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Synthesis, Characterization, and NO Release Study of the *cis*- and *trans*-$[Ru(Bpy)_2(SO_3)(NO)]^+$ Complexes

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Aiming at the development of novel NO donors, the synthesis and reactivity of the *trans*-$[Ru(bpy)_2(SO_3)(NO)](PF_6)$ and *cis*-$[Ru(bpy)_2(L)(NO)](PF_6)_n$ complexes (L = imidazole, isonicotinamide, or sulfite ions) were investigated. The complexes were characterized and the NO release capabilities were evaluated. The rate constant for NO dissociation for the *cis*-$[Ru(bpy)_2(SO_3)(NO)]^+$ complex is $k = 2.0{\cdot}10^{-3}\ s^{-1}$. The *cis* and *trans*- $[Ru(bpy)_2(SO_3)(NO)]^+$ complexes showed promising physical-chemical properties towards nitric oxide generation, and great stability towards hydroxide attack in the 2.0–9.0 pH range. The reduction potential of the $\{RuNO\}^{6/7}$ process of the *cis* and *trans*-$[Ru(bpy)_2(SO_3)NO]^+$ complexes ($E_{1/2}$ = –0.14 and –0.34 V vs. Ag/AgCl) is appropriated for reduction in vivo by biological reducing agents, with consequent NO release.

Introduction

Nitric oxide (NO) has a critical role in physiological and pathophysiological processes in the cardiovascular system.[1–3] NO insufficiency limits NO-mediated signal transduction of normal or protective physiological processes. Dysfunction of the normally protective endothelium is found in several cardiovascular diseases, including hypertension, coronary heart disease, and arterial thrombotic disorders.[4] The endothelial dysfunction leads to nitric oxide (NO) deficiency, which has been implicated in the underlying pathobiology of many of these disorders.[5] Exogenously administered NO donors have provided the basis for a broad field of pharmacotherapeutics in cardiovascular medicine. Pharmacological compounds that release NO have been useful tools for evaluating the pivotal role of NO in cardiovascular physiology and therapeutics. NO donors are pharmacologically active substances that spontaneously release or are metabolized to NO or its redox partners. For example in cases of emergency hypertension or heart attacks, NO needs to be administered due to its vasodilator effect. Sodium nitroprusside, $Na_2[Fe(CN)_5NO]{\cdot}2H_2O$, is part of a class of compounds that release NO spontaneously and is the unique NO metal complex used clinically.[6] Problems associated with the use of $Na_2[Fe(CN)_5NO]{\cdot}2H_2O$ include susceptibility to photolysis and oxidative breakdown through the action of the immune system, both of which lead to the release of cyanide.[7] There are also Ru-edta complexes that can control the levels of circulating NO such as K[Ru(Hedta)Cl] that exists in equilibrium with its aqua species.[8] This aqua species has been shown to be useful in some conditions such as septic shock (also for diabetes, arthritis, inflammation, and epilepsy) where excessive production of NO can be a problem. This can lead to arteries responding poorly to vasoconstrictor drugs during treatment.[9]

Many drugs have direct vasoactive effects and have been used to treat ischemic heart disease, heart failure, and hypertension for many years. Nevertheless, undesirable effects such as the short therapeutic half-life, adverse hemodynamic effects, and drug tolerance limit the use of most of them.[5] To overcome these limitations, the development of novel NO donors are of great interest. The literature[10–12] has pointed out that it is possible to modulate the NO reduction potential by coordination to transition metals and by the π character of the spectator ligands. Hence, the correct establishment of the NO reduction potential in a specific compound is an important requirement for its use as a metallodrug.[10] With this purpose in mind, we have investigated the synthesis and reactivity of the *trans*-$[Ru(bpy)_2(SO_3)(NO)](PF_6)$ and *cis*-$[Ru(bpy)_2(L)(NO)](PF_6)_n$ complexes, where L = imidazole (imN), isonicotinamide (isn), or sulfite ions (SO_3^{2-}), and bpy = 2,2′-bipyridine. The complexes were characterized in order to infer the correct composition and coordination modes of the ligands, and to evaluate their NO release capabilities.

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Results and Discussion

The electronic spectra of the *cis*-$[Ru(bpy)_2L(NO_2)]^{+/-}$ nitro complexes (L = imN, isn, SO_3^{2-}) are dominated by intense (bpy)π*←dπ(Ru) MLCT bands (around 410 nm) and by the bpy intraligand bands, which are characteristic of the Ru^{II}-bipyridine complexes.[13,14] The bands observed in the 320–326 nm range for the *cis*-$[Ru(bpy)_2L(NO)]^{n+}$ and *trans*-$[Ru(bpy)_2(SO_3)(NO)]^+$ nitrosyl complexes (Table 1) are attributed to the (bpy)π*←dπ(Ru) MLCT transition. These bands are shifted to a higher energy range when compared with the spectra of the corresponding nitro complexes. This effect can be explained by the strong (NO^+)π*←dπ(Ru) backbonding interaction, which causes a large stabilization of the molecular orbitals that have a high contribution of the metal dπ levels. Unfortunately, the (NO^+)π*←dπ(Ru) transitions were not detected in the spectra probably because of superposition with those of (bpy)π*←dπ(Ru) transitions. These bands are expected to appear in the higher energy region of the spectra because of the stabilization of the dπ levels by the (bpy)π*←dπ(Ru) backbonding interaction. In fact, the (NO^+)π*←dπ(Ru) transition is observed in the range of 420–480 nm for $[Ru(NH_3)_4(NO)X]^{n+}$ complexes (X = OH^-, Cl^-, Br^-, and I^-), with a very weak intensity ($\varepsilon < 50\ mol^{-1}\cdot cm^{-1}\cdot L$).[15,16] The intense bands observed at about 290 nm are assigned to the π*←π (bpy) transition, which commonly appears in the spectra of bis(2,2′-bipyridine)ruthenium(II) complexes.[17]

Table 1. Electronic spectra data for the nitro and nitrosyl-bis(2,2′-bipyridine)ruthenium(II) complexes, in 0.10 M CF_3CO_2Na aqueous solution.

