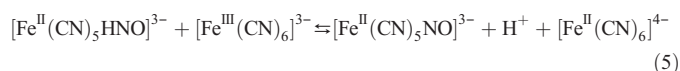
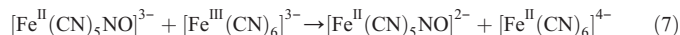
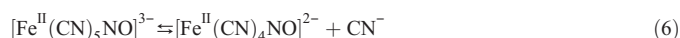


Fig. 5a reveals the exponential decay of  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{HNO}]^{3-}$  under an excess of  $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$ , and Fig. 5b depicts the linear behavior of  $k_{\text{obs}}$  against  $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$ , from which the following rate-law is obtained:  $-\text{d}[\text{Fe}^{\text{II}}(\text{CN})_5\text{HNO}]^{3-}/\text{dt} = k_4 \times [\text{Fe}^{\text{III}}(\text{CN})_6]^{3-} \times [\text{Fe}^{\text{II}}(\text{CN})_5\text{HNO}]^{3-}$ , with  $k_4 = 70 \text{ M}^{-1} \text{ s}^{-1}$  at 25.0 °C.

According to the stoichiometry and the rate law, we propose that the oxidation of  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{HNO}]^{3-}$  to NP occurs in successive mono-electronic steps, with the intermediacy of  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{NO}]^{3-}$ . In principle, we assume that the first electron transfer, reaction (5), is comparatively slower than the second one.



We may consider that reaction (5) is an additional example of the cross reactions between pentacyano(L)-complexes containing different L ligands (viz., py, 4NH<sub>2</sub>py, CN<sup>-</sup>), as detailed in Table 2 [72,73]. Reaction (5) can be distinguished by the fact that HNO is a redox-active ligand, as suggested by the properties of the CV wave in Fig. 3. Reaction (5) should be followed by a step involving the further oxidation of  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{NO}]^{3-}$ . As this ion could not be detected by UV-vis spectroscopy, we infer that it reacts rapidly giving the very stable nitroprusside product through the rapid steps (6) and (7).<sup>2</sup>

