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Incorporation of a Designed Ruthenium Nitrosyl in PolyHEMA Hydrogel and Light-Activated Delivery of NO to Myoglobin

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A ruthenium nitrosyl with 4-vinylpyridine (4-vpy) as one ligand, namely, $[Ru(Me_2bpb)(NO)(4\text{-vpy})](BF_4)$ (**1**), has been synthesized and structurally characterized. This diamagnetic $\{Ru–NO\}^6$ nitrosyl is photoactive and readily releases NO upon exposure to low-intensity (5–10 mW) UV light (quantum yield at 300 nm = 0.18). Radical-induced copolymerization of 2-hydroxyethyl methacrylate (HEMA) and ethyleneglycol dimethacrylate (EGDMA) in the presence of **1** has afforded a **1**-pHEMA, a transparent hydrogel in which **1** is covalently attached to the polymer backbone. Exposure of **1**-pHEMA to UV light (5–10 mW) results in rapid release of NO (detected by NO electrode) that can be delivered to biological targets such as myoglobin. The photoactivity of **1**-pHEMA is strictly dependent on exposure to UV light.

Introduction

In recent years, nitric oxide (NO) has been shown to play various physiological and pathological roles in a cell- and concentration-dependent manner.[1−3] For example, NO participates in neurotransmission, blood pressure control, and inflammatory responses.[4−6] Although the production of endogenous NO from arginine by the enzyme nitric oxide synthase (NOS) occurs in nanomolar levels in endothelial and neuronal cells, macrophages produce micromolar concentration of NO at infected or malignant sites. The discovery of the biological effects of NO has prompted research in the area of development of exogenous NO donors that can deliver NO at desirable locales for treatment of various diseases.[7−9] Such attempts have afforded novel NO donors such as diazeniumdiolates[10,11] and *S*-nitrosothiols[12] that release NO in cellular environment via enzymatic or pH-dependent processes. However, lack of control on NO release remains the fundamental problem associated with these systemic NO drugs, as well as traditional ones like glyceryl trinitrate and amyl nitrite; the drug goes everywhere in the body, and NO is released through multiple pathways. This inexorability of NO release becomes a major issue in many biomedical applications.

Research to date strongly suggest that the metal nitrosyls (NO complexes) belong to a promising class of NO donor that can provide NO under various conditions including exposure to light of convenient frequency.[13−16] For example, sodium nitroprusside (marketed as Nipride) has been widely used in hospitals to lower blood pressure during hypertensive episodes and congestive heart failure. During the past 4 years,

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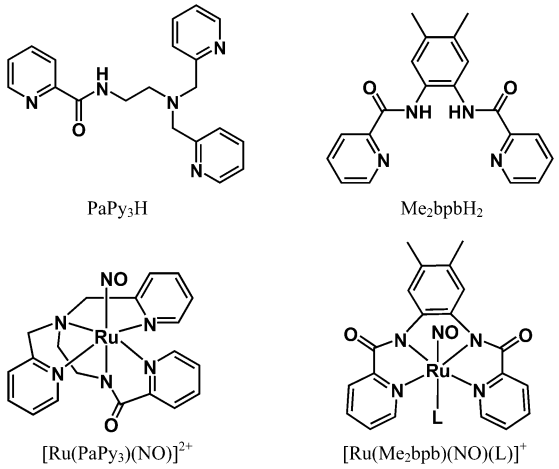

Figure 1. Structures of $PaPy_3H$ and Me_2bpbH_2 ligands (top) and the two ruthenium complexes $[Ru(PaPy_3)(NO)]^{2+}$ and $[Ru(Me_2bpb)(NO)(L)]^+$ (bottom).

we have developed a series of designed metal nitrosyls that rapidly release NO under low intensity (W to mW) illumination. The first generation metal (M = iron, manganese, and ruthenium) nitrosyls with the designed coligand $PaPy_3H$ (*N*,*N*-bis(2-pyridylmethyl)amine-*N*-ethyl-2-pyridine-2-carboxamide, Figure 1) exhibit excellent photolability of NO under low-intensity visible (in case of iron and manganese) and UV (in case of ruthenium) light.[17−20] Although the iron nitrosyl $[Fe(PaPy_3)(NO)](ClO_4)_2$ has limited stability in aqueous medium, both $[MnPaPy_3NO](ClO_4)$ and $[RuPaPy_3NO](BF_4)_2$ are indefinitely stable in water and aqueous buffer. Very recently, we have successfully incorporated $[MnPaPy_3NO](ClO_4)$ within a polyurethane-coated silicate-based sol−gel matrix.[21] This nitrosyl−polymer hybrid rapidly releases NO upon exposure to visible light (quantum yield = 0.25 at 532 nm light, 10 mW).