Complexes	(bpy)π*←dπ(Ru) MLCT, λ [nm] (ε, $mol^{-1}\ L\cdot cm^{-1}$)
cis-$[Ru(bpy)_2(imN)(NO_2)]^+$	412 ($7.30\cdot 10^3$)
cis-$[Ru(bpy)_2(isn)(NO_2)]^+$	408 ($9.04\cdot 10^3$)
cis-$[Ru(bpy)_2(SO_3)(NO_2)]^-$	405 ($1.02\cdot 10^3$)
cis-$[Ru(bpy)_2(imN)(NO)]^{3+}$	325 sh (8.92×10^3)
cis-$[Ru(bpy)_2(isn)(NO)]^{3+}$	321 sh ($1.11\cdot 10^4$)
cis-$[Ru(bpy)_2(SO_3)(NO)]^+$	326 ($8.27\cdot 10^3$)
trans-$[Ru(bpy)_2(SO_3)(NO)]^+$	320 sh ($3.08\cdot 10^4$)

The electronic spectra of the nitrosyl complexes were observed to be pH dependent. As the pH of the solutions containing these complexes is increased, new intense bands with a maximum in the 400–450 nm range are observed. These bands can be assigned to the (bpy)π*←dπ(Ru) MLCT transition of the nitro species formed according to the chemical equilibrium displayed in Reaction (1).[18–20] Similar behavior was observed in the spectra of the *trans*-$[Ru(NH_3)_4(L)(NO)]^{3+}$ complexes.[21]

The pH vs. absorbance plots for all nitrosyl complexes showed only one inflection (Figure 1), indicating a unique equilibrium assigned to the $[NO]^+ \rightleftharpoons [NO_2]^-$ interconversion, with both species present in equal concentrations at pH = 1.45 for L = isn, pH = 5.54 for L = imN, and pH = 10.32 for L = SO_3^{2-}. The calculated K_{eq} equilibrium constants are displayed in Table 2 together with $\nu(NO^+)$ and $E_{1/2}\{RuNO\}^{6/7}$ values for correlation purposes. The electrophilic character of coordinated nitrosyl has been associated with the ν(NO) frequency.[22] The ν(NO) values reflect the electron density at the nitrogen atom of the nitrosyl group, which is the target for the nucleophilic attack. The literature has established that complexes with ν(NO) greater than 1860 cm^{-1} are reactive OH^-.[16] It has also been observed that the equilibrium constant [Reaction (1)] is greater as the ν(NO) frequency is more positive. Therefore, the K_{eq} values might also reflect the electrophilic character of the nitrosyl complex involved in the chemical equilibrium.

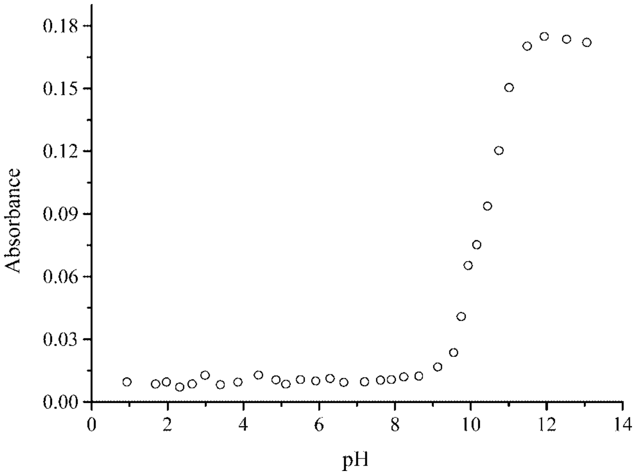


Figure 1. Spectrophotometric titration of $[Ru(bpy)_2(SO_3)(NO)]^+$ complex, λ_{max} = 410 nm; μ = 1.0 M CF_3CO_2Na aqueous solution.

Table 2. Values of K_{eq} for the equilibrium (*1*), $\nu(NO^+)$, and $E_{1/2}$ $\{RuNO\}^{6/7}$ for the $[Ru(bpy)_2(L)(NO)]^{n+}$ complexes, pH = 2.0; *pH = 1.0.

