Photoinduced Electron Transfer between the Cationic Complexes $Ru(NH_3)_5pz^{2+}$ and *trans*-$RuCl([15]aneN_4)NO^{2+}$ Mediated by Phosphate Ion: Visible Light Generation of Nitric Oxide for Biological Targets†

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The photochemical behavior of the tetraazamacrocyclic complex *trans*-$RuCl([15]ane)(NO)^{2+}$ ($RuNO^{2+}$) in a 10 mM phosphate buffer solution, pH 7.4, and in the presence of $Ru(NH_3)_5pz^{2+}$ ($Rupz^{2+}$) is reported. Irradiation (436 nm) of an aqueous solution containing both cationic complexes as PF_6^- salts labilizes NO from $RuNO^{2+}$ with a quantum yield (ϕ_{NO}) dependent on the concentration of $Rupz^{2+}$ with a maximum value of ϕ_{NO} ($1.03(11) \times 10^{-3}$ einstein mol^{-1}) found for a solution with equimolar concentrations (5×10^{-5} M) of the two complexes in phosphate buffer solution. The quantitative behavior of this system suggests that the two cations undergo preassociation such that photoexcitation of the visible absorbing $Rupz^{2+}$ is followed by electron or energy transfer to $RuNO^{2+}$, which does not absorb appreciably at the excitation wavelength, and this leads to NO release from the reduced nitrosyl complex. Notably, the NO release was not seen in the absence of phosphate buffer; thus, it appears that phosphate ions mediate NO generation, perhaps by facilitating formation of a supramolecular complex between the two ruthenium cations. Reexamination of the cyclic voltammetry of $Rupz^{2+}$ showed that the electrochemical behavior of this species is also affected by the presence of the phosphate buffer.

Introduction

There has long been interest in developing methodologies for delivering the bioregulatory diatomic nitric oxide to physiological targets for possible therapeutic applications.[1] Photochemical techniques for delivery are attractive because they allow control of the location and timing of the photoexcitation leading to NO release.[2] In this context, various metal complexes have been examined as photochemical NO generators,[3,4] among them the macrocycle ruthenium nitrosyl *trans*-$RuCl([15]aneN_4)(NO)^{2+}$ ($RuNO^{2+}$, $[15]aneN_4$ = 1,4,8,12-tetraazacyclopentadecane) (e.g., eq 1). Such saturated amine ruthenium nitrosyl complexes have high thermal stability; however, their minimal absorptivity in the visible range precludes effective NO photogeneration in that wavelength range. Thus, the present study was initiated with the goal of developing a mechanism for sensitizing the visible light photochemistry of this system.

$$trans\text{-}RuCl([15]aneN_4)(NO)^{2+} \xrightarrow[H_2O]{h\nu\ (355nm)} trans\text{-}RuCl([15]aneN_4)(H_2O)^{2+} + NO\ (RuNO^{2+}) \quad (1)$$

Solutions of the pentaammine ruthenium(II) complex ion $Ru(NH_3)_5(pz)^{2+}$ ($Rupz^{2+}$, pz = pyrazine) display a strong metal-to-ligand charge transfer (MLCT) absorption band centered at 472 nm.[5] A deaerated solution (pH 7.4 phosphate buffer (PB)) of $Rupz^{2+}$ is relatively photoinactive when irradiated at 436 nm, although it does show a small quantum yield (ϕ_d) for bleaching of the MLCT band owing to limited photoaquation processes. Similar photolysis of $RuNO^{2+}$ solutions shows little photochemistry owing to the absence of absorptions in the wavelength range 400−800 nm; however, a solution containing both $Rupz^{2+}$ and $RuNO^{2+}$ gives a very different situation. Visible range photolysis of such $RuNO^{2+}/Rupz^{2+}$ mixtures leads to NO labilization and the formation of a dinuclear complex (eq 2). Furthermore, this behavior is specific to PB solution, indicating that phosphate is somehow mediating the photochemistry, a likely mechanism being the formation of a one-to-one supramolecular assembly of $RuNO^{2+}$ and $Rupz^{2+}$ in solution.

$$RuNO^{2+} + Rupz^{2+} \xrightarrow[h\nu = 436\ nm]{H_2O} Ru\text{-}pz\text{-}Ru^{4+} + NO \quad (2)$$

Reported here is a quantitative study of this photoreaction. Also described are rat aorta dilation experiments confirming that biologically relevant concentrations of NO are photochemically released from these supramolecular assemblies.

Experimental Section

Apparatus. The UV−visible spectra were recorded on a Hitachi U-3501 and HP 3854 spectrophotometer. Infrared (IR) spectra were recorded on a Protegé 460 series FT-IR spectrometer, using solid samples pressed in KBr pellets. Low-resolution

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mass spectra were obtained using a VG Fisons Platform II single quadrupole mass spectrometer with an electrospray ionization source run with a Fisons Masslinks data system. The pH measurements were made using a Corning model 430 pH meter.

The electrochemical study was performed with a potentiostat−galvanostat Autolab PGSTAT 30 model, which consists of a conventional three-electrode cell with a Pt wire auxiliary electrode, a Ag/AgCl reference electrode, and a glassy carbon working electrode. The supporting electrolyte was 0.1 M KCl solution.

In most cases, photolysis of the ruthenium complexes was performed in phosphate buffer solutions at pH 7.4 with ionic strength of 0.1 M adjusted with KCl solution. The light source was the output from a 200 W, high-pressure mercury lamp passed through an IR filter and a 436 nm interference filter to select the desired irradiation wavelength λ_{irr}. A sample of known volume in a 1.0 cm square quartz cuvette at 25.0 ± 0.1 °C was irradiated while stirring for defined time periods. The UV−visible spectrum was recorded after each irradiation period. Light intensity was determined by standard potassium ferrioxalate actinometry before each photolysis experiment. Nitric oxide release was detected and measured using an amperometric NO sensor from Innovative Instruments, Inc. coupled to a computer.

