

Luminescence Lifetime Standards for the Nanosecond to Microsecond Range and Oxygen Quenching of Ruthenium(II) Complexes

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A rapid and reproducible method for determining the temperature dependence of luminescence lifetimes has been developed. With the use of this method, a set of standards for the excited-state lifetime oxygen quenching of several ruthenium(II) transition metal complexes was established. With the use of three solvents of different viscosities and two metal complexes with widely different lifetimes, an overlapping range of ca. 100 ns to 6 μ s was obtained. The decays are pure single exponentials, which means that they can be used reliably with both phase and pulsed lifetime instruments. For a pure single-exponential decay, a properly operating phase shift instrument will give the same lifetime as a time domain instrument. With the use of a thermal deactivation model and a three-parameter temperature-dependent oxygen quenching constant, the lifetime temperature-dependent data was well fit by a simple six-parameter equation that covers the temperature range of 10–50 °C and oxygen pressures from 0 to 1 atm of oxygen with excellent precision (ca. <1%). This permits both laboratory and field calibration of instruments.

Luminescence-based sensors are becoming increasingly important in the industrial, environmental, biological, and health areas.^{1–19} Many of these sensor systems are based on luminescent

transition metal complexes.^{1–8,12–18} Both intensity and lifetime methods are used. Intensity methods are subject to errors from source fluctuation, detector drift, geometry changes, and photo-decomposition.¹⁹ To alleviate these problems, internal standards that have different emission spectra that are not affected by the analyte can be incorporated. In contrast, lifetime approaches have the advantage that they are inherently self-referencing. The lifetime directly provides the desired information and is largely independent of the perturbations that affect intensity methods. Lifetime methods have become increasingly popular, but at present, low-cost lifetime instrumentation is phase shift based and limited to lifetimes greater than 100 ns. This largely limits their use to systems based on metal complexes such as platinum metals (Ru, Ir, Os, and Pt).

The popularity of luminescent platinum metal complexes rests on their long-lived excited states coupled with strong visible absorptions that aid in sensor design. Their lifetimes and spectral characteristics are generally well suited for low-cost LED-based phase shift instrumentation or pulsed instruments.²⁰ The primary disadvantage of phase shift instruments is that they generally provide only a single phase shift at one frequency, which allows calculation of a lifetime. However, since complete decays are not available for examination, there is little warning of instrument malfunction.

Using a series of fluorophores in liquid solution at 20 °C, a group of nine independent laboratories developed a set of lifetime standards with lifetimes ranging from 89 ps to 31.2 ns.²¹ A set of 20 fluorescence lifetime standard/solvent combinations was compiled. These standards should prove extremely useful in the testing and calibration of fluorescence lifetime instruments such as fluorescence lifetime imaging microscopy (FLIM) where the lifetimes of the most commonly used fluorophores are a few

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nanoseconds or less. These standards would be useless, however, in calibrating metal complex based sensors, due to the long-lived excited states of metal complexes. Lakowicz reported a few lifetime standards for this class of molecules, but it was not comprehensive.²² Further, there is also a practical need for standards that are not limited to laboratory conditions, where temperature and oxygen pressure can be easily controlled, but are applicable over a wide range of temperatures and oxygen pressures. It would therefore be useful to have a set of standards that could be used to rapidly check the performance of instruments both in the laboratory and under the much more extreme condition of field or plant work. Also, in spite of the wide use of these systems, there is still little detailed fundamental information on such things as the temperature dependence of quenching.

We wished to develop a simple method for determining the temperature and oxygen quenching properties of metal complexes. Exploiting this technology, we wanted to develop a set of reliable standards that could be used to calibrate phase shift and pulsed lifetime based instruments. Our systems were optimized for use with platinum metal complexes having lifetimes in the 0.1–6 μ s range. Further, the standards should be usable over a wide range of temperatures and air pressures so that they could be used to check the operation of instrumentation under a variety of field conditions. Finally, we wanted to develop models that would allow precise descriptions of the pressure and temperature dependences of luminescent complexes.

