Photochemical release of nitric oxide from a regenerable, sol-gel encapsulated Ru–salen–nitrosyl complex

Jeane Bordini,[a] Peter C. Ford[b] and Elia Tfouni*[a]

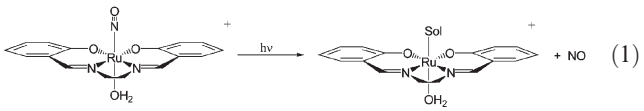
Received (in Cambridge, UK) 24th May 2005, Accepted 28th June 2005
First published as an Advance Article on the web 25th July 2005
DOI: 10.1039/b507407j

Light actviation leads to release of NO from a silicate sol-gel material SG-RuNO prepared from the ruthenium complex, $[Ru(salen)(OH_2)(NO)]^+$ (salen = *N,N′*-bis-(salicylidene)ethylenediaminato); after photochemical NO photolabilization, SG-RuNO can be regenerated from the spent material *via* the subsequent reaction with aqueous nitrite.

Nitric oxide (NO) is generated endogenously in mammals and plays key roles in blood pressure regulation, in the nervous system and in immune response to pathogens.[1] Furthermore, NO over- or under-production accompanies numerous pathological states,[2] and there is continuing interest in therapeutic applications of NO scavengers and donors.[3] Described here is the photochemical release of nitric oxide from a ruthenium nitrosyl complex, $[Ru(salen)(OH_2)(NO)]^+$ (**1**, salen = *N,N′*-bis-(salicylidene)ethylenediaminato), impregnated into a silicate sol-gel (**SG-RuNO**). Such materials have optical transparency, stability and porosity and may be used in association with optical fibers to provide the opportunity for controlled NO release at specific target sites using laser photoexcitation.[4] We also report that after NO photolabilization, **SG-RuNO** can be regenerated by reaction of the NO depleted material **SG-Ru** with aqueous NO_2^-, either directly or after reduction with Eu^{2+}.

Although other materials have been reported to be precursors for NO release,[5,6] the unique feature of the present system is that the photochemical NO release gives an encapsulated transition metal complex that can be regenerated by the reaction with nitrite. Such regeneration is especially relevant in the context that NO_2^- is the largest pool of NO_x species in the body that may serve as a potential source of NO in mammalian systems.[7] Thus, if one can access bio-available NO and use photochemical strategies to deliver this to the target upon demand, the procedure could have valuable therapeutic applications. The system described here provides a proof-of-concept demonstration of this strategy.

We have previously demonstrated the photolability of ruthenium salen nitrosyl complexes [Ru(salen-Y)(X)(NO)] (X = Cl^- or ONO, salen-Y = various substituted salen ligands or analogs) in solution phase studies (*e.g.*, eqn. (1)).[8,9] Related systems were exploited in subsequent studies by Borovik *et al*,[6] who immobilized the ruthenium nitrosyl complexes by modifying the salen ligand to allow for copolymerization with ethyleneglycol dimethylacrylate. In our current studies we accomplish this task by entrapping the soluble complex $[Ru(salen)(OH_2)(NO)]^+$ in a porous silicate sol-gel to prepare an immobilized but regenerable NO source that can be activated by light.



(1)

SG-RuNO was prepared by mixing a solution of tetraethoxysilane (2.5 mL), water (0.4 mL) and 0.1 mol L^{-1} hydrochloric acid (0.6 mL) for 5 min. To this mixture was added a solution of $[Ru(salen)(OH_2)(NO)](NO_3)$ (22 mg, 4.6×10^{-5} mol) in 4 mL acetonitrile.[10] The resulting mixture reacted to form a gel, was cured, dried, and aged at room temperature under vacuum for 5 days, with a load of 24.5 mg (51.3 μmol) of complex per gram of material. The brownish-red vitreous material was then washed with dimethylformamide to remove surface adsorbed complexes. The incorporation of **1** into the sol-gel matrix was confirmed by the FTIR spectra of pulverized **SG-RuNO** in KBr pellets, which displayed a broad ν_{NO} band at 1856 cm^{-1} (Fig. 1a) shifted ~20 cm^{-1} from that of $[Ru(salen)(OH_2)(NO)](NO_3)$ (1836 cm^{-1} in KBr).