Poly(2-hydroxyethyl methacrylate) (pHEMA) hydrogels, crosslinked with ethyleneglycol dimethacrylate (EGDMA), have been extensively used in biomedical research because of its optical transparency.[22] We therefore decided to incorporate the nitrosyls developed in our laboratory into pHEMA hydrogel. Out of the three photoactive nitrosyls, only $[RuPaPy_3NO](BF_4)_2$ (Figure 1) survived the radical polymerization step that affords the pHEMA hydrogel. The resulting nitrosyl−pHEMA hydrogel showed efficient photorelease of NO under low-intensity UV light (λ_{max} = 365 nm, 10 mW). Unfortunately, the macroporous structure of this hydrogel allows leakage of $[RuPaPy_3NO](BF_4)_2$ from the polymer matrix, an effect that is highly undesirable because of the toxicity of the Ru-containing photoproducts. While such leakage can be prevented by the application of polyurethane coating to the pHEMA hydrogel, we decided to design a new ruthenium nitrosyl that could be covalently attached to the pHEMA backbone.

A close look at the structure of $[RuPaPy_3NO]^{2+}$ reveals that the Ru center is coordinatively saturated, and hence this nitrosyl has no easy point of attachment to other structures. We therefore selected one of our second generation of ruthenium nitrosyls derived from the tetradentate ligand Me_2bpbH_2 (Figure 1).[23] As shown in Figure 1, the ligand is planar and hence allows isolation of a variety of photoactive nitrosyls of the type $[Ru(Me_2bpb)(NO)(L)]^+$ (L = pyridine or other similar ligand). Since the presence of 4-vinylpyridine at the axial coordination site (trans to the NO) permits incorporation of the nitrosyl into the backbone of the crosslinked pHEMA structure, we have now synthesized the 4-vinylpyridine (4-vpy) analogue $[Ru(Me_2bpb)(NO)(4\text{-vpy})](BF_4)$ (**1**). The vinyl group of the 4-vinylpyridine ligand of **1** gets *covalently attached* to the pHEMA backbone during radical polymerization of the hydrogel. In this paper, we describe the synthesis, structure, and NO photolability of $[Ru(Me_2bpb)(NO)(4\text{-vpy})](BF_4)$ (**1**) and the **1**-pHEMA nitrosyl−hydrogel conjugate. The results of successful transfer of NO from the **1**-pHEMA nitrosyl−hydrogel conjugate to myoglobin is also described. Borovik and co-workers have recently reported covalent attachment of metal−salen (salen = *N*,*N*′-bis(salicylidene)-1,2-ethylenediminato(2−)) complexes in EGDMA-based porous materials 4-vinylbenzyloxy links and the utility of their NO-adducts as NO donors.[24−26]

Experimental Section

Materials. 4,5-Dimethyl-1,2-diaminobenzene, picolinic acid, triphenyl phosphite, HEMA, and EGDMA were purchased from Aldrich Chemical Co. and used without further purification. 2,2′-Azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044) was procured from Wako Pure Chemical Industries Ltd. and used as received. NO gas was purchased from Spectra Gases Inc. and was purified by passing through a long KOH column prior to use. $RuCl_3 \cdot xH_2O$ (Aldrich Chemical Co.) was treated several times with concentrated HCl to prepare the starting metal salt, $RuCl_3 \cdot 3H_2O$. All solvents were purified, dried, or both by standard techniques and distilled. Horse heart myoglobin (Mb) was purchased from Aldrich and used as received. The ligand 1,2-bis(pyridine-2-carboximido)-4,5-dimethylbenzene (Me_2bpbH_2) and the corresponding ruthenium nitrosyl, $[Ru(Me_2bpb)(NO)(Cl)]$, were synthesized by following the published procedures.[23]