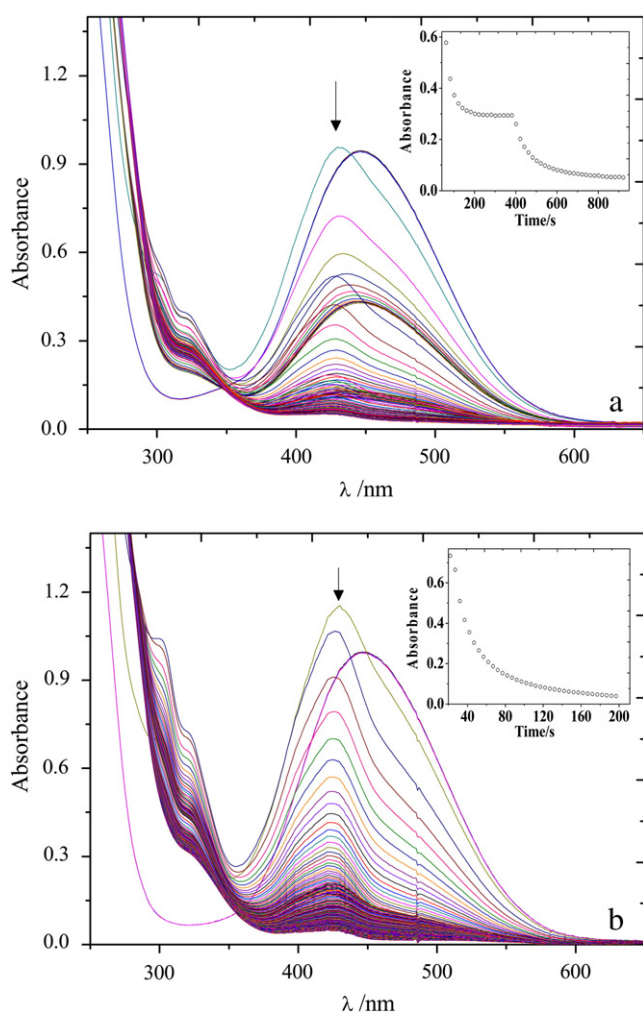


By applying the Marcus model to reaction (5) [72,73], we can estimate a value for the self-exchange rate constant for oxidation of complex (I)<sup>3</sup> [74,75]. The simplified Marcus equation is  $k_{12} = (k_{11} \times k_{22} \times K_{12})^{1/2}$ , where  $k_{12} = 70 \text{ M}^{-1} \text{ s}^{-1}$  and  $K_{12} = 160$  (estimated from the redox potentials for the hexacyanoferrate(III,II) couple (0.45 V) [71], and the  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{HNO}]^{3-}/[\text{Fe}^{\text{II}}(\text{CN})_5\text{NO}]^{3-}, \text{H}^+$  couple (0.32 V, Fig. 3). The self-exchange value for the hexacyanoferrate(III,II) couple is  $5 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$  [73], and in this way we obtain a value for  $k_{22}$ , the self-exchange rate constant for the  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{HNO}]^{3-}/[\text{Fe}^{\text{II}}(\text{CN})_5\text{NO}]^{3-}, \text{H}^+$  couple =  $6.2 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$ . In the cross-reactions between pentacyano(L)ferrates with non-redox active ligands, the self-exchange rate constants are much faster, of ca.  $10^5 \text{ M}^{-1} \text{ s}^{-1}$ , corresponding to “metal-centered” redox mechanisms [72,73]. The low value for  $k_{22}$  associated with oxidation of the HNO ligand can be related to a proton-coupled electron transfer (PCET) reaction evolving with a great reorganization energy, influenced by the geometrical changes when going from HNO to NO.

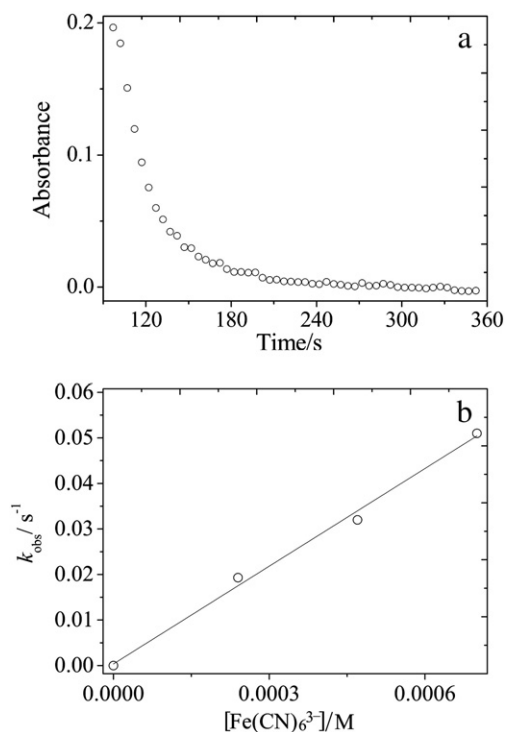
<sup>1</sup> As reported in [65], while  $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$  absorbs at 417 nm and 303 nm, the band at ca. 425 nm appearing as a product in Fig. 4 can be assigned to  $[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}$ , in addition to the shoulder at 320 nm. The consumption of  $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$  is confirmed by the nearly complete decay of the 417 nm band and the absence of its characteristic absorption at  $\approx 300$  nm.

<sup>2</sup> Reaction (6) comprises a rapidly established equilibrium, with values of  $K_6 = 6.8 \times 10^{-5} \text{ M}$ ,  $k_6 = 2.8 \times 10^2 \text{ s}^{-1}$ , and  $k_{-6} = 4 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$  [41]. At pH 6, cyanide protonation favors the predominance of the tetracyano- over the pentacyano-complex.

<sup>3</sup> On the basis of finding cation catalysis, it has been demonstrated that these reactions may be considered as outer-sphere as far as the cyanometallate ions are concerned but in which a partially desolvated cation bridges the reacting anions [74]. The predictions of Marcus theory are satisfied considering the uncatalyzed electron transfer path, by suppressing the cation-catalyzed pathway. The rate constants commonly cited in the literature are in fact conditional values at a given concentration of cation, as in the present case ( $[\text{K}^+] = 0.05 \text{ M}$ ) [75].