When vitreous **SG-RuNO** was irradiated with light from a 150 W xenon lamp (IR filtered), the ν_{NO} band at 1856 cm^{-1} decreased (Fig. 1), consistent with photochemical labilization of the coordinated NO. (This behavior parallels the IR spectral changes seen for the photolysis of 10^{-4} mol L^{-1} aq. $Ru(salen)(OH_2)(NO)](NO_3)$ in pH 7 phosphate buffer solution recorded with a MB Bomem 102 FTIR spectrometer using a ZnSe ATR crystal.) Irradiation of the solid for 3 h leads to a color

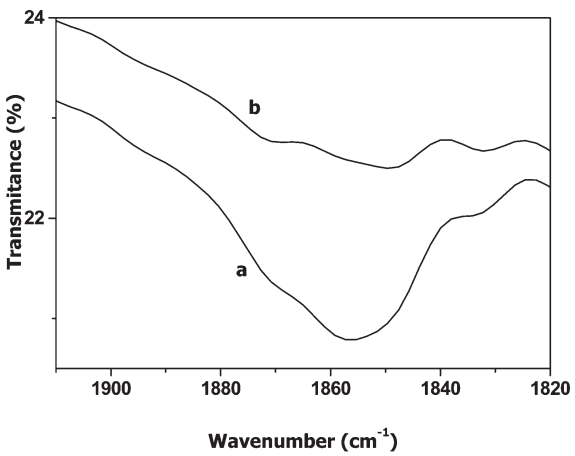


Fig. 1 Infrared transmittance spectral changes, in the ν_{NO} region, of solid SG-RuNO: (a) before photolysis, (b) after 3 h irradiation.

[a]*Departamento de Química - Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes, 3900, 14040-901, São Paulo, Brazil. E-mail: eltfouni@usp.br*
[b]*Department of Chemistry and Biochemistry, University of California, Santa Barbara, Santa Barbara, CA, 93106-9510, USA*

change from brownish-red to green (Fig. 2, top) corresponding to a broad absorption increase in the 500–700 nm region of the optical spectrum (Fig. 2, bottom). As we have demonstrated previously,[8,9] these spectral changes indicate the formation of a Ru^{III}–salen photoproduct. NO photolabilization from solutions of $[Ru(salen)(OH_2)(NO)]^+$ and other [Ru(salen)(X)(NO)] complexes gives the respective solvento species [Ru(salen)(X)(sol)] which display such visible range absorptions.

The photolabilization of nitric oxide from **SG-RuNO** was quantitatively confirmed by trapping the NO. A 96 mg sample of the "dry" solid **SG-RuNO** was placed in the cuvette and a solution (3 mL) of $[Ru(Hedta)(OH_2)]$ (7.4×10^{-5} mol L^{-1}) was added into a glass reservoir connected to the cuvette under an argon atmosphere. $[Ru(Hedta)(OH_2)]$ reacts with NO to form [Ru(Hedta)(NO)], which shows a characteristic absorption band at 250 nm.[11] Upon 3 h photolysis of the **SG-RuNO** in the cuvette, the absorption change in the $[Ru(Hedta)(OH_2)]$ solution corresponded to the trapping of 25 nmol of NO as [Ru(Hedta)(NO)].

The NO depleted sol-gel material **SG-Ru** can be converted back to the nitrosyl form **SG-RuNO** by reaction with nitrite. Regeneration was accomplished by reaction of **SG-Ru** with a 5.0×10^{-2} mol L^{-1}, pH 1 ($HClO_4$), aqueous solution of the reducing agent Eu^{2+}, followed by immersion of the product in a 1.0 mol L^{-1} sodium nitrite solution (*ca.* 1 day). Alternatively, this was also achieved, albeit more slowly (*ca.* one week), by using 1.0 mol $\cdot L^{-1}$ sodium nitrite solution only. The regeneration of **SG-RuNO** was confirmed by observing the reappearance of its

