

Generating a Warm Glow: Lanthanide Complexes Which Luminesce in the Near-IR

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This article reviews and expands upon our observations of neodymium and ytterbium-centered luminescence in the near-IR. A variety of neodymium (III) and ytterbium (III) complexes with aminocarboxylate ligands was synthesized and their photophysical properties were investigated in aqueous solutions. Metal-centered emission was observed in the near-IR for complexes of both ions and time-resolved studies were used to show how quenching of the excited states is dependent on both inner and outer sphere coordinated water molecules.

KEY WORDS: Luminescence; neodymium; ytterbium; solution; near-IR.

INTRODUCTION

The time-resolved luminescence of lanthanide complexes has been of interest for some years [1–4]. However, interest has centered almost-exclusively on the luminescence of complexes of Eu(III) and Tb(III). In addition, the extinction coefficients for the lanthanide ions tend to be very small—rendering their use in biological systems problematic. Sensitized luminescence, involving energy transfer from an aryl chromophore, has been widely used to increase the intensity of metal centred emission and render them suitable for use in biological imaging applications. In the majority of cases, the energy transfer process has been shown to be mediated by the aryl triplet state [2], though examples are known where the excited singlet state transfers energy directly to the metal center [5,6]. Lanthanide complexes have the advantage of possessing long luminescent lifetimes, but they are excited using long-wavelength UV radiation in the

same range ($\lambda < 360$ nm) as biological molecules. This limits the utility of such complexes for direct *in vivo* applications. An additional problem arises from the relatively large energy gap between the emissive and the ground states ($>20,000$ cm^{-1}), which requires the use of aromatic antennae with relatively high triplet-state energies to sensitize the metal efficiently [7].

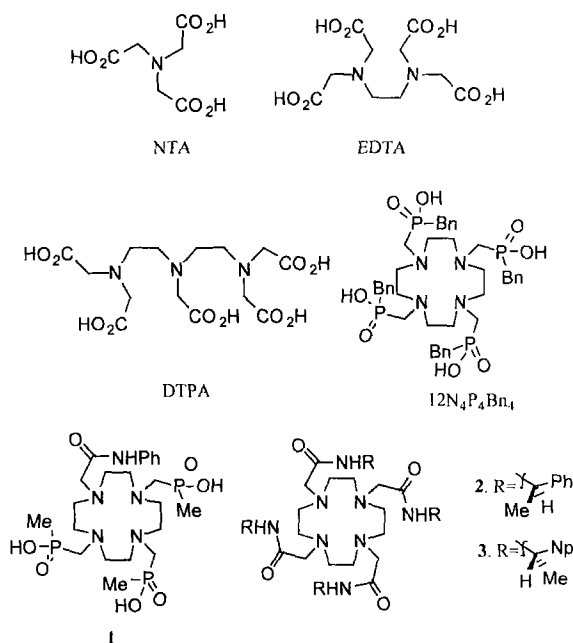
We have been investigating the luminescence of lanthanides which emit at longer wavelengths, i.e., those which have relatively small energy gaps between the excited state and the ground state. This allows the use of antennae with much lower triplet-state energies, greatly reducing the competitive excitation of biological chromophores, potentially leading to improved tissue penetration and image resolution in the fluorescence microscopy of biological systems.

In our initial investigations, the time-resolved luminescence of neodymium (III) was studied by deconvolution of the detector response from a nitrogen-cooled germanium diode, allowing the resolution of lifetimes as short as 20 ± 4 ns [8]. We have also recently extended this approach to the luminescence from ytterbium, which also offers the possibility of long-wavelength luminescence with an emission centered at 980 nm [9]. Ytterbium

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Scheme 1. Structures of the ligands.

luminescence in the solution state has been largely neglected until recently, and the few investigations published have concentrated on measurement of the emission spectra of simple systems with no time resolution [10,11].

In this paper we compare and contrast the luminescence from ytterbium and neodymium and discuss the effect of inner and outer sphere solvent molecules on the nonradiative quenching of the excited states of the metal ions.