**Fig. 4.** Oxidation of  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{HNO}]^{3-}$  (I) with  $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$ . a) UV-vis spectra after the sequential addition of 1 + 1 equivalents of  $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$  to (I). The spectrum centered at  $\lambda_{\text{max}} = 445$  nm corresponds to complex (I), before adding  $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$ . Inset: Successive exponential traces at 490 nm. b) UV-vis spectra after the addition of 2 equivalents of  $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$  to  $2.5 \times 10^{-4} \text{ M}$  (I).  $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-} = 2.5 \times 10^{-4} \text{ M}$  (final concentration per equivalent);  $1.25 \times 10^{-3} \text{ M}$   $[\text{CN}^-]_{\text{initial}}$ ; pH 6,  $3.0 \times 10^{-3} \text{ M}$   $\text{H}_2\text{PO}_4^-/\text{H}_2\text{PO}_4^-$ ;  $10^{-3} \text{ M}$  [EDTA];  $I = 0.1 \text{ M}$  (NaCl). T = 25.0 °C. Inset: Exponential decay at 490 nm.



**Fig. 5.** a) Exponential decay of  $7.25 \times 10^{-5}$  M  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{HNO}]^{3-}$  in the presence of  $4.7 \times 10^{-4}$  M  $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$ , at 490 nm. b) Observed rate constants for the decay of  $7.25 \times 10^{-5}$  M  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{HNO}]^{3-}$ , against the concentration of  $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$  ( $2.5$ – $7.0 \times 10^{-4}$  M);  $10^{-3}$  M  $[\text{CN}^-]_{\text{initial}}$ ; pH 6,  $3.0 \times 10^{-3}$  M  $\text{HPO}_4^{2-}/\text{H}_2\text{PO}_4^-$ ;  $10^{-3}$  M [EDTA];  $I = 0.1$  M (NaCl);  $T = 25.0$  °C.

It has been found that the  $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$  ion reacts with free HNO (derived from Angeli's salt, at pH 7) forming  $[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}$  and NO. By assuming a second order rate law, we estimate a value of  $k = \text{ca. } 13 \text{ M}^{-1} \text{ s}^{-1}$ , at 37 °C, from the published results [76]. This value is within an order of magnitude as the one presently reported for bound HNO, thus suggesting a similar mechanism, namely a direct attack of  $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$  on the ligand. In fact, electron-interchange reactions on nitrosyl-complexes are expectedly ligand centered, as anticipated by the electronic structures comprising low-energy lowest unoccupied molecular orbitals (LUMO), which have been described as having a dominant nitrosyl-character [77].

### 3.6. Unreactivity of (I) toward weaker oxidants: nitroprusside and methyl viologen

By mixing either NP ( $E_{\text{NO}^+/\text{NO}} = 0.05$  V) [60], or methyl viologen ( $E_{\text{MV}^{2+}/\text{MV}^+} = -0.44$  V) [53], with complex (I), we found no reaction in the minute time scale. Instead, a very slow spontaneous decomposition of (I) evolved, with the same rate than that described in Section 3.2. The negative result with NP as an oxidant reveals