Complexes	K_{eq}	$\nu(NO^+)$ [cm^{-1}]	$E_{1/2}$, $\{RuNO\}^{6/7}$ [V]
cis-$[Ru(bpy)_2(isn)(NO)](PF_6)_3$	$1.80\cdot 10^{25}$	1948	+0.34*
cis-$[Ru(bpy)_2(imN)(NO)](PF_6)_3$	$1.42\cdot 10^{16}$	1944	+0.20
cis-$[Ru(bpy)_2(SO_3)(NO)](PF_6)$	$1.74\cdot 10^7$	1911	–0.14
trans-$[Ru(bpy)_2(SO_3)(NO)](PF_6)$	$6.42\cdot 10^3$	1881	–0.34

From the data presented in Table 2, the proportionality between the K_{eq}, $\nu(NO^+)$, and $E_{1/2}$ parameters is clearly observed. Compounds in which the NO^+ character is favored [larger $\nu(NO^+)$] present large K_{eq} values in the whole series. In fact, the greater the electron deficiency of the NO moiety resulting from the π-acid character of the $[Ru(bpy)_2L]^{2+}$ group, the larger will be the driving force for reaction of

$$[Ru(bpy)_2(L)(NO)]^n_{(aq)} + 2\,OH^-_{(aq)} \xrightleftharpoons{K_{eq}} [Ru(bpy)_2(L)(NO_2)]^{(n-2)}_{(aq)} + H_2O_{(aq)} \qquad (1)$$

the coordinated NO^+ with OH^- in Reaction (1). The $E_{1/2}$ values are in accordance with the $\nu(NO^+)$ and K_{eq} data, and reflect well the influence of electronic effects on the reactivity of the nitrosyl metal complexes. In addition, a good linear correlation between $E_{1/2}$ $\{RuNO\}^{6/7}$ and $\nu(NO^+)$ is observed for the series of nitrosyl complexes. From Figure 2, it is observed that the lower redox potential of the $\{RuNO\}^{6/7}$ redox couple gives also a lower $\nu(NO^+)$, which is in agreement with the decrease seen in the electronic density of the NO^+ group because of the higher π-acceptor character of the L ligand.

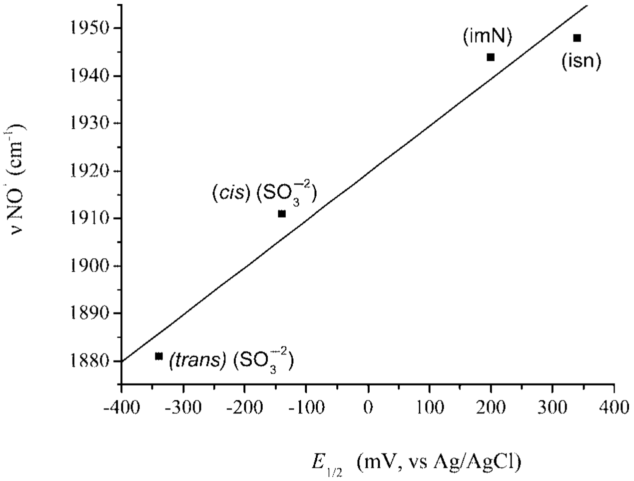


Figure 2. $\nu(NO^+)$ vs. $E_{1/2}$ $\{RuNO\}^{6/7}$ plot for the $[Ru(bpy)_2(L)NO]^{n+}$ complexes.

Generally, when a good σ-donor ligand is coordinated to nitrosyl-metal complexes in the *trans* position in relation to the NO^+ moiety, an increase of the $NO^+ \leftarrow M$ backbonding interaction occurs and the $\nu(NO^+)$ stretching shifts to a lower frequency in the infrared (IR) spectrum. Conversely, the electron-withdrawing capability of π-acid ligands reduces the $NO^+ \leftarrow M$ backbonding interaction causing an opposite effect on the $\nu(NO^+)$.[23] It should be noted that the $\nu(NO^+)$ peak of free NO is located at 1876 cm^{-1}, but in the metal complexes it is very sensitive to the binding properties and oxidation states of the metal ion. The literature reports that the $\nu(NO^+)$ frequencies for *cis*-$[Ru^{II}(bpy)_2(NO)L]$ complexes (L = N_3^-, Cl^-, NO_2^-, NH_3, py, CH_3CN) have been observed in the 1923–1970 cm^{-1} range.[17] For $[Ru^{II}(NH_3)_5(NO)]^{3+}$[24] and *trans*-$[Ru^{II}(NH_3)_4(NO)L]$[25] complexes [L = imidazole, L-histidine, py, isonicotinamide, pyrazine, $P(OEt)_3$], $\nu(NO^+)$ values lie in the 1909–1941 cm^{-1} range, whereas for L = SO_3^{2-}, the $\nu(NO)$ value is 1871 cm^{-1}. In the case of *trans*-$[Ru^{II}(py)_4(NO)L]$[26] complexes, for L = Cl^- and Br^-, $\nu(NO^+)$ = 1910 and 1901 cm^{-1}, respectively, but for L = OH^-, $\nu(NO^+)$ = 1860 cm^{-1}. Also, $\nu(NO^+)$ = 1860 cm^{-1} is observed for the *trans*-$[RuCl([15]aneN_4)NO]^{2+}$ complex.[27] In such examples, the ruthenium center has been considered as mainly Ru^{II}, thus giving a predominantly NO^+ character to the nitrosyl ligand.