EXPERIMENTAL

Neodymium and ytterbium salts were purchased from Aldrich, with the exception of neodymium (III) perchlorate, which was obtained by the pyrolysis of the acetate at 800°C and dissolving the residue in 70% perchloric acid followed by drying *in vacuo*. Normal solvents were HPLC grade; methanol was freshly distilled from magnesium methoxide. Deuteriated solvents were obtained from Aldrich and used as received. Complexes of the ligands nitrilotriacetic acid (NTA; Aldrich), ethylenediaminetetraacetic acid (EDTA; Aldrich), and diethylenetriaminepentaacetic acid (DTPA; Aldrich) were prepared by mixing solutions of the ligand sodium salts with neodymium nitrate solutions (Scheme I). Nd $12N_4P_4Bn_4$ was prepared analogously to the Eu complex [12] by heating neodymium acetate with an aqueous solution of 1,4,7,10-tetraazacyclodecane-1,4,7,10-tetrakis-

(methylenebenzylphosphinic acid) ($12N_4P_4Bn_4$) for 1 h at pH 6 and recrystallizing the product from boiling water. The preparation of ytterbium complexes of azamacrocycle-derived ligands has been described elsewhere [9,13].

Neodymium samples were excited by the third harmonic of a Q-switched Nd:YAG laser (Spectra Physics GCR-150-10; 10 Hz, 355 nm). The radiation was delivered to the sample via a liquid light guide and typical pulse energies at the sample were in the range 0.1–2 mJ with a pulse duration of *ca.* 6 ns. For the ytterbium complexes, direct excitation experiments were performed using 970-nm radiation selected from the output of an OPO driven by a Nd:YAG laser. For indirect excitation experiments, the second, third, and fourth harmonics of a Q-switched Nd:YAG laser (Spectra Physics GCR-150-10) operating at 10 Hz were used as appropriate to the sensitising chromophore. Interference from stray 532- and 1064-nm radiation in the excitation beam was avoided by the use of optical filters. The luminescence was collected at 90° and focused onto the entrance slits of a 300-mm-focal length monochromator, (Bentham TM300V); the bandpass of the monochromator was set to 5 nm. The selected radiation was detected by a liquid nitrogen-cooled germanium photodiode/amplifier (North Coast EO-817P) operating in high-sensitivity mode. Under these conditions this detector has a rise time of *ca.* 200 ns and a FWHM response of 400 ns. The signal was captured and averaged by a digital storage oscilloscope (Tektronix TDS320) and transferred to a PC for analysis. Typical decays were recorded as the average of eight laser shots. Instrument response functions were obtained using the fluorescence from a solution of a red laser dye (DCM), $\tau_f = 2.2$ ns. The lifetime of this dye is very short compared to the metal ion and is used as an alternative to a scattering sample to provide an instrument response profile for the apparatus. This is justified on the grounds that the fluorescence lifetime of the dye is much shorter than the rise time of the detector and the lifetime of the neodymium emission and has been discussed elsewhere [8]. The decays were analyzed by iterative deconvolution and nonlinear least-squares analysis of the instrument response profile with a single exponential function and, in all cases, gave good-quality fits as judged by the randomness of the residuals and a satisfactory reduced chi-square [14]. This method of data analysis is more typically applied to data obtained using time-correlated single-photon counting, where the statistics of the noise are very well characterized and sophisticated criteria of fit can be used. The application of such fits to our systems is justified in Ref. 8. A typical fitted decay is illustrated in Fig. 1.