EXPERIMENTAL SECTION

Materials. Our samples consisted of one of two Ru(II) metal complexes in several analytical grade solvents. The ruthenium complex tris-(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) dichloride, $[\text{Ru}(\text{dpp})_3]\text{Cl}_2$, was from GFS Chemicals Inc. The second complex, tris-(2,2'-bipyridine)ruthenium(II) dichloride, $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$, was synthesized by a standard method,²³ but it is also available from GFS Chemicals and others. The solvents chosen for this experiment were ethylene glycol (Sigma-Aldrich or Fisher Scientific), glycerol (Aldrich), and double-distilled water. Because of its low solubility $[\text{Ru}(\text{dpp})_3]\text{Cl}_2$ was not measured in water. The sample concentrations for these experiments were approximately 45 μM for $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$ in ethylene glycol, 18 μM solution $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$ in water, 40 μM solution of $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$ in glycerol, and 10 μM solution of $[\text{Ru}(\text{dpp})_3]\text{Cl}_2$ in ethylene glycol.

Experimental Setup. The experimental setup used is shown in Figure 1. Samples were excited with a short, 3 ns, pulse from a N_2 laser (Laser Science, VSL 337). A saturated CuSO_4 solution was placed at the output of the N_2 laser to remove plasma emissions. The beam was focused onto the sample, and a Hamamatsu R928 photomultiplier tube (PMT) was used to detect the luminescence. A complementary red pass filter was used over the PMT to eliminate scattered excitation light. The photomultiplier output was recorded on a digital oscilloscope (Tektronix TDS 2022 two-channel storage oscilloscope, 200 MHz, 2GS/s) interfaced to a desktop PC through an IEEE 488 interface. A 50 Ω load resistor was used. Sample temperature was monitored with a thermocouple (Type T, Omega Engineering, Inc.) that was

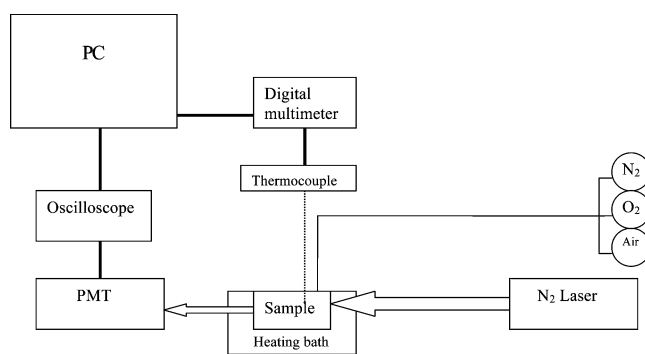


Figure 1. Schematic diagram of experimental setup.

immersed directly in the sample. An electronic cold junction compensator was used. The voltage was read with a Keithley 2000 digital multimeter interfaced to the PC through an IEEE 488 interface. The samples were placed in a well-stirred water bath heated with a variac-controlled immersion heater. The sample was first cooled below 10 $^{\circ}\text{C}$ and then heated at approximately 0.5 $^{\circ}\text{C}/\text{min}$ to collect data.

During data collection, the well-stirred sample was sparged with compressed N_2 , air, or O_2 gas bubbled directly into the sample with a frit to maintain the equilibrium oxygen concentration during the measurement. For water solutions, the gas was water saturated by using a bubbler to minimize evaporation. Glycol and glycerol have negligible vapor pressure, and evaporation was not an issue.

Experimental Procedure. The lifetimes of the Ru(II) complexes in a variety of solutions were measured at different temperatures. Separate measurements under nitrogen, oxygen, and air were performed. Solutions were bubbled, with the respective gas, for at least 40 min to allow the systems to equilibrate. The sample was then immersed in an ice bath and then cooled to less than approximately 8 $^{\circ}\text{C}$. The sample was allowed several minutes to stabilize at this low temperature, and then the temperature was slowly raised. Starting at a predetermined temperature (typically 10 $^{\circ}\text{C}$), a decay curve was collected at every 0.5 $^{\circ}\text{C}$. At each temperature, 100 decays were collected and averaged. The oscilloscope does not average except at 8-bit resolution, so to exploit the enhanced resolution of averaging, each decay was transferred to the computer and averaged in software. One hundred averages raises the effective resolution to approximately 12 bits.²⁴ This has the advantage of allowing each transient to be analyzed and to reject large pulses that saturated the ADC. Each temperature and its corresponding average decay were saved in separate LabView files. Finally, once the temperature reached 50 $^{\circ}\text{C}$, the experiment was terminated. The sample was cooled, and the next gas was bubbled through the solution.