Synthesis of $[Ru(Me_2bpb)(NO)(4\text{-vpy})](BF_4)$ (1). A batch of 0.100 g (0.196 mmol) of $[Ru(Me_2bpb)(NO)(Cl)]$ was placed in 25 mL of acetonitrile. To this, a solution of 0.050 g (0.235 mmol) of $AgBF_4$ in 3 mL of acetonitrile was added with stirring, and then the reaction mixture was heated to reflux for 20 h. Next, it was filtered through a cintered glass crucible containing a layer of diatomaceous earth to remove precipitated AgCl. A batch of 0.030 g (0.285 mmol) of 4-vinylpyridine was then added to the deep

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brown filtrate, and the mixture was once again heated to reflux for an additional 12 h. After a second filtration to remove additional precipitated AgCl, the volume of the acetonitrile solution was reduced to 10 mL by short path distillation. The concentrate was left at −20 °C over night. It was filtered one more time to remove trace amount of a dark solid, and toluene was allowed to diffuse into the filtrate. Dark brown needles of $[Ru(Me_2bpb)(NO)(4\text{-vpy})](BF_4)$·toluene (**1**·toluene) were isolated in 64% yield. Anal. Calcd for $C_{34}H_{31}BF_4N_6O_3Ru$ (**1**·toluene): C, 53.76; H, 4.12; N, 11.07. Found: C, 53.46; H, 4.16; N, 10.98. Selected IR frequencies (KBr disk, cm^{-1}): 1872 (ν_{NO}, s), 1836 (vs), 1596 (vs), 1483 (m), 1356 (m), 1083 (s). Electronic absorption spectrum in acetonitrile: λ_{max} 392 (ϵ = 7 800 M^{-1} cm^{-1}), 276 nm (ϵ = 34 000 M^{-1} cm^{-1}). ^1H NMR (500 MHz, CD_3CN, from TMS): δ 9.21 (q, 2H), 8.48 (s, 2H), 8.30 (t, 2H), 8.13 (d, 2H), 8.05 (d, 2H), 7.96 (m, 2H), 7.29 (d, 2H), 6.58 (dd, 1H), 6.04 (d, 1H), 5.62 (d, 1H), 2.35 (s, 6H).

Incorporation of $[Ru(Me_2bpb)(NO)(4\text{-vpy})](BF_4)$ into pHEMA Hydrogel. A batch of 0.020 g (0.060 mmol) of 2,2′-azobis-[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044) was dissolved in 5.7 g (320 mmol) of deionized water by sonication. Next, a batch of 10 g (77 mmol) of HEMA and a batch of 0.50 g (2.5 mmol) of EGDMA were added to this solution sequentially, and the mixture was sonicated thoroughly after each addition. To keep one reference polymer sample without the nitrosyl, an aliquot of 2.7 g of the prepolymer mixture was taken out and placed in a polystyrene cuvette. The remaining mixture was made 0.1 mM with respect to **1** by addition of a solution of **1** in ethanol, and it was put into five cuvettes (2.7 g in each). These prepolymer samples were then thoroughly degassed by bubbling nitrogen through them, followed by sonication. Finally, the cuvettes were placed in an oil bath at 50 °C and heated for 30 min. The hydrogels thus obtained were transparent solids that can be sliced easily with the aid of a razor blade. The blank hydrogel was colorless, while the nitrosyl-containing gels were pale yellow.

Physical Measurements. The apparent rate of NO release (loss) from **1**-pHEMA nitrosyl−hydrogel conjugate was studied by electronic absorption spectroscopy, while the quantum yield was determined by standard ferrioxalate actinometry. An Oriel Apex monochromator illuminator (150 W xenon lamp) with an Oriel 1/8 m Cornerstone monochromator was used as the light source. Absorption spectra were recorded on a Cary 50 Varian spectrophotometer. Infrared spectra were obtained with a Perkin-Elmer 1600 FTIR spectrophotometer. Electron paramagnetic resonance (EPR) spectra were monitored on a Bruker ELEXSYS 500 spectrometer. A Varian 500 MHz spectrometer was employed to record the ^1H NMR spectra at 298 K. The NO amperogram was recorded with an amiNO-2000 electrode (part of an inNO nitric oxide measuring system, Innovative Instruments, Inc.). Diffraction data were collected on a Bruker SMART 1000 system. Mo Kα (0.71073 Å) radiation was used, and the data were corrected for absorption. The structure was solved by direct methods (standard SHELXS-97 package).