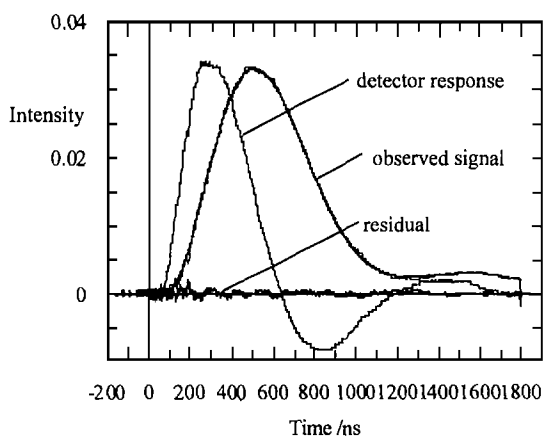


Fig. 1. Decay curve obtained by excitation of the ytterbium complex of dibenzoylmethane in MeOH at 355 nm and residuals for fitted curves generated by convolution of the instrument response function with a single-exponential function, $\tau = 500 \pm 5$ ns.

RESULTS AND DISCUSSION

The emission spectrum from neodymium in aqueous solution shows three bands, centered at 880, 1055, and 1330 nm, corresponding to the ${}^4F_{3/2} > {}^4I_{9/2}$, ${}^4I_{11/2}$, and ${}^4I_{13/2}$, respectively. The uncorrected spectrum is shown in Fig. 2. The lifetimes obtained by monitoring the bands at 880 and 1330 nm were identical within experimental error to those observed at 1055 nm and the kinetic measurements reported in this paper were obtained at either 880 or 1055 nm. The ytterbium (III) ion has a relatively simple spectrum: there is only one excited state (${}^2F_{5/2}$), which emits and absorbs around 980 nm. The emission spectrum (${}^2F_{5/2} > {}^2F_{7/2}$) of the dibenzoylmethane complex of ytterbium is shown in Fig. 3.

The lifetime of the decay from neodymium nitrate in water was found to be independent of concentration

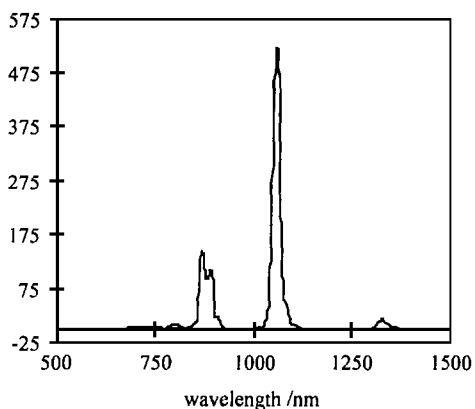


Fig. 2. Uncorrected emission spectrum of neodymium nitrate in H_2O . Excitation wavelength, 355 nm.

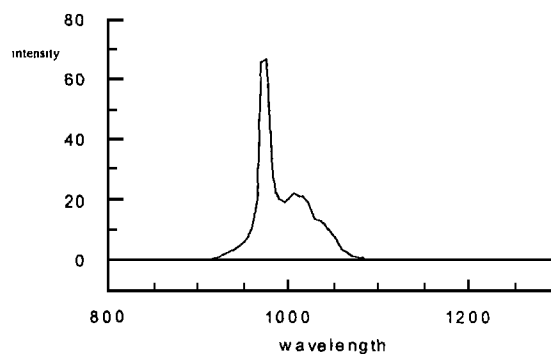


Fig. 3. Uncorrected emission spectrum of the ytterbium complex with dibenzoylmethane. Excitation wavelength, 355 nm.

over the range $0.01\text{--}1.00$ mol dm^{-3} , showing that there is no reduction in lifetime due to ion aggregation effects; $\tau = 29$ ns [8]. In D_2O the lifetime of the emission increases to 152 ns, due to the less efficient deactivation of the excited state of the metal ion via an electronic–vibrational energy transfer to the solvent. In the case of water the energy gap between the emissive ${}^4F_{3/2}$ state and the ${}^4I_{15/2}$ ground state corresponds to two quanta of vibrational energy for an O–H bond stretch. With the heavier isotope the coupling between the energy levels is less efficient and the Franck–Condon factor for the relaxation process is reduced, effectively increasing the lifetime of the emission. For the aqueous system we investigated this effect further using mixtures of H_2O and D_2O to study the lifetime dependence on solvent composition. A linear relationship was observed between $1/\tau$ vs mole fraction of D_2O . Assuming that neodymium has a similar degree of solvation to the other lanthanides in water, we can estimate a value for the A_{Ln} factor described by Horrocks and Sudnick: $A_{Nd} = 3.6 \times 10^{-7}$ s [8,15]. This experimentally derived factor quantifies the efficiency of quenching of the excited state by O–H oscillators, europium and terbium, which are quenched less efficiently by higher harmonics of the O–H oscillator, with A factors of 1.05×10^{-3} and 4.2×10^{-3} respectively.