Each decay curve was fit by nonlinear least-squares in LabView. All the decays were single exponential. A complete data set for each sample consisted of decay times versus temperature for each of the three oxygen concentrations. The nonlinear models for fitting the oxygen temperature dependence of the lifetimes (see below) were fit by nonlinear least-squares using PSI Plot (Poly Software International) or a MathCAD routine.

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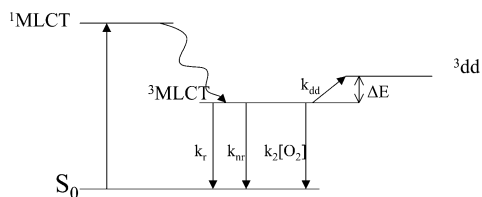


Figure 2. Lifetime temperature dependence model.

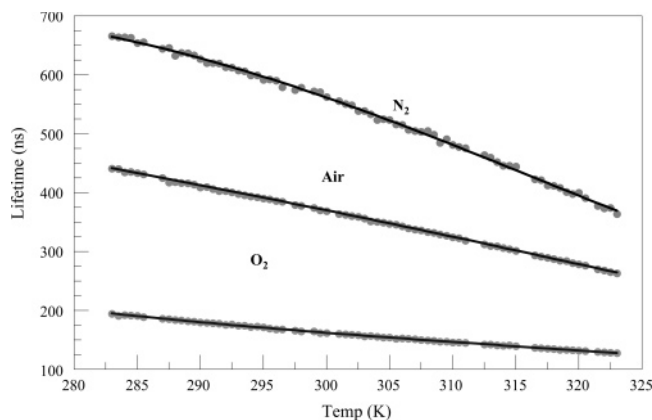


Figure 3. Lifetime temperature dependence of $[\text{Ru}(\text{bpy})_3]^{2+}$ in distilled water purged with N_2 , air, O_2 , all at approximately 1 atm partial pressure. The actual pressure was used in the modeling. The dots are data points, while the solid black lines represent the fit to the model.

RESULTS AND DISCUSSION

The model used for interpreting the temperature dependence is shown in Figure 2 where k_r is the radiative rate constant, k_{nr} is the nonradiative rate constant, and k_{dd} is the rate constant for thermally activated quenching via the nonluminescent d–d state. This model adopts the accepted temperature dependence for thermal deactivation of the emitting metal-to-ligand charge transfer (MLCT) state via a nonluminescent d–d excited state. The lifetime of the MLCT state, in nitrogen, air, and oxygen, is given by

$$\tau_0 = 1/(k_0 + k_{dd}) \quad (1)$$

$$\tau_{\text{air}} = 1/(k_0 + k_{dd} + k_2[\text{O}_2]) \quad (2)$$

$$\tau_{\text{oxygen}} = 1/(k_0 + k_{dd} + k_2[\text{O}_2]) \quad (3)$$

$$k_0 = k_r + k_{nr} \quad (4)$$

$$k_{dd} = A \exp(-\Delta E/kT) \quad (5)$$

$$[\text{O}_2] = K_h P_{\text{oxygen}} \quad (6)$$

where τ_0 is the lifetime in nitrogen-purged samples and, therefore, in the absence of a bimolecular quencher, k_0 is the sum of the radiative and nonradiative rate constants, τ_{air} is the lifetime in air-purged samples, k_2 is the bimolecular quenching rate constant, $[\text{O}_2]$ is the quencher concentration, K_h is the Henry's law constant for oxygen solubility, and P_{oxygen} is the oxygen pressure. τ_{oxygen} is the lifetime in oxygen-purged samples, A is a pre-exponential factor, ΔE is the energy difference between the emitting charge-transfer state and the thermally populated d–d state, k is the