On the basis of these discussions, the very intense peaks observed in the IR spectra of the nitrosyl complexes at 1948, 1944, 1911, and 1882 cm^{-1} for the *cis*-$[Ru(bpy)_2(isn)(NO)](PF_6)_3$, *cis*-$[Ru(bpy)_2(imN)(NO)](PF_6)_3$, *cis*-$[Ru(bpy)_2(SO_3)(NO)](PF_6)$, and *trans*-$[Ru(bpy)_2(SO_3)(NO)](PF_6)$ complexes, respectively, are assigned to $\nu(NO^+)$. The most relevant features in the IR spectra of the nitro complexes are the strong absorptions at 1340 and 1280 cm^{-1} attributed respectively to the symmetric and asymmetric $\nu(NO_2)$ stretching modes of the coordinated nitro ligand.[28] It is important to point out that the infrared spectrum for the product isolated from Reaction (1) for L = SO_3^{2-} at pH 12.0 does not show the $\nu(NO)$ frequency value, characteristic of the NO^+ form. Instead of the band at 1911 cm^{-1}, this spectrum presented bands at 1321 and 1271 cm^{-1}, which are associated with the asymmetric and symmetric stretching mode of the nitro ligand (NO_2). This result supports the proposed equilibrium suggested by Reaction (1), in agreement with that reported in the literature.[21,23]

To better characterize the nitrosyl complexes, their ^1H NMR, 2D COSY, and HMQC spectra were acquired. Figure 3 illustrates the HMQC spectrum for the *cis*-$[Ru(bpy)_2(imN)(NO)]^{3+}$ complex. The presence of all signals of the N-heterocyclic ring suggests that the imN and isn ligands are coordinated to the metal center through the nitrogen atom.[10] The appearance of 16 signals for the hydrogen atoms of the two bipyridine molecules suggests that all hydrogen atoms are nondiamagnetically equivalent,[29,30] strongly indicating the *cis* configuration for the $[Ru(bpy)_2(isn)(NO)]^{3+}$, $[Ru(bpy)_2(imN)(NO)]^{3+}$, and $[Ru(bpy)_2(SO_3)(NO)]^+$ complexes. On the other hand, the ^1H NMR spectrum of the *trans*-$[Ru(bpy)_2(SO_3)(NO)]^+$ complex shows only four signals at δ = 8.85 ppm (d; H_6, H_{11}), δ = 8.57 ppm (d; H_3, H_8), δ = 8.40 ppm (t; H_5, H_{10}), and δ = 7.87 ppm (t; H_4, H_9), which is characteristic of the *trans* configuration of the bipyridine ligands.[31]

The cyclic voltammograms (Figure 4) of the nitro complexes show only one reversible redox process. The data are summarized in Table 3.

Because of the π-acceptor character of the imidazole, isonicotinamide, and nitro moieties, all values of $E_{1/2}$ for the $Ru^{III/II}$ redox couple are more positive than that for the $[Ru(bpy)_2Cl_2]$ complex in aqueous solution.[32]

The electrochemical studies of the nitrosyl complexes were performed by using cyclic voltammetry and square wave voltammetry techniques. Figure 5 presents the cyclic voltammogram of the *cis*-$[Ru(bpy)_2(SO_3)(NO)]^+$ complex in an aqueous medium, at pH = 7.0. The electrochemical data for all complexes are illustrated in Table 2.

By applying a negative scan potential to the working electrode, starting from +0.90 V, only one reversible electrochemical process characteristic of the $\{RuNO\}^{6/7}$ redox couple was observed (Figure 5, dotted line). With a positive scan potential, a second electrochemical process is observed at 0.72, 0.70, and 0.62 V for L = isn, imN, and SO_3^{2-}, respectively. This process is strongly dependent on the $NO^{+/0}$ redox couple, as can be observed in Figure 5 (solid line), and is assigned to the $Ru^{III/II}$ redox process of the $[Ru(bpy)_2(L)(H_2O)]^{2+}$ complexes, which are generated by NO dissociation from the $[Ru(bpy)_2(L)(NO)]^{n+}$ nitrosyl compounds. These results suggest the existence of a chemi-