Delivery of NO to Myoglobin. Horse heart myoblobin (Mb) was dissolved in phosphate buffer (20 mM, pH 7) and reduced with sodium dithionite (λ_{max} moved to 434 nm from 410 nm) in the presence of two disks of 1-pHEMA nitrosyl−hydrogel conjugate. The solution of the reduced Mb-containing polymer disks was then exposed to UV light (λ_{max} = 300 nm, 5 mW). The spectrum of Mb−NO (λ_{max} = 420 nm) appeared within 2 s. The NO transfer was complete within 5 s. No change in the spectrum of the reduced Mb was observed in absence of the UV light (over a period of 2 h).

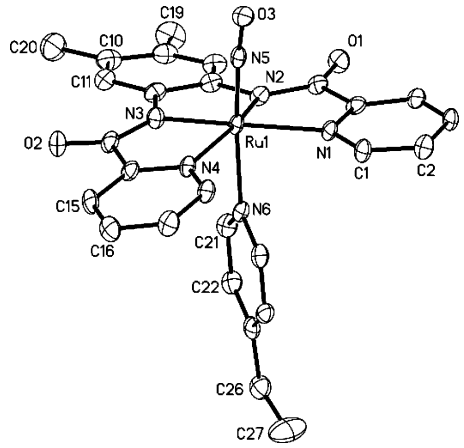

Figure 2. Thermal ellipsoid (probability level 50%) plot of $[Ru(Me_2bpb)(NO)(4\text{-vpy})]^+$ (cation of **1**). H atoms are omitted for the sake of clarity.

Table 1. Summary of Crystal Data and Intensity Collection and Structural Refinement Parameters for $[Ru(Me_2bpb)(NO)(4\text{-vpy})](BF_4)$·toluene (**1**·Toluene)

formula	$C_{34}H_{31}BF_4N_6O_3Ru$
mol wt	759.53
cryst color, habit	brown needle
T (K)	90(2)
cryst syst	triclinic
space group	$P\bar{1}$
a (Å)	7.9672(13)
b (Å)	14.871(2)
c (Å)	15.172(3)
α (deg)	112.833(2)
β (deg)	100.060(2)
γ (deg)	90.316(2)
V (Å^3)	1625.9(5)
Z	2
d_{cacld} (g cm^{-3})	1.551
abs coeff (μ, mm^{-1})	0.551
GOF[a] on F^2	1.054
R_1[b] (%)	4.64
R_w[c] (%)	12.02

[a] GOF = $\{[\sum w(F_0^2 - F_c^2)^2]/(M - N)\}^{1/2}$ (M = no. of reflections, N = no. of parameters refined). [b] $R_1 = \sum||F_0| - |F_c||/\sum|F_0|$. [c] $R_w = \{[\sum w(F_0^2 - F_c^2)^2]/[\sum w(F_0)^2]\}^{1/2}$.

Results and Discussion

Structure of $[Ru(Me_2bpb)(NO)(4\text{-vpy})](BF_4)$ (1). The structure of $[Ru(Me_2bpb)(NO)(4\text{-vpy})]^+$ (cation of **1**) is shown in Figure 2. The short the Ru−N(O) bond distance (1.765(3) Å) and nearly linear Ru−N−O bond angle (172.2(3)°) are very similar to those observed with the $\{Ru-NO\}^6$ nitrosyl $[RuMe_2bpb(NO)(py)](BF_4)$ (1.758(2) Å, 170.0(2)°) reported by us in a previous account.[23,27] The orientation and accessibility of the vinyl group of the axial 4-vpy ligand, clearly noticeable in Figure 2, confirms that the nitrosyl could be easily attached to the pHEMA framework (vide infra).

Properties of $[Ru(Me_2bpb)(NO)(4\text{-vpy})](BF_4)$ (1). Coordination of the carboxamido nitrogen to the Ru center in **1** is evidenced by the N−O stretching frequency (ν_{NO}) at 1872 cm^{-1}.[20,23] The dark brown complex is stable in air and shows no sign of decomposition under visible light. The clean ^1H NMR spectrum of **1** in CD_3CN (Figure S1, Supporting

(27) The $\{M-NO\}^n$ notation used in this paper is that of Feltham and Enemark. See Enemark, J. H.; Feltham, R. D. *Coord. Chem. Rev.* **1974**, *13*, 339−406.