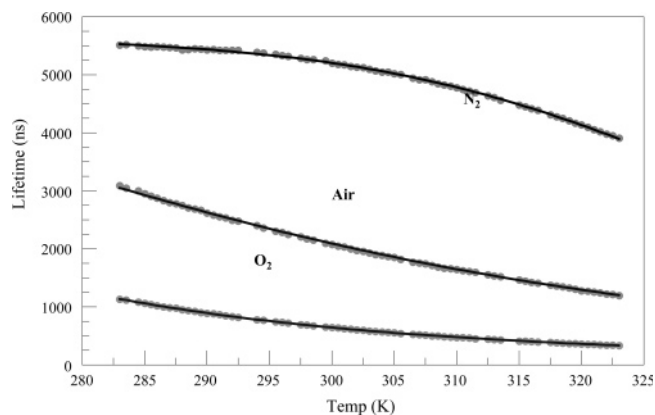


Figure 4. Lifetime temperature dependence of $[\text{Ru}(\text{dpp})_3]^{2+}$ in ethylene glycol purged with approximately 1 atm of N_2 , air, and O_2 . The dots are data points, while the solid black lines represent the fit to the model.

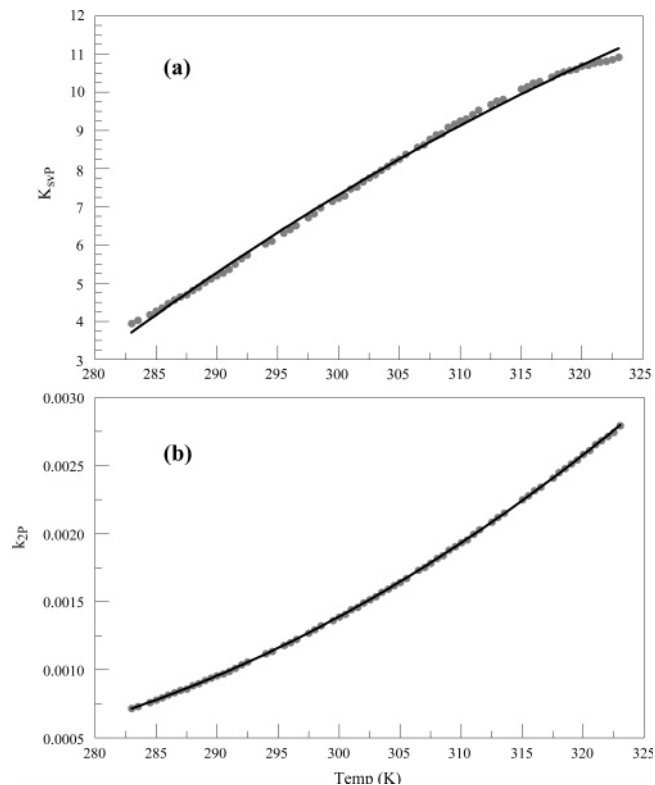


Figure 5. (a) Temperature dependence of K_{SVP} . The dots are calculated pressure-based Stern–Volmer constants, K_{SVP} , for each temperature, while the solid black line represents a second-order polynomial fit of $K_{\text{SVP}}(T)$. (b) Temperature dependence of k_{2P} . The dots are the defined k_{2P} calculated using the temperature-dependent pressure-based Stern–Volmer constant divided by the unquenched temperature-dependent luminescent lifetime, while the solid black line is the second-order polynomial fit of $k_{2P}(T)$.

Boltzmann constant, and T is temperature. K_h and k_2 are assumed to be the only temperature-dependent quantities in our systems.

Figures 3 and 4 show the lifetime temperature and oxygen pressure dependence of $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$ in water and $[\text{Ru}(\text{dpp})_3]\text{Cl}_2$ in ethylene glycol, respectively. The dots are data points, while the solid black lines represent the fit to the model described below. As expected by the thermally activated decay path, the lifetime decreases as the temperature is raised. Oxygen quenching is also pronounced except for the very viscous glycerol, but even there

Table 1. Fitting Parameters for the Lifetimes^a

complex	solvent	k_0 10^{-5} (s^{-1})	K_{dd} 10^{-12} (s^{-1})	E (cm^{-1})	B 10^{-6} ($s \cdot atm$) $^{-1}$	C 10^{-4} ($s \cdot atm \cdot K$) $^{-1}$	D 10^{-1} ($s \cdot atm$) $^{-1}$ (K) $^{-2}$	SD (ns)
Ru(bpy) ₃ ²⁺	ethylene glycol	7.609	55.18	3834	30.66	-24.6	49.61	4.6
	distilled water	13.26	2.657	3238	-37.21	23.78	-32.95	2.1
	glycerol	7.693	169.7	4115	5.572	-3.935	6.95	4.4
Ru(dpp) ₃ ²⁺	ethylene glycol	1.774	249.6	4894	34.70	-27.09	53.29	10.5

^a The number of significant figures on the fitting parameters is provided to give reliably computed lifetimes, and no physical significance should be attributed to their number or certainty.