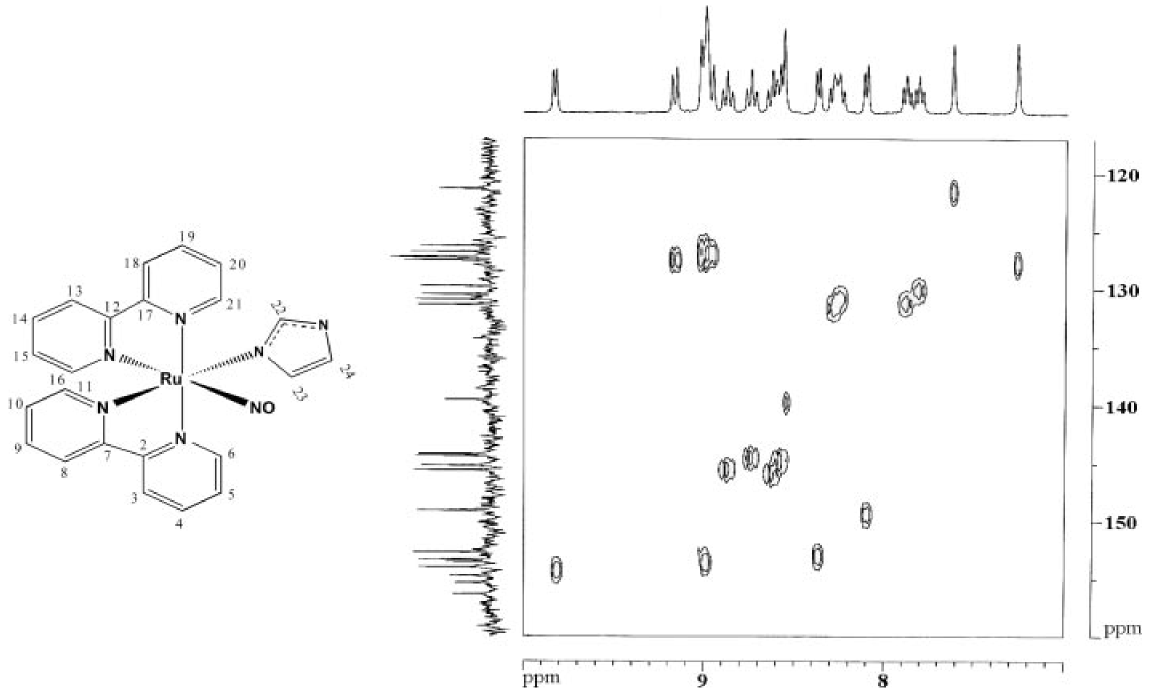


Figure 3. HMQC spectrum of *cis*-$[Ru(bpy)_2(imN)(NO)]^{3+}$ in CD_3COCD_3.

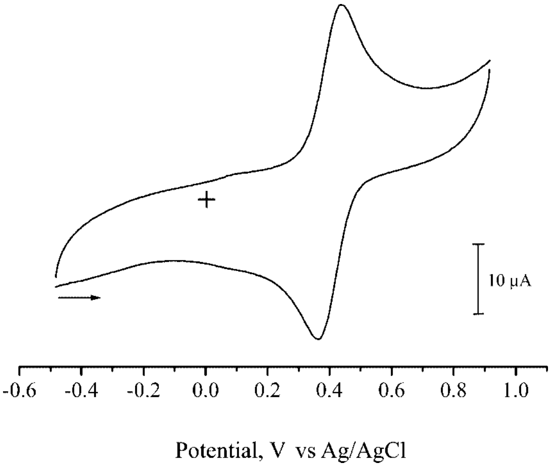


Figure 4. Cyclic voltammograms at 0.10 Vs^{-1} of the *cis*-$[Ru(bpy)_2(SO_3)(NO_2)]^-$ complex in 0.10 M CF_3CO_2Na aqueous solution, pH 7.0.

Table 3. $E_{1/2}$ values for the $Ru^{III/II}$ redox couple in $[Ru(bpy)_2L^1L^2]^{+/2+}$ complex ions.

L^1, L^2	$E_{1/2}$ [V]
isn, NO_2^-	+0.97
imN, NO_2^-	+0.75
SO_3^{2-}, NO_2^-	+0.45
SO_3^{2-}, H_2O	+0.38

cal reaction that precedes the oxidation of the metal center in the aqua complexes. A reasonable interpretation of the electrochemical results is proposed in Reactions (2), (3), and (4).

$$[Ru^{II}(bpy)_2(L)(NO^+)]^{3+} + e^- \xrightleftharpoons{-0.14} [Ru^{II}(bpy)_2(L)(NO^0)]^{2+} \quad (2)$$

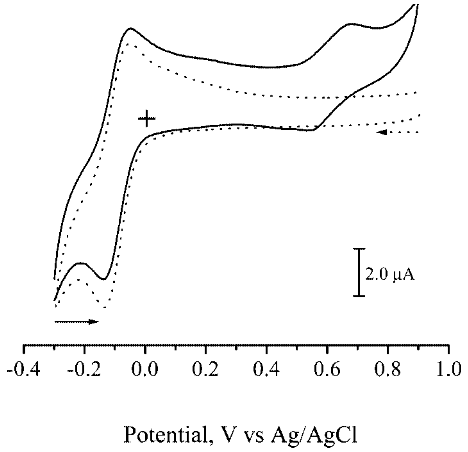


Figure 5. Cyclic voltammograms at 0.10 Vs^{-1} of the *cis*-$[Ru(bpy)_2(SO_3)(NO)]^+$ complex in 0.10 M CF_3CO_2Na aqueous solution, pH 7.0, scan starting at –0.3 V (solid line) and at 0.9 V (dotted line). + → i = 0.0 µA and E = 0.0 V.

$$[Ru(bpy)_2(L)(NO^0)]^{2+} + H_2O \xrightarrow{k_{NO}} [Ru(bpy)_2(L)(H_2O)]^{2+} + NO^0 \quad (3)$$

$$[Ru^{II}(bpy)_2(L)(H_2O)]^{2+} \xrightleftharpoons{+0.62} [Ru^{III}(bpy)_2(L)(H_2O)]^{3+} + e^- \quad (4)$$

The chemical scheme of the reaction proposed in Reaction (3) is corroborated by the redox process observed in the 0.72–0.62 V range, which is characteristic of the aqua complex, illustrated in Figure 5 (solid line).

Aiming to reinforce this assignment, the square wave voltammograms for the *cis*-$[Ru(bpy)_2(L)(NO)]^{n+}$ complexes were acquired by a change in the time polarization of the working electrode, at –0.30 V. For all nitrosyl complex series, it was observed that the peak current related to the $Ru^{III/II}$ redox process increases with an increase in time polarization. Figure 6 illustrates the electrochemical behavior of the *cis*-$[Ru(bpy)_2(SO_3)(NO)]^+$ complex.