Chemicals and Reagents. $RuCl_3 \cdot nH_2O$, pyrazine (pz), 1,-10′-phenanthroline, and 1,4,8,12-tetrazacyclopentadecane ([15]-aneN_4) were purchased from Aldrich Chemicals as high-purity reagents. Vaseline (Chemco), propylene glycol (Synth), and Paramul J (Galena) were used as supplied. Nanopure H_2O was used for all experiments. The $[Ru(NH_3)_5(pz)](PF_6)_2$ and *trans*-$[RuCl(15]aneN_4)NO](PF_6)_2$ salts were synthesized by published procedures.[4,5] The positive ion ESI mass spectrum of the Ru−NO complex (molecular weight 671.0) in aqueous solution showed a *m*/*z* peak at 526.0 (calcd 526.0) assigned to $\{Ru(C_{11}N_4H_{26})(NO)Cl(PF_6)\}^+$ and a *m*/*z* peak at 380.0 (calcd 380.0) assigned to $\{Ru(C_{11}N_4H_{25})(NO)Cl\}^+$. The ruthenium pyrazine complex (MW 557.0) shows two main peaks at *m*/*z* 412.0 and 395.0, which correspond to $\{[Ru(NH_3)_5(pz)](PF_6)\}^+$ (calcd 412.0) and $\{[Ru(NH_3)_4(pz)](PF_6)\}^+$ (calcd 395.0), respectively.

NO Sensor Calibration. The calibration curve of the NO-selective electrode was prepared by using several dilutions of a known volume of a saturated nitric oxide solution in 10.0 mL of deaerated acetate buffer solution (pH = 7.4). For each volume added, the current value in nA was recorded. The NO concentration was calculated according to the reported molar fraction solubility of NO (2.1×10^{-3} mol L^{-1} at 25 °C).[4a,7]

Preparations of Water/Oil (w/o) Emulsion. The w/o emulsion consisted of Vaseline (5 wt %), propylene glycol (4), Paramul J (8), and 0.01 M of phosphate buffer solution at pH 7.4 (up to 100). Briefly, the oil and the water phases, which contained 10^{-3} M each of $RuNO^{2+}$ and $Rupz^{2+}$ complexes, were heated separately to 60.0 ± 0.1 °C and mixed together while stirring until cooled to room temperature to form a gel.

In Vitro Diffusion Studies. The diffusion of $RuNO^{2+}$, $Rupz^{2+}$, and $RuNO^{2+}/Rupz^{2+}$ compounds from these w/o emulsions was assessed using a Franz diffusion cell (Figure S1, Supporting Information).[7,8] On the day of the experiment, the cellulose acetate membrane (diffusion area of 0.882 cm^2) was mounted, and the formulated emulsion (1.50 g) was applied to the surface of the membrane. (In order to achieve higher reproducibility, the membranes were prehydrated for 2 h before applying the formulation.) The receptor compartment was filled with 6.0 mL of receptor medium (pH 7.4 PB solution), and this was under constant stirring and maintained at 37.0 ± 0.1 °C by a water jacket. Samples were periodically withdrawn (1.0 mL each hour) from the receptor compartment (up to 12 h), replaced with the same amount of fresh buffer solution, and analyzed by UV−visible spectroscopy. Cumulative concentration of actives released was calculated as a function of the membrane surface area. The experiment was carried out six times for each preparation, and the average release was calculated for each release point. The average release plus/minus the standard deviation was expressed for each time point and displayed graphically. Significance was determined using Student's *t*-test, and *P* values <0.05 were considered to be significant.

Pharmacological Assays. This study was performed in accordance with the Ethical Animal Committee of University of São Paulo (Campus of Ribeirão Preto).

Vessel Preparations. Male Wistar rats (180−200 g) were killed by decapitation, and the thoracic aorta was quickly removed, dissected free and cut into rings 4 mm long. The endothelium was mechanically removed by gently rolling the lumen of the vessel on a thin wire. The aortic rings were connected between two stainless-steel stirrups, which consist of an isometric force transducer (F-60 force displacement transducer) and a fixed support in the chamber in order to record the tension on a polygraph (Figure S2, Supporting Information). The rings were placed in a 10 mL organ chamber containing Krebs solution with the following composition (mmol/L): NaCl 130, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 14.9, glucose 5.5, and $CaCl_2$ 1.6. The solution was maintained at pH 7.4 under an atmosphere of 95% O_2 and 5% CO_2 at 37 °C. The rings were initially stretched to a basal tension of 1.5 g, then allowed to equilibrate for 60 min in the bath fluid that was changed every 15−20 min. Endothelial integrity was qualitatively assessed by the degree of relaxation caused by acetylcholine (ACh, 1 μmol/L) following contraction induced by phenylephrine (0.1 μmol/L), and the rings were discarded if there was any degree of relaxation in response to ACh. The tissues were washed and precontracted with the EC_{50} of phenylephrine (0.1 μmol/L). The concentration−response curves for a 1:1 mixture of $RuNO^{2+}/Rupz^{2+}$ varying from 0.1 nmol/L to 100 μmol/L were generated in the absence and presence of visible light irradiation. For the experiments performed with the w/o emulsion, the chamber was loaded with 0.166 ± 0.012 g (0.09 mg of $RuNO^{2+}/Rupz^{2+}$ system) of the delivery system in a dialysis bag. For the pharmacological assays, samples were irradiated in a photoreactor consisting of a 0.5 m^3 box lined with aluminum to increase light reflection. Three 250 W fluorescent lamps (Philips) with filters to cut off light with $\lambda < 450$ nm were used as the light source.