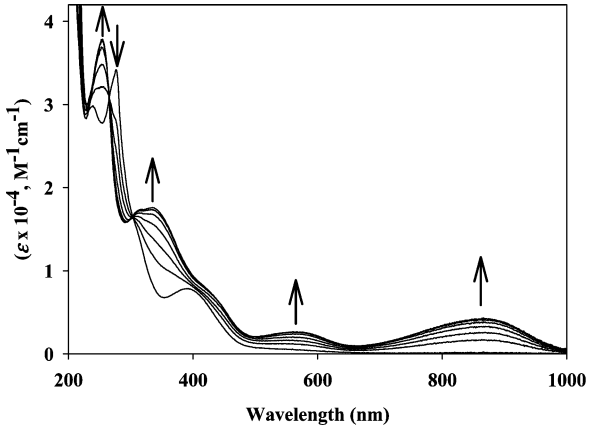

Figure 3. Changes in the electronic absorption spectrum of **1** in acetonitrile upon exposure to 10 mW UV light (360 nm)

Table 2. Selected Bond Distances (Å) and Angles (deg) of **1**·Toluene

Ru−1	2.132(3)	Ru−N2	1.989(3)
Ru−N3	1.988(3)	Ru−N4	2.129(3)
Ru−N5	1.756(3)	Ru−N6	2.135(3)
C6−O1	1.223(4)	C13−O2	1.235(5)
N5−O3	1.149(4)		
N1−Ru−N2	80.26(12)	N1−Ru−N3	162.48(12)
N1−Ru−N4	115.16(12)	N1−Ru−N5	89.40(12)
N1−Ru−N6	85.37(11)	N2−Ru−N3	83.06(12)
N2−Ru−N4	160.84(12)	N2−Ru−N5	98.78(13)
N2−Ru−N6	87.59(12)	N3−Ru−N4	80.23(12)
N3−Ru−N5	98.47(13)	N3−Ru−N6	88.60(12)
N4−Ru−N5	92.93(12)	N4−Ru−N6	82.70(11)
N5−Ru−N6	170.97(12)	Ru−N5−O3	172.2(3)

Information) confirms the $S = 0$ ground state of this {Ru−NO}^{6} nitrosyl.[20,23]

Complex **1** dissolves in water and aprotic solvents such as acetonitrile. In acetonitrile, **1** exhibits its absorption band at 392 nm ($\epsilon = 7800$ M^{-1} cm^{-1}), which resembles that of [Ru(Me$_2$bpb)(NO)(py)]$^+$ (394 nm, $\epsilon = 7200$ M^{-1} cm^{-1}) very closely. This absorption maximum is blue shifted in water (from 392 to 380 nm). Ford and co-workers have assigned this band in similar {Ru−NO}^{6} nitrosyls as d_π(Ru)−π^*(NO) metal-to-ligand charge transfer (MLCT) band.[28] Previous work by us and others have demonstrated that illumination of such {Ru−NO}^{6} nitrosyls with UV light (300−450 nm) results in release of NO and formation of [Ru^{III}(ligand)(solvent)]$^{n+}$ photoproduct.[20,23,28,29] Quite in line with behavior, the solution of **1** in acetonitrile (or water) rapidly release NO when exposed to low-intensity UV light ($\lambda_{max} = 360$ nm, 10 mW). As shown in Figure 3, exposure of a solution of **1** in acetonitrile to UV light results in blue shift of the 392 nm band to 325 nm and generation of new bands with λ_{max} at 570 and 870 nm. Very similar spectral changes have been noted during photolysis of [Ru(Me$_2$bpb)(NO)(py)](BF$_4$)[23] and [Ru(salen)(NO)(Cl)].[28] In all cases, the broad low-energy band in the 700−900 nm range arises from ligand-to-Ru^{III} charge-transfer transition (LMCT).[23,28,30] The