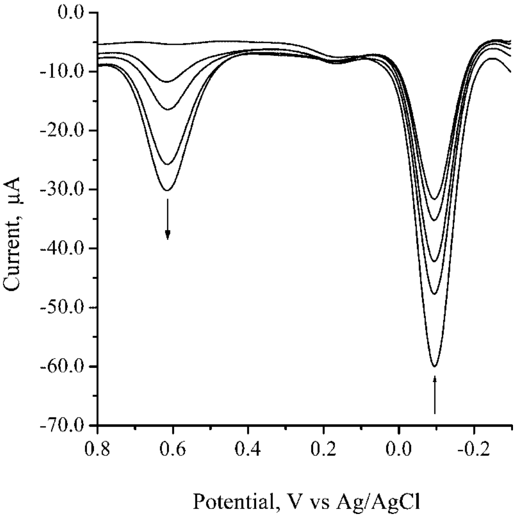


Figure 6. Square wave voltammogram at 0.10 Vs^{-1} of the *cis*-$[Ru(bpy)_2(SO_3)(NO)]^+$ complex in 0.10 M CF_3CO_2Na aqueous solution, pH 7.0, scans starting at –0.30 V.

The proposed scheme for the reaction is also supported by the electronic and FTIR spectra obtained for the $[Ru(bpy)_2(L)(NO)]^{3+}$ complexes acquired after the electrolysis experiments in which the potential was controlled at –0.30 V. Upon reduction, the electronic spectrum showed the two MLCT bands characteristic of the $[Ru(bpy)_2(L)(H_2O)]^{2+}$ complexes.[32] Furthermore, the IR spectra of all the reduced species, in the solid state, do not present the characteristic $\nu(NO^+)$ band. The rate constant of the NO dissociation, determined by the chronoamperometric method, was $k_{NO} = 2.0 \cdot 10^{-3} \cdot s^{-1}$ for L = SO_3^{2-}.

Preliminary photochemical studies of the nitrosyl complexes presented here were performed by irradiation at 355 nm wavelength. The electronic spectra changes observed during the photolysis of the *cis*-$[Ru(bpy)_2(SO_3)(NO)]^+$ complex in aqueous solution are presented in Figure 7.

From Figure 6, the appearance of the LMCT band at 450 nm is clearly observed, typical of the *cis*-$[Ru(bpy)_2(SO_3)(OH)]$ generated after the release of NO, in accordance with Scheme 1.[33]

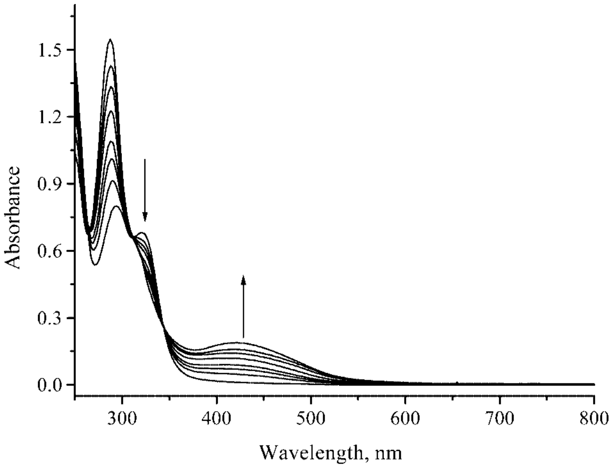


Figure 7. Spectral changes observed during the photolysis of the *cis*-$[Ru(bpy)_2(SO_3)(NO)]^+$ complex in CF_3CO_2Na aqueous solution, pH 7.0 at 355 nm.

The calculated quantum yield for photochemical NO release from the *cis*-$[Ru(bpy)_2(SO_3)(NO)]^+$ complex was 0.10 ± 0.01. A similar photochemical behavior is found for the other complexes as reported in the literature.[34,35]

In addition, the photolysis of the *cis*-$[Ru(bpy)_2(SO_3)(NO)](PF_6)$ complex dispersed in solid KBr was performed and monitored by IR spectroscopy. The successively acquired IR spectra show the decrease in the intensity of the $\nu(NO^+)$ peak at 1915 cm^{-1}, which corroborates the proposed photochemical reaction (Scheme 1). The same behav-

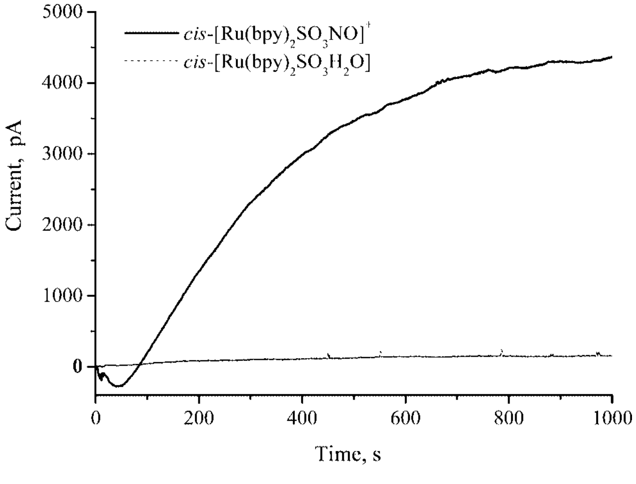
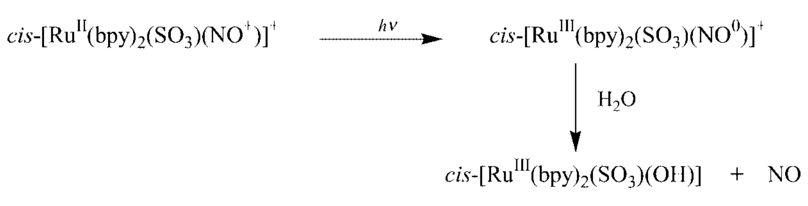