Table I shows the lifetimes of a variety of neodymium complexes in aqueous solutions. It may be seen that the observed luminescence lifetime increases on moving from simple 4-coordinate ligands such as nitrilotriacetic acid to more complex 6- and 8-coordinate ligands such as EDTA and DTPA. With ligands of higher denticity, water molecules are excluded from the inner coordination sphere and so the number of O–H oscillators proximate to the metal center is reduced. The octadentate ligand $12N_4P_4Bn_4$ is a special case in which the pendent benzyl groups prevent close approach of water molecules and hence prolong the observed luminescence [13]. In D_2O

Table I. The Luminescence Lifetimes in Aqueous Solutions of a Variety of Neodymium Aminocarboxylate Complexes^a

Ligand	$\tau_{\text{H}_2\text{O}}$ (ns)	$\tau_{\text{D}_2\text{O}}$ (ns)
NTA	45 (77) ^b	430
EDTA	53 (110) ^b	484
DTPA	75	411
12N4P ₄ Bn ₄	159	329

^a All ligands were used as their sodium salts, with the exception of the benzyl phosphinate, which was used as its H₃O⁺ salt. Errors are $\pm 10\%$.

^b Obtained using a ligand:metal ratio of 5:1.

the excited state of the metal center is quenched less efficiently, as explained above, and the observed lifetime increases accordingly. However, a general trend may be observed whereby $\tau_{\text{D}_2\text{O}}$ decreases with increasing ligand coordination. This implies that other pathways are also involved in quenching of the metal center. It is likely that the most important of these is quenching by harmonics of the C–H oscillators within the ligand skeleton [16]; the quenching contribution of the C–H oscillators would appear to be related directly to the number of such oscillators. This effect is markedly smaller than that observed for O–H oscillators, presumably because the oscillator strength of the C–H oscillator is much less than that of the O–H oscillator. The synthesis of perdeuteriated analogues of a variety of ligands is currently under way to quantify this effect and allow the relative contributions of C–H and proximate and outer sphere O–H oscillators to be established. When the value for A_{Nd} is applied to the lifetimes of neodymium complexes, the q values obtained are not those which would be expected from the evidence from solid-state structures. Owing to the diverse variety of possible quenching pathways, this is not altogether surprising, but more detailed study is necessary to allow a more appropriate breakdown of the contributions to luminescence quenching. It is hoped that this will be provided by the synthesis of the perdeuteriated analogues mentioned above.

Measurement of the lifetime of ytterbium salts in aqueous solution is more problematic, since direct excitation at 980 nm while observing emission at 980 nm leads to the emission being swamped by scattered light and renders accurate analysis of the decay impossible. Accordingly, the A_{Yb} factor cannot be determined by such a simple experiment as that used for neodymium; use of sensitizing chromophores (*vide infra*) is necessary to allow us to quantify the effect of water molecules.

Lifetimes for a variety of ytterbium (III) complexes are tabulated in Table II. These show similar behavior to the neodymium complexes; with increasing hydration of

Table II. The Luminescence Lifetimes in Aqueous Solutions of a Variety of Ytterbium Aminocarboxylate Complexes^a