Data and Statistical Analysis. Relaxant responses to the NO donor were measured from the plateau of the phenylephrine contraction and were expressed as percent reversal of the phenylephrine precontraction. Data are expressed as mean ± SEM. In each set of experiments, *n* indicates the number of rats studied. The pharmacological parameters, maximal effect (E_{max}) obtained from concentration−response curves for the $RuNO^{2+}/Rupz^{2+}$ mixtures, were used to analyze the data. E_{max} was considered as the maximal amplitude response reached in the concentration−effect curves for relaxant agents. Statistical significance was tested by the Student *t*-test, and values of $P < 0.05$ were considered to be significant.

Results and Discussion

As noted in the Introduction, the photochemical properties described below indeed suggest there is a solution interaction of the two ruthenium cations $RuCl([15]aneN_4)(NO)^{2+}$ and $Ru(NH_3)_5pz^{2+}$ that is mediated by the presence of phosphate ion.

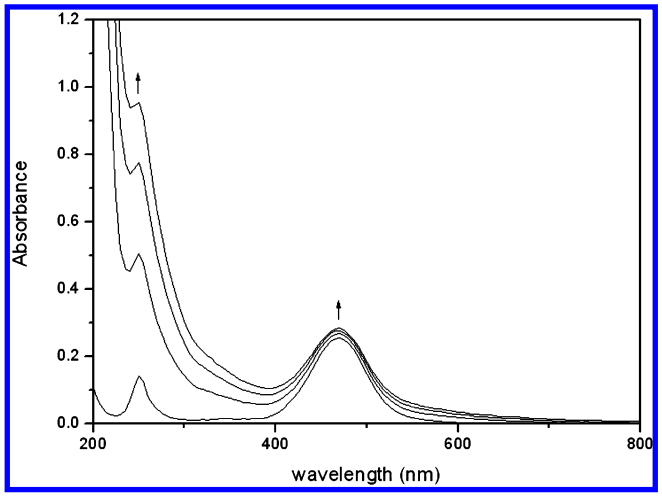


Figure 1. Electronic spectrum of $Rupz^{2+}$ (2.8×10^{-5} M) in the presence of different concentrations of $RuNO^{2+}$ (8.5×10^{-5}, 1.4×10^{-4}, and 1.8×10^{-4} M) in 10 mM pH 7.4 phosphate buffer solution.

For example, a preliminary investigation showed that 436 nm photolysis of an equimolar (5×10^{-5} M) $RuNO^{2+}/Rupz^{2+}$ mixture in pH 7.4, 10 mM PB solution leads to the release of NO but analogous photolysis of $RuNO^{2+}$ alone did not. This led us to investigate the spectral and electrochemical properties of this system to probe other indications of such complexation.

Spectroscopic Properties. The properties and photochemical reactions of the complex ions $RuNO^{2+}$ and $Rupz^{2+}$ have been described previously.[4−6,9] The visible spectrum of $Rupz^{2+}$ is dominated by a MLCT band attributed to a $d_{\pi}Ru^{II}-\pi^*(pz)$ transition and bands in ultraviolet region attributed to intraligand $\pi-\pi^*$ transitions. The MLCT band is strongly dependent on the solvent nature and in neutral water has a λ_{max} at 472 nm ($\epsilon = 10.4 \times 10^3$ M^{-1} cm^{-1}).[5] In contrast, the aqueous solution spectrum of the nitrosyl ruthenium species $RuNO^{2+}$ displays only UV bands that were characterized as $d_{\pi}(Ru^{II})-\pi^*(NO^+)$ and $\pi-\pi^*$ transitions.[4a]

The electronic spectrum of $Rupz^{2+}$ (28 μM) in phosphate buffer solution is displayed in Figure 1, as are the spectra of analogous $Rupz^{2+}$ solutions to which different concentrations of $RuNO^{2+}$ (up to 60 μM) have been added. At first glance, the spectral differences appear to be small, but careful analysis indicates that there are modest absorbance increases (but no obvious shift in the λ_{max}) of the MLCT band at 472 nm as well as a broad new absorbance in the range of 550−600 nm, perhaps due to an outer sphere intervalence charge transfer. Given that solutions of $RuNO^{2+}$ show no significant absorption in this wavelength region (see Figure S3 in the Supporting Information), these changes suggest an interaction between the two cations. No such spectral changes were noted when a solution of $Rupz^{2+}$ and $RuNO^{2+}$ was prepared without added phosphate (Figure S4 in Supporting Information).

The infrared spectrum of a 1 mM solution of $RuNO^{2+}$ in deuterium oxide (10 mM PB solution, pD 7.4) exhibited a nitrosyl stretching band ν_{NO} at 1860 cm^{-1}, similar to that observed in a KBr pellet (1860 cm^{-1}).[4a] Addition of $Rupz^{2+}$ (1 mM) to this solution had no effect on the position of this IR band.