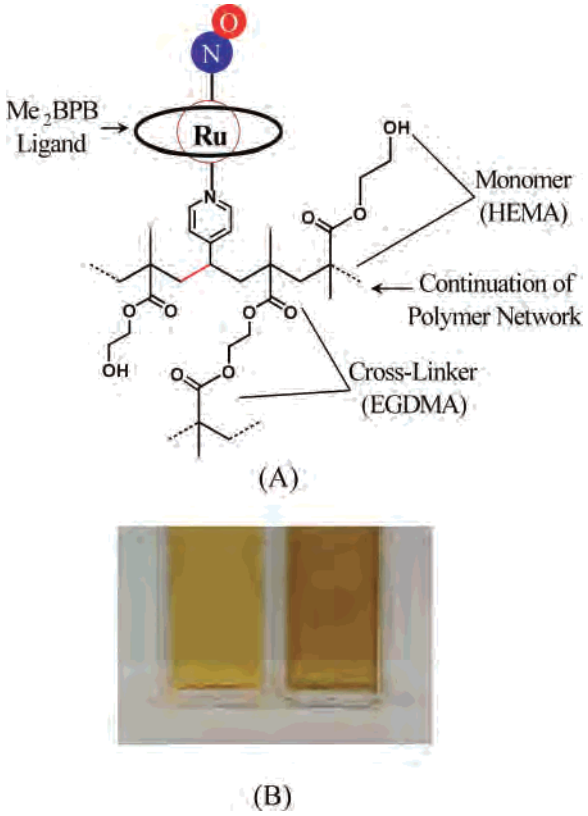

Figure 4. (A) Schematic representation of the covalent attachment of **1** in pHEMA matrix and (B) photographs of **1**-pHEMA hydrogel before (left) and after (right) photolysis.

clean conversion of the {Ru−NO}^{6} nitrosyl **1** to its photoproduct [Ru^{III}(Me$_2$bpb)(4-vpy)(MeCN)]$^+$ is indicated by isosbestic points at 303 and 266 nm in Figure 3.

1-pHEMA Hydrogel and its Properties. The nitrosyl-containing hydrogel **1**-pHEMA has been synthesized by introduction of small amounts of the nitrosyl during the radical polymerization process involving HEMA and EGDMA (crosslinking agent). The pale-yellow hydrogels (Figure 4) are transparent and rigid enough to be shaped and cut. When slices of **1**-pHEMA are kept immersed in water, *no leakage* of **1** is noted (detected by mass spectrum of the aqueous medium) even after 72 h. This confirms covalent attachment of the vinyl end of the 4-vpy ligand into the polymer backbone of **1**-pHEMA (Figure 4). The hydrogel is stable for weeks when kept moist at 4 °C.

The electronic spectrum of **1**-pHEMA resembles that of **1** in ethanol very closely (Figure 5) and attests to the fact that **1** retains its structural integrity during the copolymerization process. The slight shift of the λ_{max} from 397 (**1** in ethanol) to 390 nm presumably arises from the surrounding hydrogel environment. When **1**-pHEMA is exposed to UV light (360 nm, 10 mW), this band moves from 390 to 335 nm, and a new broad band of moderate intensity appears with λ_{max} at 760 nm. As shown in Figure 3, loss of NO from **1** gives rise to such changes in the electronic absorption spectrum of **1**.

It is therefore evident that exposure to UV light promotes photorelease of NO from **1** incorporated in the hydrogel. The photoreleased NO can be easily detected by a NO electrode

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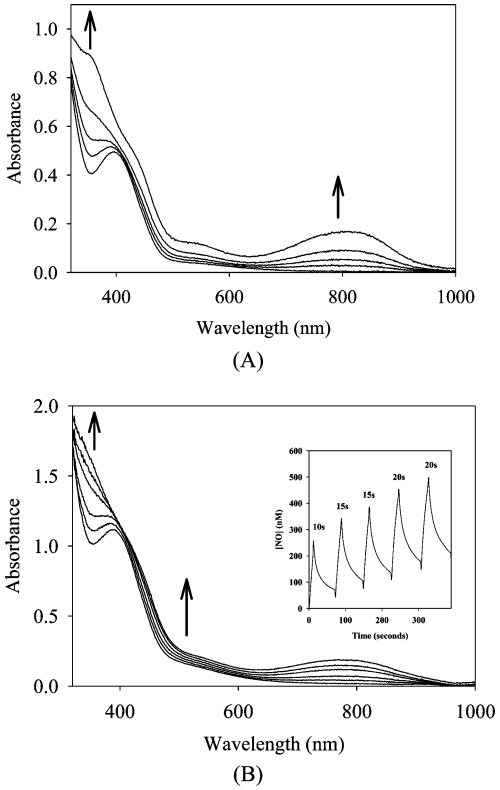

Figure 5. Changes in the electronic spectrum of (A) **1** in ethanol and (B) **1**-pHEMA hydrogel upon exposure to 10 mW UV light (350 nm). Inset of B: NO amperogram of **1**-pHEMA hydrogel.