Table 2. Optimal Lifetime Ranges of [Ru(bpy)₃]²⁺ and [Ru(dpp)₃]²⁺ in Glycol, Water, and Glycerol

complex	solvent	lifetime—low end (O ₂ high temperature) (ns)	lifetime—high end (N ₂ high temperature) (ns)
[Ru(bpy) ₃] ²⁺	ethylene glycol	~200	~1100
	distilled water	~125	~675
	glycerol	~350	~1100
[Ru(dpp) ₃] ²⁺	ethylene glycol	~300	~5500

the lifetime of [Ru(dpp)₃]²⁺ is long enough to give noticeable quenching especially at higher temperatures where the viscosity is lower.

Since oxygen is a dynamic quencher of the luminescence lifetime, we can describe the quenching at each temperature with a Stern–Volmer equation.

$$\tau_0/\tau = 1 + K_{sv}P_{\text{oxygen}} = 1 + K_{sv}K_hP_{\text{oxygen}} \quad (7)$$

$$K_{sv} = k_2\tau_0 \quad (8)$$

where ϑ is the measured lifetime, K_{sv} is the Stern–Volmer quenching constant, and K_{sv} is a pressure-based Stern–Volmer constant. The lifetime at each oxygen pressure and temperature is given by

$$\tau = \tau_0/(1 + K_{sv}P_{\text{oxygen}}) \quad (9)$$

However, the different quantities in the equations are temperature dependent. k_2 will increase, whereas ϑ_0 and K_h will decrease, with increasing temperature. Therefore, a complex temperature dependence for K_{sv} would not be surprising. Rewriting eq 9 including the temperature dependence we obtain

$$\tau(T) = 1/(k_0 + k_{dd}(T) + K_{sv}(T)P_{\text{oxygen}}/\tau_0(T)) \quad (10a)$$

$$\tau(T) = \tau_0(T)/(1 + K_{sv}(T)P_{\text{oxygen}}) \quad (10b)$$

Equation 10b allows the calculation of ϑ at any temperature and oxygen pressure if one knows $\tau_0(T)$ and $K_{sv}(T)$. Equation 1 provides a fundamental form for $\tau_0(T)$, and the oxygen-free temperature data can provide the necessary parameters to reproduce $\tau_0(T)$. Figure 5 shows the $K_{sv}(T)$ versus T for Ru(dpp)₃²⁺ in ethylene glycol.

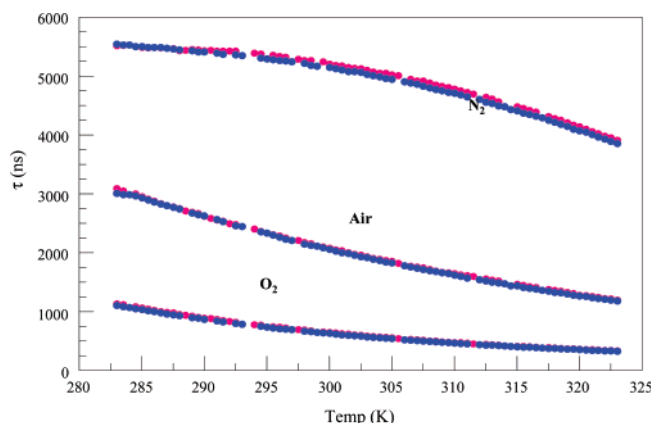


Figure 6. [Ru(dpp)₃]²⁺ in ethylene glycol purged with N₂, air, and O₂. The red and blue dots are data collected more than 1 week apart.