Figure 8. Chronoamperogram of NO release by reaction of $1.0 \cdot 10^{-4}$ M of $[Ru(bpy)_2(SO_3)(NO)]^+$ aqueous solution with cysteine, in a pH 7.4 phosphate buffer solution.



Scheme 1. Photochemical reaction of NO release by the *cis*-$[Ru(bpy)_2(SO_3)(NO)]^+$ complex.

ior has been observed for several nitrosyl ruthenium complexes.[25,27,35]

The *cis*- and *trans*-[Ru(bpy)$_2$(SO$_3$)(NO)]$^+$ nitrosyl complexes are also reduced by biological reducing agents, such as cysteine, with subsequent NO release. The signal recorded by the NO sensor was observed when a reaction with cysteine was initiated (Figure 8), which indicated the presence of free NO. These results, along with other biological experiments, are currently under investigation and will be reported in a further publication.

Conclusion

The properties of the *cis* and *trans*- [Ru(bpy)$_2$(SO$_3$)(NO)]$^+$ species reported here suggest the considerable advantages provided by these complexes because of their nitric oxide generation, when compared with other NO donors reported in the literature.[36–38] For instance, the *trans*-[Ru(NH$_3$)$_4$P(OEt)$_3$(NO)]$^{3+}$ complex is considered a promising compound that enhances neuronal firing in mice hippocampal slices.[39] Unfortunately, this complex is chemically modified by hydroxide attack on the NO$^+$ moiety, resulting in dissociation of nitrite ions in physiological conditions.[39] Since the *cis* and *trans*- [Ru(bpy)$_2$(SO$_3$)(NO)]$^+$ complexes are inert to hydroxide attack in the 2.0–9.0 pH range, and the reduction potential of the {RuNO}$^{6/7}$ redox process for the *cis*-[Ru(bpy)$_2$(SO$_3$)(NO)]$^+$ species ($E_{1/2}$ = –0.14 V vs. Ag/AgCl) is close to that of the *trans*-[Ru(NH$_3$)$_4$P(OEt)$_3$(NO)]$^{3+}$ complex,[38] the sulfite complex is potentially a better NO donor in vivo.

Experimental Section

Chemicals and Reagents: Ultrahigh purity water from a Millipore system was used throughout the experiments. RuCl$_3$·*x*H$_2$O, imidazole, isonicotinamide, cysteine, and sodium sulfite were purchased from Aldrich and used without further purification. [Ru(bpy)$_2$Cl$_2$] and *trans*-[Ru(NH$_3$)$_4$(SO$_2$)Cl]Cl were prepared according to the literature procedures.[13,14,40]

Synthesis of the Complexes: The [Ru(bpy)$_2$(SO$_3$)(H$_2$O)] complex was prepared by dissolving *trans*-[Ru(NH$_3$)$_4$(SO$_2$)Cl]Cl (0.080 g, 0.26 mmol) in water (5.0 mL) under argon. 2,2′-Bipyridine (0.082 g, 0.52 mmol) was then added, and the mixture was stirred for 2 h. The solution volume was reduced under vacuum to approximately 2 mL. Addition of ethanol (10 mL) resulted in the precipitation of the product as a black solid. The precipitation of the product was induced by the addition of ethanol (10 mL). Yield (0.107 g) >80%. C$_{20}$H$_{16}$N$_4$O$_4$RuS (511.38): calcd. C 46.92, H 3.52, S 6.25; found C 46.83, H 3.50, N 6.21.

The [Ru(bpy)$_2$(L)(NO$_2$)](PF$_6$)$_n$ complexes were prepared by following a standard procedure in all series. In a typical procedure, the reactions were performed by mixing a sample of [Ru(bpy)$_2$Cl$_2$] (0.200 g, 0.41 mmol) and L (0.49 mmol; L = imidazole, isonicotinamide, sodium sulfide) in a 1:1 ethanol/water solution (20 mL). The mixtures were kept under reflux for 1 h. To these solutions, NaNO$_2$ (0.034 g, 0.49 mmol) was added and the mixtures were then allowed to react for 2 h, under reflux. The resulting solutions were concentrated by rotary evaporation until reduction of the volume to approximately 10 mL has been achieved. The solids were precipitated by the addition of saturated aqueous NH$_4$PF$_6$ solution (2 mL). Yields (0.209 g, 0.126 g, and 0.195 g for L = imidazole, isonicotinamide and sulfite, respectively) >75%. C$_{23}$H$_{19}$F$_6$N$_7$O$_2$PRu (672.36): calcd. C 41.05, H 2.82, N 14.58; found C 41.23, H 2.79, N 14.38. C$_{26}$H$_{22}$F$_6$N$_7$O$_3$PRu (726.46): calcd. C 42.94, H 3.02, N 13.49; found C 42.65, H 2.98, N 13.32. C$_{20}$H$_{16}$N$_5$NaO$_5$Ru (562.38): calcd. C 42.67, H 2.85, N 12.45; found C 42.37, H 2.78, N 12.19.