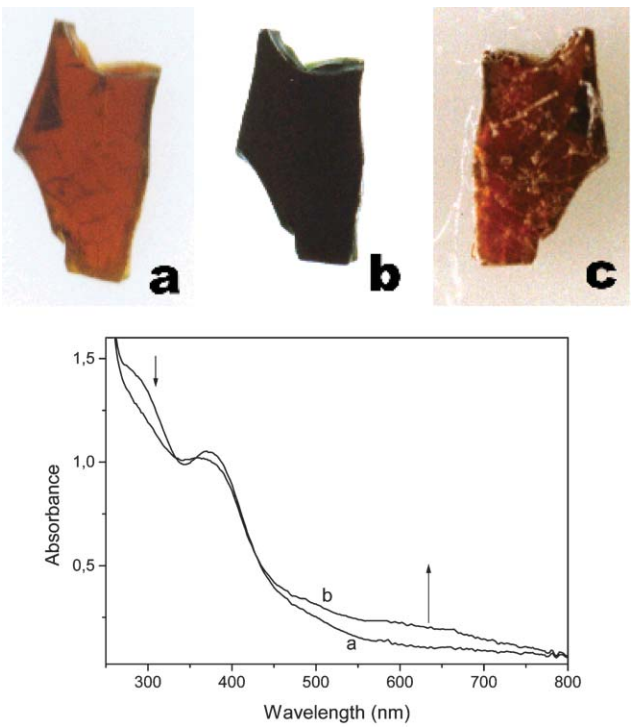


Fig. 2 Top: Sol-gel samples (dimensions: $5.0 \times 3.0 \times 0.5$ mm) (a) **SG-RuNO** (brownish-red), (b) **SG-Ru** formed by photolysis of **SG-RuNO** (green) and (c) **SG-RuNO** after regeneration (brownish-red). Bottom: UV-visible absorbance spectra of analogous sol-gels taken by grinding the material and dispersing in CCl_4. (a) **SG-RuNO** before irradiation and (b) **SG-Ru** after irradiation.

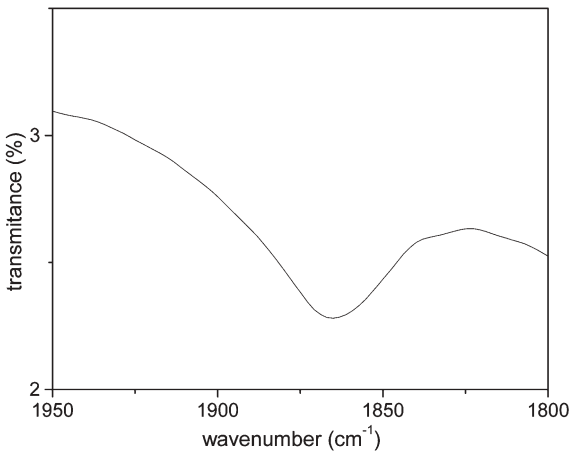


Fig. 3 Infrared spectrum of photolysed SG-RuNO after treatment with nitrite at pH 7.

characteristic red-brown color (Fig. 2, top) and of the ν_{NO} stretching band at 1856 cm^{-1} in the FTIR spectrum (Fig. 3).

Experiments to improve the NO release and regeneration of the material, including conditions closer to the physiological nitrite concentration (0.1–10 μmol L^{-1}),[12] the number of cycles for the process, as well as studies on the material porosity and brittleness, are currently underway and will be reported later.

In summary, we have shown that the ruthenium nitrosyl complex $[Ru(salen)(OH_2)(NO)]^+$ can be incorporated into a silica sol-gel to give a stable new material **SG-RuNO**. Irradiation of this material with visible light leads to the photochemical release of nitric oxide as evidenced by changes in the spectrum of the solid as well as by trapping the released NO. Furthermore, the resulting material can be converted back to **SG-RuNO** by reduction followed by reaction with NO_2^-. Thus, this system has the potential to serve as a model for a regenerable precursor for photochemical NO delivery to various biological targets once implanted in the appropriate location. Furthermore, such regeneration can be accomplished using NO_2^-, the most common form of bioavailable NO_x equivalents.

ET and JB thank the Brazilian agencies CAPES, CNPq and FAPESP and PCF thanks the US National Science Foundation (CHE-0352650) for financial support.

Notes and references

1 *Nitric Oxide: Biology and Pathobiology*, ed. L. J. Ignarro, Academic Press, San Diego, 2000.
2 *Nitric Oxide and Infection*, ed. F. C. Fang, Kluwer Academic/Plenum Publishers, New York, 1999.
3 (*a*) E. Tfouni, M. Krieger, B. R. McGarvey and D. W. Franco, *Coord. Chem. Rev.*, 2003, **236**, 57–69; (*b*) A. K. Patra, J. M. Rowland, D. S. Marlin, E. Bill, M. M. Olmstead and P. K. Mascharak, *Inorg. Chem.*, 2003, **42**, 6812; (*c*) P. G. Wang, M. Xian, X. P. Tang, X. J. Wu, Z. Wen, T. W. Cai and A. J. Janczuk, *Chem. Rev.*, 2002, **102**, 1091; (*d*) P. C. Ford and I. M. Lorkovic, *Chem. Rev.*, 2002, **102**, 993; (*e*) F. G. Marcondes, A. A. Ferro, A. Souza-Torsoni, M. Sumitani, M. J. Clarke, D. W. Franco, E. Tfouni and M. H. Krieger, *Life Sci.*, 2002, **70**, 2735; (*f*) B. F. de Barros, J. C. Toledo, D. W. Franco, E. Tfouni and M. H. Krieger, *Nitric Oxide*, 2002, **7**, 50; (*g*) A. S. Torsoni, B. F. de Barros, J. C. Toledo, M. Haun, M. H. Krieger, E. Tfouni and D. W. Franco, *Nitric Oxide*, 2000, **39**, 247; (*h*) D. R. Lang, J. A. Davis, L. G. F. Lopes, A. A. Ferro, L. C. G. Vasconcellos, D. W. Franco, E. Tfouni, A. Wieraszko and M. J. Clarke, *Inorg. Chem.*, 2000, **39**, 2294;