Electrochemistry. Figure 2 shows the cyclic voltammogram of $Rupz^{2+}$ (1.0 mM) in PB solution (10 mM) using KCl (0.1 M) as the supporting electrolyte. A reversible cathodic wave is observed at 0.45 V (vs SHE) attributed to the oxidation of Ru(II) to Ru(III) (by comparison to the literature)[5] and a second cathodic peak (which appears not to have been previously

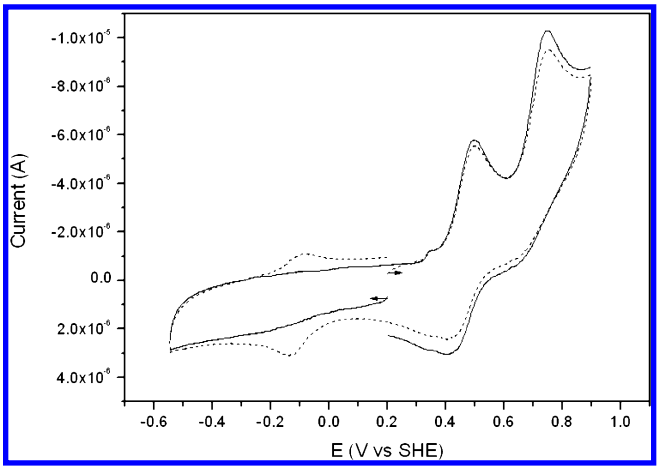


Figure 2. Cyclic voltammogram of $Ru(NH_3)_5(pz)](PF_6)_2$ (1.0 mM) in 10 mM phosphate buffer solution (pH 7.40, KCl = 0.1 M). Cathodic scan (solid line); anodic scan (dashed line). (Arrows indicate the starting point and direction of each scan.)

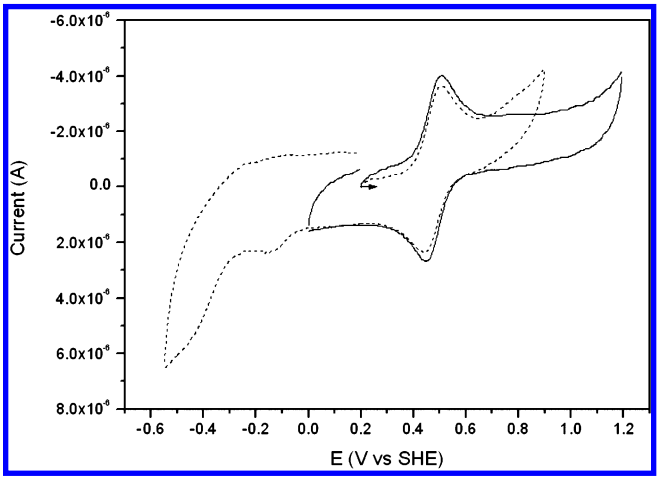


Figure 3. Cyclic voltammograms (anodic scans) of $Ru(NH_3)_5(pz)](PF_6)_2$ (1.0 mM). Solid line, in 0.1 M KCl solution; dashed line, in pH 7.4, 10 mM PB solution with $RuNO^{2+}$ (1.0 mM).

reported) at 0.75 V vs SHE. We attribute the latter to the oxidation of Ru(III) to Ru(IV). The latter oxidation is apparently followed by a chemical reaction to give a species that shows $E_{1/2} = -0.10$ V vs SHE (Figure 2). This new couple was attributed to the $Ru(NH_3)_5Cl^{3+/2+}$ reduction by comparison to the cyclic voltammogram of authentic $[Ru(NH_3)_5Cl]Cl_2$ in the same solution.

The cyclic voltammogram of $Rupz^{2+}$ did not display the second oxidation either in a KCl (0.1 M) solution unless phosphate was present or in an analogous PB/KCl solution to which $RuNO^{2+}$ (1.0 mM) was added. In the latter case, the only extra peak (−0.12 V vs SHE) seen in addition to the first oxidation of $Rupz^{2+}$ process could be attributed to the irreversible reduction of the nitrosyl ligand $RuNO^{2+}$ (Figure 3). The effect of the phosphate on the electrochemistry of $Rupz^{2+}$ suggests some type of outer sphere interaction, perhaps hydrogen bonding with an ammine group. We can draw some analogy here to the role of phosphate in facilitating the oxidation of tyrosine.[10]

The pH dependences of the two oxidations seen for $Rupz^{2+}$ in phosphate buffer solution are shown by a Pourbaix Diagram (Figure 4). The first oxidation is pH-independent at values above ~3 but shows a slope of −60 mV/pH at lower pH indicating a pH-dependent process consistent with the pK_a of 2.5 that had been determined previously by UV−visible spectroscopy.[9a] For

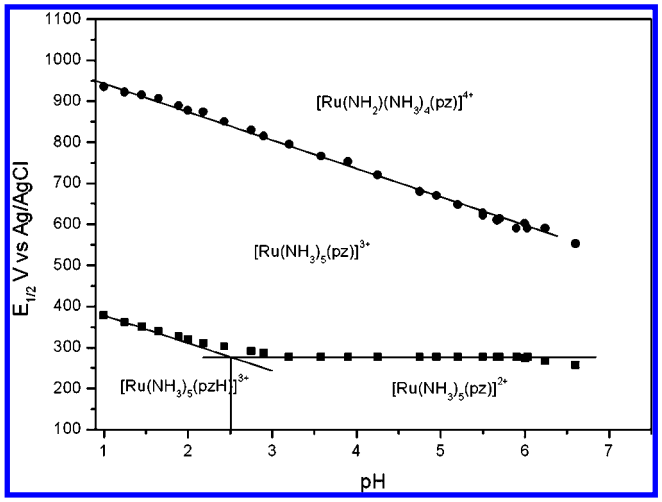


Figure 4. Pourbaix diagram for $Rupz^{2+}$ in 10 mM phosphate solution showing the pH dependencies of the first and second oxidations of $[Ru(NH_3)_5pz]^{2+}$.