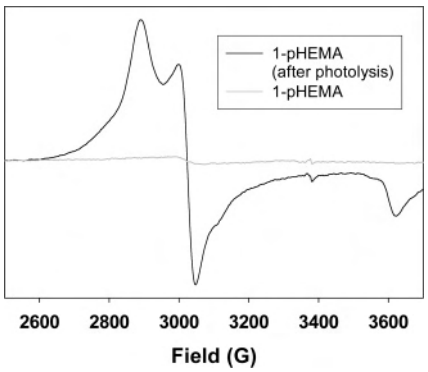

Figure 6. X-band EPR spectrum (120 K) of **1**-pHEMA hydrogel before and after exposure to 10 mW UV light (350 nm). Spectrometer settings: microwave power, 50 mW; microwave frequency, 9.49 GHz; modulation frequency, 100 kHz; modulation amplitude, 2 G.

(a typical NO amperogram is shown in the inset of Figure 5). The amount of NO is proportional to the exposure time. Since **1** is covalently attached to the polymer backbone in **1**-pHEMA and only NO diffuses out of the macroporous hydrogel, it is very reasonable to expect that such material could be used in photodelivery of NO to biological targets. Such delivery will be devoid of any toxicity arising from the photoproducts because of containment of all Ru-containing species inside the polymer framework. Indeed, the EPR spectrum of the photolyzed hydrogel confirms confinement of the Ru(III) photoproduct $[Ru^{III}(Me_2bpb)(4\text{-}vpy)(solv)]^+$ (Figure 6) in all the photolysis experiments. Also, no leaching of any ruthenium complex out of the

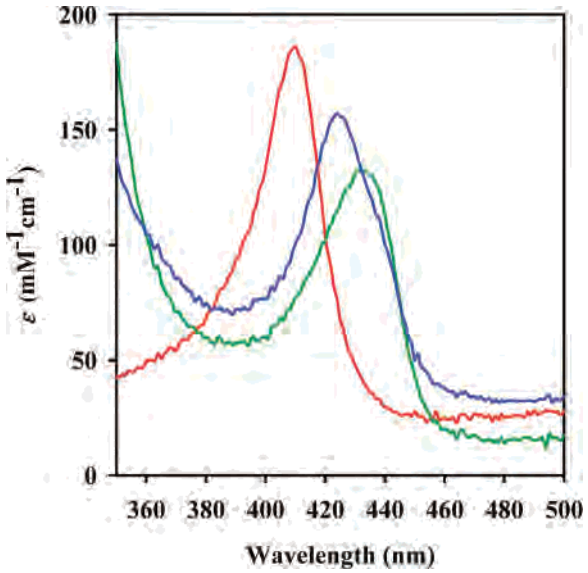

Figure 7. Transfer of NO from **1**-pHEMA hydrogel to reduced Mb in phosphate buffer upon exposure to low-intensity UV light (300 nm, 5 mW): red trace, Met−Mb; green trace, a mixture of reduced Mb and two slices of **1**-pHEMA (and a slight excess of sodium dithionite) under room light (kept for 30 min); blue trace, the same mixture upon exposure to UV light for 5 s.

photolyzed hydrogel in water has been observed even after 72 h (checked by mass spectrometry).

The apparent rate of NO loss from **1**-HEMA gel ($8.5 \pm 0.2 \times 10^{-4}$ s^{-1}) approaches that of **1** in ethanol ($1.2 \pm 0.2 \times 10^{-3}$ s^{-1}) under identical conditions (360 nm UV light, 10 mW, starting absorbance value of 0.8). This result verifies that covalent attachment of **1** in pHEMA matrix does not alter its efficiency of photorelease of NO to a great extent. Results of quantum yield measurements also support this fact. For example, the quantum yields of **1** (solution in ethanol) and **1**-pHEMA at 300 nm (ϕ_{300}) are 0.18 and 0.11, respectively. The high quantum yield value of the **1**-pHEMA hydrogel is quite noteworthy.