(*i*) S. P. Fricker, E. Slade, N. A. Powell, O. J. Vaughan, G. R. Henderson, B. A. Murrer, I. L. Megson, S. R. Bisland and F. N. Flitney, *Br. J. Pharmacol.*, 1997, **122**, 1441; (*j*) Y. Chen and R. E. Shepherd, *J. Inorg. Biochem.*, 1997, **54**, 183; (*k*) J. Bourassa, W. DeGraaf, S. Kudo, D. A. Wink, J. B. Mitchell and P. C. Ford, *J. Am. Chem. Soc.*, 1997, **119**, 2853–2860.

4 (*a*) G. Stochel, A. Wanat, E. Kulis and Z. Stasicka, *Coord. Chem. Rev.*, 1998, **171**, 203–220; (*b*) P. C. Ford, J. Bourassa, K. M. Miranda, B. Lee, I. M. Lorkovic, S. Boggs, S. Kudo and L. Laverman, *Coord. Chem. Rev.*, 1998, **171**, 185–202.

5 (*a*) L. K. Keefer, *Nat. Mater.*, 2003, **2**, 357–358; (*b*) M. H. Schoenfisch and M. E. Robbins, *J. Am. Chem. Soc.*, 2003, **125**, 6068–6069; (*c*) M. H. Schoenfisch, S. M. Marxer, A. R. Rothrock, B. J. Nablo and M. E. Robbins, *Chem. Mater.*, 2003, **15**, 4193–4199; (*d*) M. M. Collinson and A. R. Howells, *Anal. Chem.*, 2000, **72**, 21, 702–709 A; (*e*) R. B. Bhatia, C. J. Brinker, A. K. Gupta and A. K. Singh, *Chem. Mater.*, 2000, **12**, 8, 2434–2441.

6 A. S. Borovik, T. M. Reed and J. T. Mitchell-Koch, *Angew. Chem., Int. Ed.*, 2004, **43**, 2806–2809.

7 (*a*) K. Cosby, K. S. Partovi, R. P. Patel, C. D. Reiter, S. Martyr, B. K. Yang, M. A. Waclawiw, G. Zaloa, X. Xu, K. T. Huang, H. Shields, D. B. Kim-Shapiro, A. N. Schechter, R. O. Cannon, III and M. T. Gladwin, *Nat. Med.*, 2003, **9**, 1498–1505; (*b*) J. Rodriquez, R. E. Maloney, T. Rassaf, N. S. Bryan and M. Feelisch, *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 336–341.

8 J. Bordini, D. L. Hughes, J. D. D. Neto and C. J. Cunha, *Inorg. Chem.*, 2002, **41**, 5410–5416.

9 (*a*) C. F. Works and P. C. Ford, *J. Am. Chem. Soc.*, 2000, **122**, 7592; (*b*) C. F. Works, C. J. Jocher, G. D. Bart, X. Bu and P. C. Ford, *Inorg. Chem.*, 2002, **41**, 3728.

10 [Ru(salen)(OH_2)(NO)](NO_3)] was prepared from [Ru(salen)(Cl)(NO)] as described previously as described in ref. 8.

11 A. Wanat, T. Schneppensieper, A. Karocki, G. Stochel and Rudi van Eldik, *J. Chem. Soc., Dalton Trans.*, 2002, 941–950.

12 (*a*) A. Dejam, C. J. Hunter, A. N. Schechter and M. T. Gladwin, *Blood Cells, Mol. Dis.*, 2004, **32**, 423–429; (*b*) P. Kleinbongard, A. Dejam, T. Lauer, T. Rassaf, A. Schindler, O. Picker, T. Scheeren, A. Gödecke, J. Schrader, R. Schulz, G. Heusch, G. A. Schaub, N. S. Bryan, M. Feelisch and M. Kelm, *Free Radical Biol. Med.*, 2003, **35**, 790–796; (*c*) H. Moshage, B. Kok, J. R. Huizenga and P. L. Jansen, *Clin. Chem.*, 1995, **41**, 892–896; (*d*) S. Kage, K. Kudo and N. Ikeda, *J. Anal. Toxicol.*, 2002, **26**, 320–324.