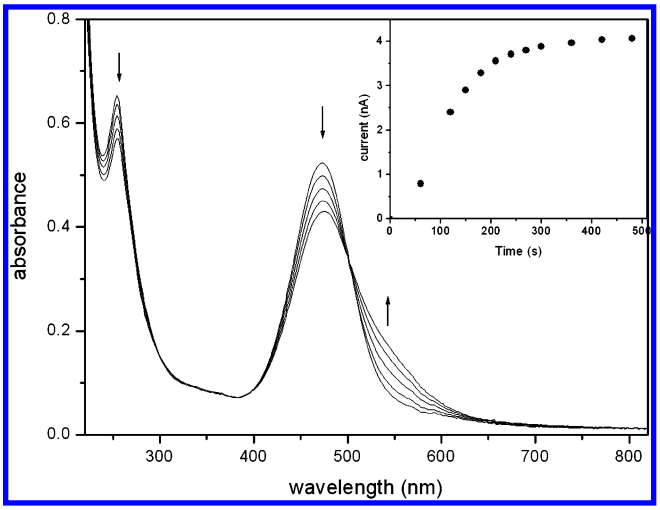


Figure 5. Spectral changes recorded upon continuous 436 nm photolysis of equimolar solutions of $Ru(NH_3)_5pz^{2+}$ and $RuCl(^{15}aneN_4)(NO)^{2+}$ (each 5.0×10^{-5} M) under argon in pH 7.40, 10 mM phosphate buffer solution (ionic strength = 0.11 M) adjusted with KCl. $T = 25.0 \pm 0.1$ °C. Inset: The NO measurement with the NO sensor during photolysis of the complex mixture.

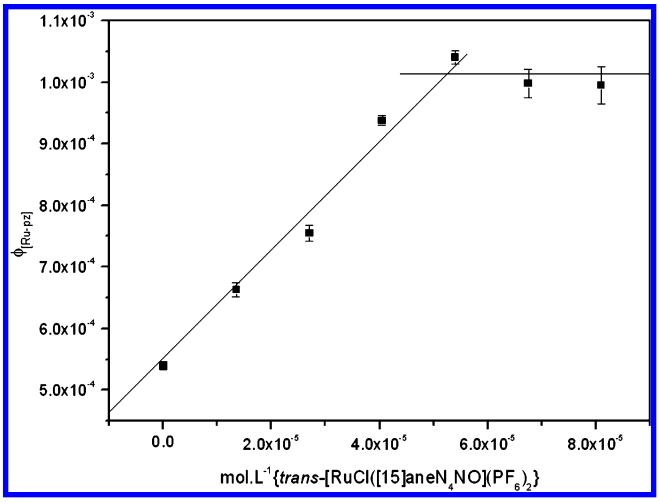


Figure 6. Quantum yields, Φ_d, for beaching of the MLCT band of $Ru(NH_3)_5(pz)^{2+}$ (5×10^{-5} M) upon photolysis in pH 7.40, 10 mM phosphate buffer solution as a function of the concentrations of $RuCl([15]aneN_4)(NO)^{2+}$ (both as the PF_6^- salts). ($\mu = 0.11$ M, adjusted with KCl. $T = 25.0 \pm 0.1$ °C.)

the second oxidation, there is a linear relationship between the $E_{1/2}$ and pH over the range 1.0−6.5. This would be consistent with a proton-coupled electron transfer; for example, the oxidation of the Ru(III) complex $Ru(NH_3)_5(pz)^{3+}$ to the deprotonated Ru(IV) complex $Ru(NH_2)(NH_3)_4(pz)^{3+}$. The chemical reaction subsequent to this step may be pyrazine release accelerated by the amide group.[11] The role of PB solution in facilitating this process may involve hydrogen bonding between a coordinated ammine of $Rupz^{2+}$ and phosphate. When $RuNO^{2+}$ was added to the solution, the second oxidation of $Rupz^{2+}$ was somehow inhibited or shifted to higher potential.

Photochemistry under Continuous Photolysis. These studies were done under an argon atmosphere to avoid other photochemical reactions involving O_2 previously noted for $Rupz^{2+}$.[12] Solutions of $Rupz^{2+}$ alone displayed slow decreases in the strong MLCT absorption upon irradiation at 436 nm in pH 7.4 aqueous phosphate buffer solution (10 mM) with a quantum yield for disappearance (ϕ_d) of $<\sim 5 \times 10^{-4}$ mol/einstein. However, photolysis of an analogous solution of $Rupz^{2+}$ (0.05 mM) that also contained $RuNO^{2+}$ (0.05 mM) demonstrated much larger and faster changes in the electronic spectra, including decreases in the MLCT absorbance at 472 nm and the rise in a new absorption at ∼550 nm with an isosbestic point at 500 nm (Figure 5). There was no reaction when either solution was maintained in the dark.

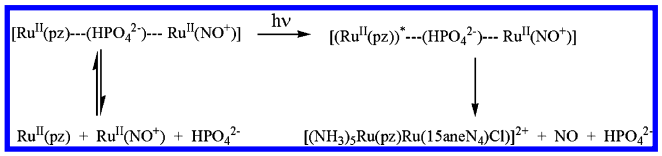
Given the relatively high extinction coefficient necessary to detect significant absorption changes at these concentrations, we attribute the new band at ∼550 nm to a Ru(II) → pyrazine MLCT transition and suggest that the species responsible is the dinuclear, mixed-valent ion $[(NH_3)_5Ru(\mu\text{-pz})Ru(15\text{-ane})Cl]^{2+}$. The latter has been synthesized independently from the reaction of $Ru(NH_3)_5pz^{2+}$ with $Ru(15\text{-ane})(Cl)(H_2O)^{2+}$ and shows a strong MLCT band ($\epsilon = \sim 12.5 \times 10^3$ M^{-1} cm^{-1}) at 550 nm (Figure S4, Supporting Information).