**Table 2**  
Rates of electron-transfer reactions ( $k_{\text{et}}$ ) with pentacyano(L)ferrate systems, at 25.0 °C.

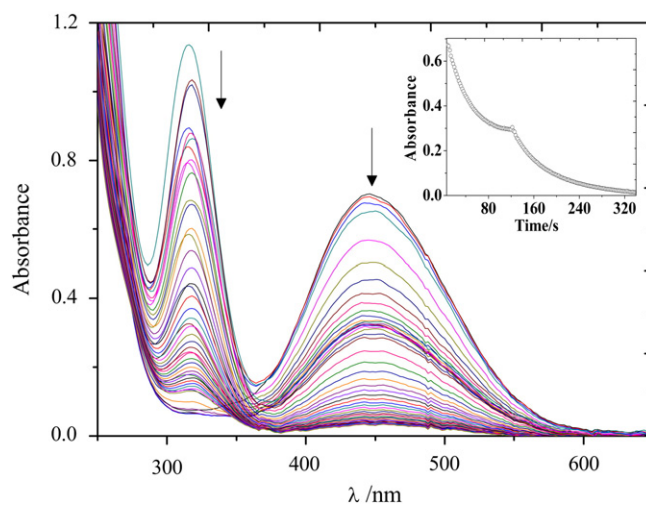
| Reaction   | $k_{\text{et}}$ , $\text{M}^{-1} \text{ s}^{-1}$ | Ref.      |
|--|--|-----------|
| $\text{Fe}^{\text{III}}(\text{CN})_6^{3-} + \text{Fe}^{\text{II}}(\text{CN})_5\text{NH}_2\text{py}^{3-}$                     | $4.3 \times 10^{5a}$                             | [72]      |
| $\text{Fe}^{\text{III}}(\text{CN})_6^{3-} + \text{Fe}^{\text{II}}(\text{CN})_5\text{py}^{3-}$                                | $3.4 \times 10^{4b}$                             | [73]      |
| $\text{Fe}^{\text{III}}(\text{CN})_6^{3-} + \text{Fe}^{\text{II}}(\text{CN})_5\text{HNO}^{3-}$                               | $70^c$   | This work |
| $\text{Fe}^{\text{III}}(\text{CN})_5\text{NH}_2\text{py}^{2-} + \text{Fe}^{\text{II}}(\text{CN})_5\text{NH}_2\text{py}^{3-}$ | $7 \times 10^{5d}$                               | [72]      |
| $\text{Fe}^{\text{III}}(\text{CN})_5\text{py}^{2-} + \text{Fe}^{\text{II}}(\text{CN})_5\text{py}^{3-}$                       | $7 \times 10^{5a,d}$                             | [73]      |
| $\text{Fe}^{\text{III}}(\text{CN})_6^{3-} + \text{Fe}^{\text{II}}(\text{CN})_6^{4-}$   | $5 \times 10^{3c}$                               | [73]      |

<sup>a</sup>  $[\text{LiClO}_4] = 0.05$  M.

<sup>b</sup>  $[\text{KNO}_3] = 0.05$  M.

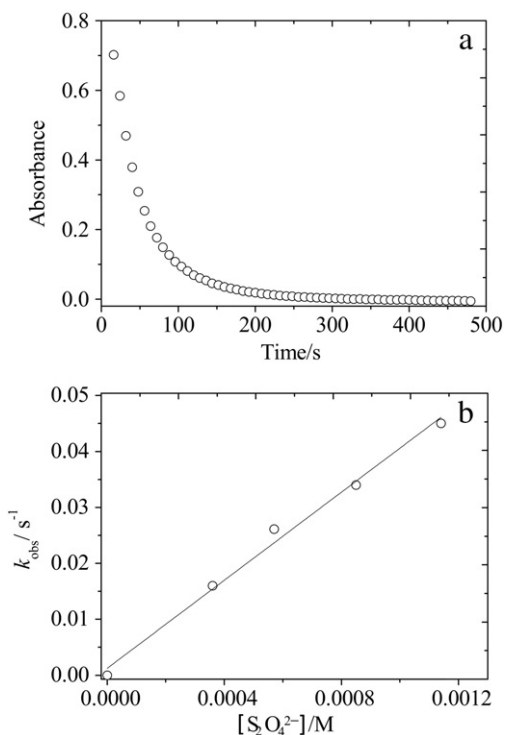
<sup>c</sup>  $[\text{K}^+] = 0.05$  M.

<sup>d</sup> Self exchange rate constants calculated using the Marcus relationship.