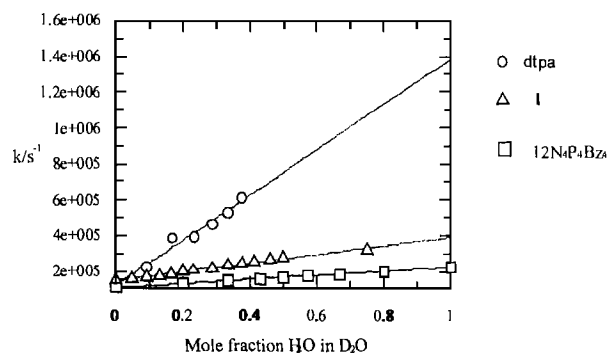
Ligand	$\tau_{\text{H}_2\text{O}}$ (μs)	$\tau_{\text{D}_2\text{O}}$ (ms)	λ_{ex}
NTA ^b	8.1	—	970
EDTA ^b	2.18	10.9	970
DTPA	0.73	8.23	970
12N ₄ P ₄ Bn ₄	4.53	9.03	266
1	1.87	6.70	266
2	0.70	7.48	266
3	0.83	5.00	266

^a For excitation at 970 nm, decay curves were obtained using a 1050-nm interference filter with a 40-nm bandpass with the aid of a polarizer to block scattered light from the laser source. Errors are $\pm 10\%$.

^b Obtained using a ligand:metal ratio of 5:1.

the metal center; the excited state is more efficiently quenched by O–H oscillators. It must be noted, however, that the observed lifetimes are significantly longer for the ytterbium complexes than for analogous neodymium complexes. A number of factors may contribute to this, particularly less efficient overlap between the ²F_{5/2} excited state and the second harmonic of the O–H, N–H, and C–H oscillators. Furthermore, the radius of the Yb³⁺ ion is considerably smaller than that of the Nd³⁺ ion, which would suggest that ytterbium (III) is far less likely to form nine coordinate complexes than neodymium since the extra size of the Nd ion may allow closer approach of water molecules to the metal center.

Figure 4 shows some typical plots of $1/\tau$ vs mole fraction of H₂O in D₂O. It may be seen that these are straight lines. Extrapolation of these lines can be used to establish the lifetime in H₂O for ligands where there is no chromophore and direct excitation of the metal center is necessary, such as in the complexes with EDTA and DTPA. The lifetime of the NTA complex of ytterbium could not be determined by these means since the large

**Fig. 4.** Effect of variation of mole fraction H₂O in D₂O for a variety of aminocarboxylate complexes of ytterbium.

number of bound-water molecules means that efficient quenching occurs at high mole fractions of D₂O and that insufficient data were obtained to generate an effective straight line. Although the crystal structure of the ytterbium complex of 12N₄P₄Bn₄ shows that there are no inner sphere water molecules [13], there is still clearly a quenching effect by added water. It is possible to assign residual quenching effects to an outer sphere contribution and therefore to use the slope of the line to estimate the magnitude of *B* for the equation

$$q = A(k_{\text{H}_2\text{O}} - k_{\text{D}_2\text{O}}) - B$$

where *q* is the number of water molecules and *A* and *B* are constants describing the inner and outer sphere contributions, respectively [9]. *B* is a function primarily of complex hydrophobicity and the availability of local H-bonding donor/acceptor groups. A hydrophobic complex such as 12N₄P₄Bn₄ sets a lower limit to the value of *B*; more hydrophilic complexes might be expected to have a higher value. Having established *B* it is possible to use the values obtained for the other complexes (whose inner sphere hydration, *q*, is known by other methods) to obtain a meaningful value for *A*, thus circumventing the problems with direct excitation described earlier. It is possible to assign values to *A* and *B* of 1000 and 250 ns, respectively. These values can be used to determine the inner and outer sphere coordination contributions within other, less well-characterized lanthanide complexes and hence provide useful structural information for the design of reagents for MRI.

CONCLUSION

Both neodymium and ytterbium complexes offer considerable potential for use as signaling probes *in vitro* and *in vivo*. They combine the potential for long-wavelength absorption with emission lines in water windows, maximizing the potential signal-to-noise ratio in imaging experiments. The excited states of both metals are quenched efficiently by energy transfer to the vibrational harmonics of O–H oscillators in proximate water mole-

cules and by N–H and C–H oscillators in the ligand backbone. Further experiments are under way to quantify the effect of C–H oscillators.

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