The photolysis-induced absorbance changes noted in Figure 5 were also accompanied by the generation of nitric oxide, as determined by a NO-specific electrode (inset Figure 5). Notably, 436 nm photolysis of $RuNO^{2+}$ alone under otherwise identical conditions showed that NO is not generated in the absence of added $Rupz^{2+}$ as a photosensitizer. The latter result was not surprising, given the absence of significant absorbance in the spectrum of $RuNO^{2+}$ at that wavelength.

The situation was dramatically different in the absence of the phosphate buffer. When the analogous photochemical experiment was performed with an equimolar $Rupz^{2+}/RuNO^{2+}$ solution in 0.1 M KCl with no added phosphate, there were few absorbance changes, even after lengthy photolysis (30 min). In other words, since $Rupz^{2+}$ is the only chromophore with significant absorbance at the 436 nm irradiation wavelength, the photochemistry (or lack thereof) of the complex reverts to that seen in the absence of $RuNO^{2+}$ when phosphate is not present.

The ϕ_d attributed to consumption of $Rupz^{2+}$ was determined from the absorption changes (ΔAbs) at 472 nm. Figure 6 is a plot of the ϕ_d measured for different concentrations of $RuNO^{2+}$. A limiting value of $1.03(11) \times 10^{-3}$ mol einstein^{-1} was achieved when $[RuNO^{2+}]$ and $[Rupz^{2+}]$ were equimolar (0.05 mM). The quantum yield determined for NO production (ϕ_{NO}) using the NO electrode for an analogous equimolar solution of $[RuNO^{2+}]$ and $[Rupz^{2+}]$ is $9.8(3) \times 10^{-4}$ mol einstein^{-1}, that is, within experimental uncertainty of that measured from the ΔAbs values. These results suggest that consumption of $Rupz^{2+}$ is related in one-to-one stoichiometry to the production of NO. A corollary of that observation is that the low-yield photoreaction

SCHEME 1



seen for solutions of $Rupz^{2+}$ alone (ligand aquation) appears to be suppressed by the process leading to NO labilization from $Rupz^{2+}/RuNO^{2+}$ mixtures in phosphate solution.

Taking into account the above data, we propose the photochemical pathway described in Scheme 1 in which phosphate facilitates formation of a supramolecular complex between the dications $Rupz^{2+}$ and $RuNO^{2+}$, probably via hydrogen bonding. The formation constant for this supramolecular species must be large, given the stoichiometric behavior in the photochemistry noted in Figure 6. Excitation of the $Rupz^{2+}$ chromophore of this species leads to NO release, bleaching of the MLCT band of $Rupz^{2+}$, and formation of the pyrazine bridged dimer. We speculate that this is the result of electron transfer from $Rupz^{2+}$ to $RuNO^{2+}$, followed by NO loss, then reaction of the $Ru^{III}pz$ moiety with the $Ru^{II}(15\text{-ane})(Cl)(H_2O)^{2+}$ product of NO dissociation. The MLCT excited state is a very strong reductant with the $[Ru(NH_3)_5pz^{3+}]/[Ru(NH_3)_5pz^{2+}]^*$ potential estimated as -2.13 V vs SHE.[12] By comparison, the reduction potential of $RuNO^{2+}$ is -0.10 V vs SHE; thus, electron transfer from $[Rupz^{2+}]^*$ to $RuNO^{2+}$ would be quite favorable. Furthermore, it is known that reduction of $RuNO^{2+}$ leads to the loss of NO.[4a] Alternatively, energy transfer from $[Rupz^{2+}]^*$ to form a spectroscopically silent, but labile, excited state of $RuNO^{2+}$ would serve the same purpose.

It is notable, especially in the context of possible phototherapeutic applications in living organisms, that the photolysis of the $RuNO^{2+}/Rupz^{2+}$ mixtures in phosphate media under aerated conditions also displayed NO release; however, the ϕ_{NO} in aerated solution (as determined with the NO electrode) was much larger than in deaerated media. We believe that the NO quantum yield increase is associated with production of superoxide, which has been shown to be a photoproduct of the visible light photolysis of $Rupz^{2+}$ in oxygenated medium.[12] This reaction is under further study.

Photophysical Measurements. A recent study from these laboratories reexamined the MLCT excited-state lifetime of $Rupz^{2+}$ by transient absorption measurements using ps flash photolysis methods.[12] In pH 7.4, 10 mM PB solution deaerated with argon, photoexcitation (400 nm) of $Rupz^{2+}$ led to transient bleaching of the MLCT absorption band, followed by complete recovery of the initial spectrum. The lifetime measured from the exponential decay was 112 ($\pm$10) ps.[12,15] Analogous flash photolysis of a $RuNO^{2+}/Rupz^{2+}$ solution (1.0 mM each) under otherwise identical conditions also led to transient bleaching ($\lambda_{max} \sim 490$ nm) of the MLCT band that decayed back to baseline (within the observation limits) (Figure 7). (This is expected, since the quantum yield for permanent photochemistry is small.) Notably, the measured lifetime (132 $\pm$ 11 ps) of the transient bleaching seen for the $RuNO^{2+}/Rupz^{2+}$ solutions at 1:1 and 2:1 ratios was longer than that recorded for $Rupz^{2+}$ alone.[12] Although the difference is but marginally outside the experimental uncertainty, this result was counterintuitive given that a model involving dynamic bimolecular energy or electron transfer from $[Rupz^{2+}]^*$ to $RuNO^{2+}$ would be expected to give a shorter, not longer, lifetime than that of $[Rupz^{2+}]^*$ alone. The longer τ suggests that the species detected by flash photolysis of solutions of $Rupz^{2+}$ alone and of $RuNO^{2+}/Rupz^{2+}$ mixtures are not identical. In the latter case, the longer lifetime may correspond to that of the supramolecular complex between $RuNO^{2+}$ and $Rupz^{2+}$ also suggested by the spectroscopic, electrochemical, and quantum yield patterns seen above.