**Fig. 6.** UV-vis spectra upon addition of 2 equimolar aliquots of  $\text{S}_2\text{O}_4^{2-}$  to  $1.75 \times 10^{-4}$  M (I). Inset: Exponential traces for the successive additions of reductant.  $10^{-3}$  M  $[\text{CN}^-]_{\text{initial}}$ ; pH 6,  $10^{-3}$  M  $\text{HPO}_4^{2-}/\text{H}_2\text{PO}_4^-$ ;  $10^{-3}$  M [EDTA];  $I = 0.1$  M (NaCl).  $T = 25.0$  °C.

that the comproportionation reaction between bound HNO and  $\text{NO}^+$  to yield  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{NO}]^{3-}$  is not feasible (if this were not the case, NP could not accumulate as a quantitative product in the thermal decomposition reaction). The result is consistent with thermodynamic constraints, avoiding the occurrence of a reaction like (2) when  $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}$  ( $E = 0.45$  V) is replaced by much weaker oxidants. The unreactivity of (I) toward methyl viologen is particularly remarkable, given the reported behavior of  $\text{Mb}^{\text{II}}\text{HNO}$  [48], which was shown to react in a few minutes. Therefore, we must conclude that (I) is a poor-to-moderate reductant, with  $E^\circ \approx 0.3$  V, and a similar reactivity than free HNO, showing a minor influence of coordination. Remarkably



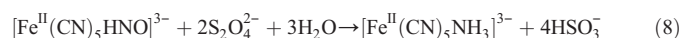
**Fig. 7.** a) Exponential decay at 445 nm in the reaction of (I) with  $\text{S}_2\text{O}_4^{2-}$ .  $1.75 \times 10^{-4}$  M (I);  $10^{-3}$  M  $[\text{CN}^-]_{\text{initial}}$ ;  $5.7 \times 10^{-4}$  M  $[\text{S}_2\text{O}_4^{2-}]$ ; pH 6,  $10^{-3}$  M  $\text{HPO}_4^{2-}/\text{H}_2\text{PO}_4^-$ ;  $10^{-3}$  M [EDTA];  $I = 0.1$  M (NaCl).  $T = 25.0$  °C. b) Linear dependence of  $k_{\text{obs}}$  for the reduction of (I) under identical conditions, with varying concentrations of  $[\text{S}_2\text{O}_4^{2-}] = 3.6$ – $12 \times 10^{-4}$  M.

however, free HNO has been reported as a strong reductant, with  $E^{\circ}(\text{NO}, \text{H}^+/\text{HNO}) = -0.55 \text{ V}$  at pH 7 [7].

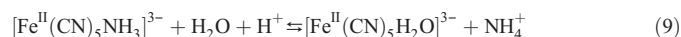
### 3.7. Reactivity of (I) toward reductants: dithionite anion

The addition of dithionite to (I) (molar ratio = 2/1) led to the complete decay of the absorbance at 445 nm (Fig. 6). The initial spectrum showed two maxima, at 315 and 445 nm, corresponding to the dithionite anion ( $\epsilon_{315} = 6900 \text{ M}^{-1} \text{ cm}^{-1}$ ) [55], and to complex (I) ( $\epsilon_{445} = 4000 \text{ M}^{-1} \text{ cm}^{-1}$ ) [52], respectively. By adding an equimolar concentration of dithionite, approximately half of the initial concentration of (I) was consumed. A complete decay of both absorbance values was observed when the rest of the reducing agent was added.

Fig. 7a shows an exponential decay of the absorbance at 445 nm upon addition of a slight excess of dithionite to (I), according to the rate law:  $v = -d[\text{Fe}^{\text{II}}(\text{CN})_5\text{HNO}^{3-}]/dt = k_{\text{obs}} \times [\text{Fe}^{\text{II}}(\text{CN})_5\text{HNO}^{3-}]$ . Fig. 7b shows a linear dependence between  $k_{\text{obs}}$  and the concentration of dithionite, indicating that  $k_{\text{obs}} = k_2 [\text{S}_2\text{O}_4^{2-}]$ . Thus, the rate law translates to  $v = -d[\text{Fe}^{\text{II}}(\text{CN})_5\text{HNO}^{3-}]/dt = k_2 [\text{S}_2\text{O}_4^{2-}] \times [\text{Fe}^{\text{II}}(\text{CN})_5\text{HNO}^{3-}]$ . This neat behavior of the reduction reaction involving 4 electrons, strongly suggests the conversion of the HNO ligand into  $\text{NH}_3$ , according to reaction (8).