There is no good fundamental expression for $K_{sv}(T)$, but for standards one only needs a fitting equation that accurately reproduces the data. We initially tried a second-order polynomial fit of $K_{sv}(T)$ versus T (Figure 5a), which works moderately well, but it has trouble fitting the low- and high-temperature limits perfectly. However, if we define k_2 in terms of pressure, k_{2P} , we have

$$k_{2P} = K_{sv}(T)/\tau_0(T) \quad (11)$$

which gives a much smoother curve that is essentially perfectly fit by a second-order polynomial (Figure 5b). Using the three parameters for the fit to $\tau_0(T)$ and the three polynomial coefficients (B , C , and D), we can then generate $\tau(T)$ at all pressures and temperatures from

$$\tau(T) = \tau_0(T)/(1 + (k_{2P}(T)\tau_0(T)P_{\text{oxygen}})) \quad (12)$$

$$k_{2P}(T) = B + CT + DT^2 \quad (13)$$

$\tau_0(T)$ is given by the three-parameter eq 1.

The fits in Figures 3 and 4 are derived from the six parameters needed in eqs 1–3 and 13. The fits are essentially perfect. The standard deviations for all three pressures and all temperatures are 10.5 ns for the [Ru(dpp)₃]²⁺ in glycol and 2.1 ns for [Ru(bpy)₃]²⁺ in water. Comparable fits are obtained for the remaining systems. Table 1 shows the parameters for the best fits for the different systems along with standard deviations for each data set. One should put no fundamental significance on the constants B , C , and D . They are merely fitting parameters that

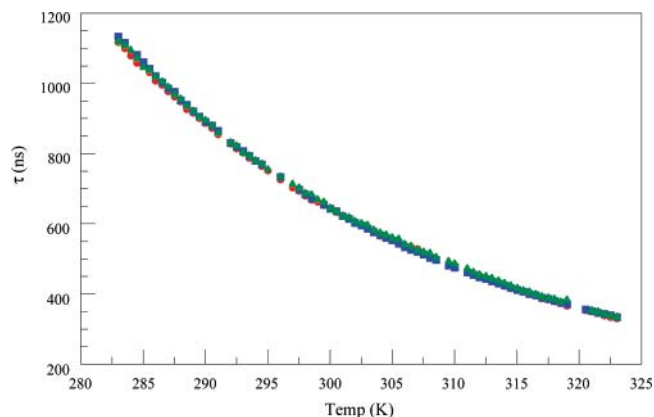


Figure 7. O₂-purged Ru(dpp)₃²⁺ in ethylene glycol samples of varying concentrations (3 μM—green triangles, 10 μM—blue squares, 24 μM—red circles).

accurately describe the data. As a guideline to the best system for given lifetime ranges, Table 2 summarizes the operational range for each system. Thus, the user can select the system that best matches his analytical reagent and the operating conditions of his instrument.

If a setup such as the one described in our experiments is to be used in real-world applications, then the system responses must be reproducible as well as not easily influenced by slight variations in sample preparation or measurements. Figure 6 shows the lifetime temperature dependence of [Ru(dpp)₃]²⁺ in ethylene glycol. The two sets of data correspond to experiments performed on the same sample more than 1 week apart. As you can see from

Figure 6, the two separate experiments agree well. Figure 7 shows the effect of concentration variations for Ru(dpp)₃²⁺ in O₂-purged ethylene glycol samples (3, 10, and 24 μM). The differences in the lifetimes of the three samples over the temperature range of 283–323 K are negligible. This is not surprising as we would not expect appreciable self-quenching in these systems.

CONCLUSION

A method was developed that allowed the rapid and efficient determination of the temperature and oxygen dependence of excited-state lifetimes in a variety of systems. It was used to characterize two Ru(II) complexes in several solvents. These systems are useful lifetime standards. Each system can be fit with high precision over the range of 10–50 °C and 0–1 atm of oxygen with a simple, six-parameter equation. The applied model fits extremely well for all systems. The systems covered a wide range of lifetimes from a little more than 100 ns to ~5.5 μs. This allows potential users the flexibility of being able to choose the appropriate system for checking system calibration either in the laboratory or in the field.

ACKNOWLEDGMENT

We thank the National Science Foundation for support through NSF CHE 04-100061 and 03-353939. B. A. DeGraff is pleased to acknowledge the support of the Dreyfus Foundation.

Received for review June 18, 2007. Accepted September 17, 2007.

AC0712796