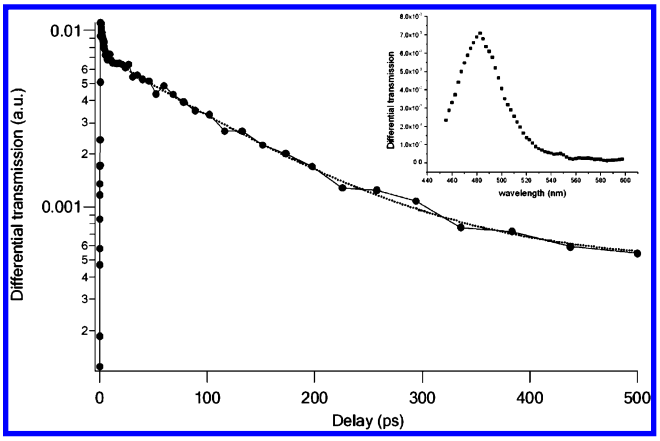


Figure 7. Lifetime measurement of the transient bleaching after excitation at 400 nm with an ultrafast laser. Inset: Transient difference spectrum (transmission mode) recorded by flash photolysis of an equimolar (1 mM) mixture of $Ru(NH_3)_5(pz)^{2+}$ and $RuCl([15]aneN_4)(NO)^{2+}$ (PF_6^- salts) in pH 7.40, 10 mM PB solution under argon.

Photoinduced Production of a Vasodilator. Although the present system leads to photosensitized NO release using visible light, the complexity of using a multicomponent system ($Rupz^{2+}$, $RuNO^{2+}$, *and* phosphate) makes practical application a challenge. In this context, we describe the use of water/oil emulsified gels to encapsulate the ruthenium species with the goal of reducing dissociation of the supramolecular complex. These ruthenium-containing emulsions, prepared as described in the Experimental Section, proved to be stable for at least 60 days according to their UV$-$vis spectra. Photolysis of a solution prepared from 1.0 g of the emulsion containing $Rupz^{2+}$, $RuNO^{2+}$, and phosphate showed NO release (Figure S6 in Supporting Information).

The diffusion of the ruthenium species out of these emulsions was examined using a Franz diffusion cell apparatus (Figure S1), which is a method used to study diffusions of transdermal drug delivery systems.[8] The donor and receptor compartments were separated using a cellulose acetate dialysis membrane (mol wt cutoff 10 000 Da) supported by a stainless steel filter grid. The receptor compartment contained pH 7.4, 10 mM PB solution. The donor compartment was loaded with 1.50 g of emulsion and placed in close contact with the membrane, which was covered with a glass lid to prevent water evaporation. Samples from the receptor phase were removed periodically, and the ruthenium concentration was analyzed using the UV$-$visible spectrum. The release profiles (Figure 8) show that the amount of ruthenium complex released from the emulsion is composition-dependent. After 12 h, the percentage of ruthenium released was 34.8, 10.4, and 14.3% for the $Rupz^{2+}$, $RuNO^{2+}$, and $RuNO^{2+}/Rupz^{2+}$ emulsions, respectively.

For the $RuNO^{2+}/Rupz^{2+}$ system, the spectra were consistent with both complexes' being released into the solution. The release data can be fitted to a zero-order release model,[8a] and the respective flux release rates for the $RuNO^{2+}$, $Rupz^{2+}$ and $RuNO^{2+}/Rupz^{2+}$ systems were calculated as 0.0335 (94), 0.0151 (11) and 0.013 1(15) mg cm^{-2} h^2. Notably, the release rate of $Rupz^{2+}$ is around three times faster than that for $RuNO^{2+}$, perhaps because the latter is more hydrophobic and, thus, has stronger interaction with the emulsion. The significantly slower diffusion rate for $Rupz^{2+}$ when $RuNO^{2+}$ and phosphate are all

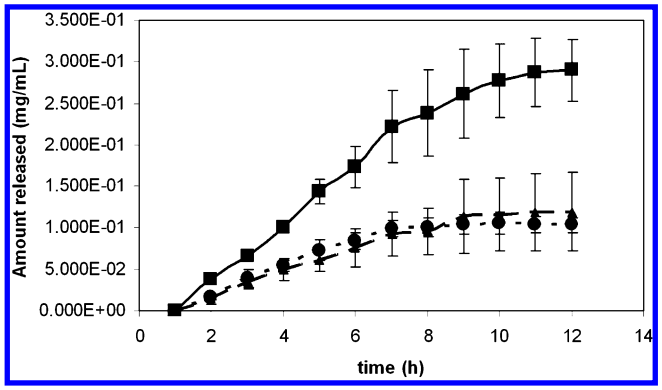


Figure 8. In vitro release profile of $Rupz^{2+}$ (■), $RuNO^{2+}/Rupz^{2+}$ (▲), and $RuNO^{2+}$ (●) from w/o emulsion at 37.0 °C. Data represent mean ± SD, $n = 4$.