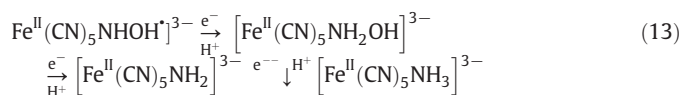
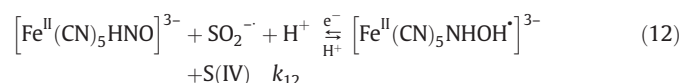
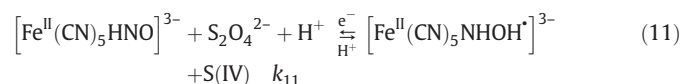
Reaction (9) shows the rapid aquation of complex  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{NH}_3]^{3-}$  ( $t_{1/2} = 40 \text{ s}$ , reaction (9)) [78], yielding quantitatively  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{H}_2\text{O}]^{3-}$  ( $\lambda_{\text{max}} = 440 \text{ nm}$ ,  $\epsilon = 640 \text{ M}^{-1} \text{ cm}^{-1}$ ) [79], as determined by the pyrazinamide test (see Experimental). This is an indirect evidence of the formation of  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{NH}_3]^{3-}$  [80].



A closer analysis of the reaction mechanism allows some additional considerations on the fate of the nitrogen containing fragment. Considering the equilibrium of the reducing agent in reaction (10), a reaction mechanism involving monoelectronic steps could be operative.



The rate limiting step can be described by two alternative monoelectronic reductions coupled to proton transfer, reactions (11) and (12), which have been proposed for the mechanisms of action of dithionite toward different transition metal complexes [55,81].



<sup>4</sup> By using either the indophenol colorimetric test, or an ion-specific electrode, we could not achieve conclusive results yielding a quantitative full reduction of bound HNO to  $\text{NH}_3$ . The results were always in defect, not higher than 50% in any case, and rather irreproducible. In view of the very revealing spectral results shown in Fig. 7b on the quantitative consumption of dithionite by complex (I), we ascribe this behavior to the interference of the cyanoferrate charged species. Also, the negative interference of sulfite in the indophenol test has been previously reported [80], and could not be overcome by distillation of ammonia at alkaline pH, probably suggesting the partial trapping of  $\text{NH}_4^+/\text{NH}_3$  in the reaction medium.

The  $[\text{Fe}^{\text{II}}(\text{CN})_5\text{NH}_2\text{OH}]^{3-}$  ion has been proposed as a 2-electron reduced intermediate in reaction (8). Though it is known to be unstable toward disproportionation [82], the reaction kinetics of the reduction process suggests that a faster competitive path is operative, and discards the disproportionation of  $\text{NH}_2\text{OH}$  as a side reaction. Intermolecular reactions, leading to N–N reduced species are unfeasible in the ca.  $10^{-4} \text{ M}$  complex solutions.

## 4. Conclusions

The HNO ligand is notoriously stable toward dissociation in complex (I). Under the reaction conditions favoring strong HNO-binding to Fe(II), no significant  $\text{N}_2\text{O}$  production could be detected. A similar inert-dissociation behavior has been also reported for  $\text{Mb}^{\text{II}}\text{HNO}$ . We conclude that the Fe(II)-HNO complexes are very robust in aqueous solutions. Referring to the biological roles of HNO, it results that a main attention should be paid to bound HNO, in addition to the possible contribution of free HNO. Probably other coordination compounds containing electron-donor coligands could be more effective in promoting HNO release, which in that case might be competitive with the redox reactivity. No stable HNO-complex could be obtained under oxidizing conditions, viz. with Fe(III), and this can be traced to the (mild) reducing properties of HNO. The chemical behavior of bound HNO differs from its deprotonated partner, which dominates at pH's > 8. As will be reported elsewhere, the pentacyanoferrate(II) complex with  $\text{NO}^-$  behaves as a stronger reductant than (I) (viz., reacting with NP and methylviologen), and can be also oxidized to bound NO and  $\text{NO}^+$ . Given the intermediate oxidation state of HNO, complex (I) can also be reduced by the strong reductant dithionite giving  $\text{NH}_3$ , though a strictly quantitative evaluation could not be assessed in the present study. Extended studies are in order comprising the reactivity of (I) with other biologically relevant redox reagents.

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