To date, at least two other groups have reported photorelease of NO from ruthenium nitrosyls encapsulated in sol−gel matrices. Bordini et al. have reported a brownish-red vitreous material **SG-RuNO** that contains ~50 micromoles of $[Ru(salen)(H_2O)(NO)]^+$ per gram of the material.[31] Upon exposure to a 150 W Xe lamp, **SG-RuNO** *slowly* releases NO (over 3 h), a step that can be reversed by reacting the photoproduct **SG-Ru** with $NaNO_2$. Borovik and co-workers have synthesized a highly cross-linked porous material P-**1**[Ru(NO)(Cl)] by copolymerizing [Ru(salen-4-vbno)(NO)(Cl)] (salen-4-vbno = salen ligand with tethered 4-vbno units[26]) with EGDMA.[25] This material exhibits an absorbance band with λ_{max} at 373 nm (in toluene). Photolysis of suspension of P-**1**[Ru(NO)(Cl)] in toluene with 370 nm light results in release of NO, and the photoproduct P-**1**[Ru(Cl)] exhibits its characteristic bands at 400 (shoulder) and 660 nm. Although no quantum yield value has been reported for P-**1**[Ru(Cl)], results of photolysis experiments and photolytic transfer of NO to $[Co^{II}(TPP)]$ in toluene indicate that P-**1**[Ru-

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(Cl)] releases NO on the order of minutes under 370 nm light (25 W). Taken together, the comparison of photolysis data of **SG-RuNO**, P-**1**[Ru(NO)(Cl)], and **1**-pHEMA indicate that the **1**-pHEMA hydrogel is clearly a superior NO-donor that operates efficiently under low-intensity (5−10 mW) UV light.

NO Delivery to Myoglobin. The NO amperogram (Figure 5B), obtained upon exposure of **1**-pHEMA to light under *aerobic* conditions, strongly suggests that the photoreleased NO does exist in solution for significant periods of time (for tens of seconds). In cellular microenvironment, the half-lifetime of NO has been reported to lie in the range of 1−500 s.[2] It is therefore reasonable to expect that the photoreleased NO from **1**-pHEMA can be delivered to biological targets such as proteins via light triggering. This has been confirmed in the present work. When two thin slices of **1**-pHEMA hydrogel (5 mm x 5 mm x 3 mm) were placed in a solution of reduced horse heart myoglobin (Mb) in phosphate buffer (20 mM, pH 7) under room light, no change in its electronic absorption spectrum was noted. However, when the mixture was exposed to low-intensity UV light (300 nm, 5 mW), the 420 nm Soret band of Mb-NO reached its maximum intensity (100% transfer) within 5 s (Figure 7, blue trace).[20,32,33] This result confirms that **1**-pHEMA can donate NO to Mb quite readily. In comparison, P-**1**[Ru(NO)(Cl)], reported by Borovik and co-workers, transfers NO to Mb (81% transfer) within 20 min under similar conditions (pH 7.2 buffer, 370 nm light).[25] We believe that this difference arises from the highly cross-linked nature of P-**1**[Ru(NO)(Cl)] compared to **1**-pHEMA (5% cross-linked).

Summary and Conclusions

The $\{Ru-NO\}^6$ nitrosyl $[Ru(Me_2bpb)(NO)(4\text{-vpy})](BF_4)$ (**1**) has been covalently attached to HEMA-based hydrogel via radical-induced copolymerization process. The transparent **1**-pHEMA nitrosyl−hydrogel conjugate readily releases NO upon exposure to low-intensity UV light (5−10 mW). Since **1**-pHEMA can transfer NO to proteins like myoglobin quite readily, it is reasonable to expect that this material could be employed in the cellular environment for light-triggered NO delivery. Meyerhoff and co-workers have reported a HEMA-based NO donor system, namely, Cu^{II}−cyclen−pHEMA (cyclen = 1,4,7,10-tetraazacyclododecane), that relies on NO release from RSNO catalyzed by Cu^{II}−cyclen imbedded in the polymer matrix.[34] This material catalytically generates NO from low-molecular-weight RSNOs (such as *S*-nitrosoglutathione) when in contact with blood and is suited for thrombo-resistant blood-contacting material for biomedical applications. However, the process of NO generation by Cu^{II}−cyclen−pHEMA is quite spontaneous and can be only controlled by the contact time. In contrast, **1**-pHEMA can deliver NO at selected locales only when triggered by UV light and hence is more controllable.

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Supporting Information Available: ^{1}H NMR spectrum of **1** (Figure S1) and X-ray crystallographic data (in CIF format) for **1**·toluene. This material is available free of charge via the Internet at http://pubs.acs.org.

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