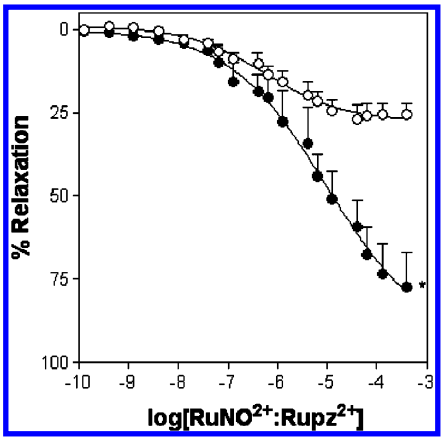


Figure 9. Effects of $RuNO^{2+}/Rupz^{2+}$ on rat thoracic aorta precontracted with phenylephrine without (○, $n = 6$) and with (●, $n = 6$) photolysis. The solution of $RuNO^{2+}/Rupz^{2+}$ in phosphate buffer was cumulatively added (0.1 nM to 400 μM). Data are means ± SEM of n experiments performed on preparations obtained from different animals

present is further support for the phosphate mediated interaction of $RuNO^{2+}$ with $Rupz^{2+}$, as suggested in Scheme 1.

The pharmacological effects on rat aorta dilation of the $RuNO^{2+}/Rupz^{2+}$ system are illustrated in Figure 9. The combination of the two compounds demonstrates concentration-dependent relaxation of denuded rat aortas precontracted with 0.1 μmol/L phenylephrine both in the dark and coupled to 470 nm irradiation. The E_{MAX} for the ruthenium species was 77 ± 10%, $n = 4$, under visible light irradiation, whereas a smaller value, 26 ± 3%, $n = 4$, was recorded in the dark. The latter may have been occasioned by reduction of $RuNO^{2+}$ by phenylephrine or by exposure to ambient light.

Similar vasorelaxation effects were observed for irradiation of the w/o emulsion of the $RuNO^{2+}$, $Rupz^{2+}$ and phosphate (Figure 10). In this context, the emulsion system could be used as an appropriate targeting delivery system, since NO can cross the barrier imposed. The entrapped ruthenium complexes induced an E_{max} of 37 ± 6% for emulsion, although the physiological solution containing both ruthenium species induced an E_{max} of 20 ± 2. The behavior of the $RuNO^{2+}/Rupz^{2+}$ system in the time-course experiments appears less effective than the concentration−effect curves. This can be possibly explained by the fact that the activation of guanylyl-cyclase is more effective in the gradually increasing concentrations of the compounds.[14] Furthermore, when the compound releases NO extracellularly, less NO may be available inside the cell to promote the guanylyl-cyclase activation and aorta relaxation. The maximum vasorelaxation was achieved around 90 min.

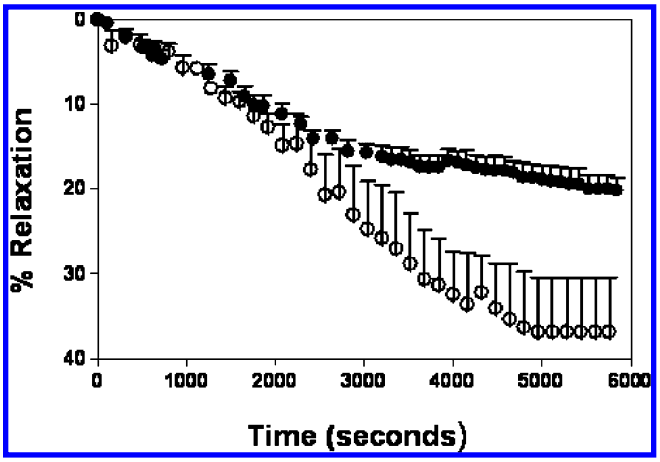


Figure 10. Temporal relaxation response for rat aorta precontracted with phenylephrine (0.1 μmol/L) upon visible light irradiation of the $RuNO^{2+}/Rupz^{2+}$ system (1.0 mM) in solution (●, $n = 4$) and in emulsion (○, $n = 4$). Relaxation responses are expressed as percent reversal of the phenylephrine-induced contraction. Data are means ± SEM of n experiments performed on preparations obtained from different animals.

Considering that only 1.8% of the $RuNO^{2+}/Rupz^{2+}$ system would have been released from the emulsion by this time, we can conclude that the observed vasodilation is the result of photolysis-induced NO release from the complexes entrapped in the emulsion.

Summary

The presence of phosphate apparently mediates the formation of a supramolecular complex between the two ruthenium amine cationic complexes $RuCl(^{15}ane)(NO)^{2+}$ and $Ru(NH_3)_5pz^{2+}$. This was shown by the effects on spectral, electrochemical, and diffusional properties and by the visible light photosensitization of NO release from $RuNO^{2+}$ upon excitation of the $Rupz^{2+}$ chromophore. The NO released from the photolysis of such $RuNO^{2+}/Rupz^{2+}$ systems is sufficient to activate the vasodilation of rat aorta. Furthermore, a system consisting of these complexes in phosphate buffer encapsulated in a pastelike water/oil emulsion has been shown to be an effective proof-of-concept model for immobilizing this complicated system in a manner that allows the photochemical delivery of NO to specific targets.

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Supporting Information Available: Figures S1 and S2 showing the apparatus used for measuring diffusion from a water/oil emulsion and for measuring induced rat aorta vasodilation. Figures S3−S5 showing the electronic spectra of $RuNO^{2+}$, of $RuNO^{2+}$, and $Rupz^{2+}$ together and of dinuclear complex $[Ru^{III}(NH_3)_5(pz)Ru^{II}([15]aneN_4)Cl]^{4+}$ in aqueous solution; and Figure S6 showing the course of photoinduced NO release from $RuNO^{2+}/Rupz^{2+}$ (1×10^{-3} M) entrapped in a matrix. This material is available free of charge via the Internet at http://pubs.acs.org.

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