The [Ru(bpy)$_2$L(NO)](PF$_6$)$_n$ complexes (L = imidazole, isonicotinamide, sulfite) were prepared by dissolving the [Ru(bpy)$_2$L(NO$_2$)](PF$_6$)$_n$ complexes (0.2 mmol) in methanol (15 mL) under an argon atmosphere. To this mixture, concentrated trifluoroacetic acid solution (2 mL) was added to promote the conversion of NO$_2$ to NO$^+$.[28] The reaction was monitored by HPLC by following the decrease of the peak at *Rt* = 6.78 min of the nitro complex at the expense of the increase of the peak at *Rt* = 3.93 min of the nitrosyl complex. After 2 h, no changes in the heights of the peaks were observed, indicating that the reaction is close to the saturation kinetics point. The solvent was then concentrated in a rotary evaporator to near 8 mL, and the solid was precipitated by the addition of ammonium hexafluorophosphate (1.0 g), collected by filtration and stored under vacuum. Yields (0.151 g, 0.160 g, and 0.107 g for L = imidazole, isonicotinamide, and sulfite, respectively) >80%. C$_{23}$H$_{23}$F$_{18}$N$_7$O$_3$P$_3$Ru (982.36): calcd. C 28.15, H 2.36, N 9.99; found C 28.23, H 2.29, N 9.68. C$_{26}$H$_{22}$F$_{18}$N$_7$O$_2$P$_3$Ru (1000.42): calcd. C 31.18, H 2.19, N 9.80; found C 31.09, H 2.12, N 9.75. C$_{20}$H$_{16}$F$_6$N$_5$OPRuS (619.98): calcd. C 35.90, H 2.39, N 10.47; found C 35.81, H 2.31, N 10.32.

Determination of the Equilibrium Constant: The literature reports of the study of some similar chemical systems that exhibit the NO$_2^-$ $\rightleftharpoons$ NO$^+$ equilibrium.[23,41] The determination of the equilibrium constant K_{eq} for Reaction (1) was carried out using the spectrophotometric method (absorbance measurements were performed at 410 nm). The total ionic strength was kept at 1.0 M with CF$_3$CO$_2$Na, according to similar studies reported for the Fe^{II} [42] and Ru^{II} [28] systems.

Determination of the Rate Constant for the NO Dissociation: The rate constant for the NO dissociation in the [Ru(bpy)$_2$(SO$_3$)(NO)](PF$_6$) complex was determined by the chronoamperometric method, according to the procedure reported in the literature.[43]

Quantum Yield: Quantum yield was determined from initial spectral changes and was plotted vs. reaction percentage and extrapolated back to 0% reaction to minimize inner filter effects. The reported quantum yield is the average of, at least, three independent experiments.

Apparatus: Electrochemical experiments were performed with an electrochemical analyzer BAS 100W from Bioanalytical Systems at 25.0 ± 0.2 °C. These were generally performed on millimolar solutions of the complexes in 0.10 M CF$_3$CO$_2$Na (pH = 7.0) aqueous solution by using a conventional three-electrode glass cell with a glassy carbon (ca. 0.13 cm^2 of geometrical area) and Pt foil as the working and auxiliary electrodes, respectively. The electrochemical experiments were performed at room temperature. The reversibility of the electrochemical processes was checked by the linearity of the peak height for the reduction process as a function of the square root of the scan rate, and the separation between the anodic (Ea) and cathodic potential peaks in the range of 50–80 mV at 25 °C.[44] NO release was measured with an ISONOP NO meter from World Precision Instruments that directly detects NO concentration by an amperometric technique.

The chromatographic analyses were performed with a Shimadzu liquid chromatograph equipped with a model LC-10AD pump and an SPD-M10A UV/Visible photodiode-array detector with a CBM-10AD interface. An ODS column (250 mm × 4.6 mm id., 5 µm particles; from Altech) was used with an isocratic elution with 20:80 acetonitrile/water containing 0.1% CF_3CO_2H, pH = 3.7. The chromatograms were taken at a constant flow rate of 1.0 $mL{\cdot}min^{-1}$. Samples for analyses were dissolved in the mobile phase and 5 µL of volumes were injected.

Elemental analyses were performed with a Perkin–Elmer CHN 2400 analyzer. Electronic spectra were acquired with a HP-8453 diode-array spectrophotometer. NMR spectra were obtained with a Bruker AVANCE DPX 500 spectrometer in D_2O and CD_3COCD_3. IR spectra were obtained in KBr pellets with a Shimadzu IR Prestige-21 spectrophotometer.

Monochromatic irradiation at 355 nm was carried out using an Oriel 200-Watt universal arc lamp source in model 68805. The irradiation wavelength was selected with an Oriel interference filter for photolysis at the appropriate wavelength. The interference filter had an average band pass of 10 nm and the collimated beam intensities ranged from $4{\cdot}10^{-8}$ to $1{\cdot}10^{-9}$ $einstein^{-1}{\cdot}cm^{-2}$ as determined by ferrioxalate actinometry. The progress of the photoreactions was monitored spectrophotometrically with an HP-8453 diode-array spectrophotometer.

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