Solution Chemistry of Europium(III) Aqua Ion at Micromolar Concentrations as Probed by Direct Excitation Luminescence Spectroscopy

by **Christopher M. Andolina**, **Ryan A. Mathews**, and **Janet R. Morrow***

Department of Chemistry, University at Buffalo, State University of New York, Buffalo, New York 14260-3000, USA (e-mail: jmorrow@buffalo.edu)

Dedicated to *Jean-Claude Bünzli* on the occasion of his 65th birthday.

Direct excitation europium(III) luminescence spectroscopy is used to study the speciation of aqueous europium(III) ions at micromolar concentrations and near neutral pH. The pH and concentration dependence of the europium(III) $^7F_0 \rightarrow {}^5D_0$ excitation peak is consistent with the formation of both mononuclear and dinuclear europium(III) hydroxide complexes at pH 6.5. Luminescence intensity and lifetime quenching studies in the presence of Nd^{III} at pH 5.0 and 6.5 support the formation of a dinuclear complex at pH 6.5. Steady state excitation and time-resolved luminescence spectroscopy are consistent with the formation of innersphere nitrate and fluoride complexes, but outersphere perchlorate and chloride complexes at pH 6.5 and 5.0.

Introduction. – The aqueous chemistry of trivalent lanthanide ions (Ln^{III}) has been studied over several decades, dating from the pioneering studies compiled by *Baes* and *Mesmer* [1] and extending to more recent studies using lanthanide luminescence and X-ray scattering techniques [2–6]. These studies show that lanthanide ions undergo H_2O ligand hydrolysis and aggregation to form mononuclear and multinuclear hydroxide complexes. Other aspects of solution chemistry of interest include the interaction of various simple anions of supporting electrolytes with lanthanide ions. Luminescence and UV/VIS absorbance spectroscopy studies show that most simple anions form outersphere complexes with Ln^{III} ions even at molar concentrations of the anion [7–10]. However, all of these studies use high concentrations of lanthanide ions ranging from molar to millimolar. In addition, most of these studies using photoluminescence spectroscopy do not control pH. Our interest in complexation of micromolar concentrations of lanthanide ions to biopolymers in aqueous conditions has led us to reexamine Eu^{III} aqueous solution chemistry by using a new MOPO (master oscillator power oscillator)/laser system, for direct excitation lanthanide photoluminescence [11]. The instrument is tunable over a broad wavelength range and facilitates direct excitation luminescence studies on Eu^{III} complexes at as low as nanomolar concentrations in aqueous solutions.

Previous studies have focused on time-resolved luminescence spectroscopy to obtain information about the coordination sphere of Eu^{III} [7][9][10][12]. Such work relies on the quenching of excited state Eu^{III} by OH oscillators of both inner and outersphere H_2O molecules. The recovered luminescence lifetimes in these experiments are not always easily interpreted, in part because outersphere and innersphere ligand quenching parameters are not well-defined for highly hydrated lanthanides.

Equations have been developed to determine the number of bound innersphere H_2O molecules from the time-resolved luminescence lifetimes equations [13–15] (see *Eqn. 1* below).

In this equation, k_{H_2O} and k_{D_2O} are the rate constants for photoluminescence decay in H_2O and D_2O, respectively, α is the quenching contribution of outersphere H_2O (0.31 ms^{-1}), and A (1.11 ms per H_2O) is a constant for nine-coordinate Eu^{III} [15]. Interpretation of Eu^{III} speciation by using photoluminescence lifetime data is made difficult by the lack of luminescence quenching parameters for terminal or for bridging OH ligands. In addition, as shown below, the various lanthanide ion complex species that arise from hydrolysis and aggregation have similar luminescence lifetimes, complicating the analysis.

Direct excitation luminescence spectroscopy of Eu^{III} complexes provides information that complements time-resolved luminescence experiments. One of the most useful transitions for determining speciation and binding studies is the $^7F_0 \rightarrow {}^5D_0$ excitation of Eu^{III}. This is because it is between two non-degenerate energy levels and, therefore, not split by the ligand fields. Thus, there is one peak for each species, and barring overlap, speciation changes are readily monitored. Excitation spectroscopy of Eu^{III} has not been fully used to probe solution chemistry of highly hydrated Eu^{III} complexes at neutral pH. Recent studies in our laboratory of Eu^{III} aqua ion ($Eu^{3+}_{(aq)}$) using the MOPO/laser system showed a change in the position of this excitation band, recorded over a concentration range of 1 mM to 50 nM for Eu^{III}, with 100 mM NaCl, at pH 6.5 [11][16]. These results suggested that there was a speciation change under these conditions. Here, we present further studies to illuminate the nature of this species by probing energy transfer from Eu^{III} to Nd^{III} to study aggregate formation. In addition, we show that simple anions, including F^-, Cl^-, I^-, NO_3^-, and ClO_4^- do modulate the $^7F_0 \rightarrow {}^5D_0$ of $Eu^{3+}_{(aq)}$ at pH 6.5 and at pH 5.0. These studies are important for the interpretation of lanthanide ion speciation of biologically relevant ligands that bind weakly to Eu^{III} with dissociation constants, K_d, in the millimolar to micromolar range and do not produce large changes in luminescence intensity. One class of biopolymer ligands of interest to us are nucleic acids [17]. DNA and RNA have unusual salt concentrating effects that can influence Eu^{III} photoluminescence properties. In order to elucidate these complexes, it is critical to understand the basic solution chemistry of micromolar lanthanide ions near neutral pH in the presence of moderately high salt concentrations.

Results. – The $^7F_0 \rightarrow {}^5D_0$ excitation luminescence spectrum of $Eu^{3+}_{(aq)}$ varies markedly over the pH range of 5 to 9 in solutions with an ionic strength, I, of 100 mM (NaCl) (*Fig. 1*). Notably, the intensity of Eu^{III} luminescence increases with increasing pH from pH 5.0 to 7.0 [17], and the position of the Eu^{III} excitation peak blue-shifts from 578.97 to 578.74 nm. Above pH 7.0, a new red-shifted peak at 579.34 nm appears as the pH increases. The presence of multiple peaks at basic pH shows that there are additional new Eu^{III} species.

The concentration dependence of the $^7F_0 \rightarrow {}^5D_0$ excitation peak at pH 6.5 is consistent with multiple species existing under these conditions. Dilute solutions of $Eu^{3+}_{(aq)}$ (<1 μM) at pH 6.5 [11][16] show an excitation peak at 579.10 nm that is red-shifted from that of solutions containing micromolar or greater concentrations of $Eu^{3+}_{(aq)}$ (578.74 nm) (*Fig. 2*). This work shows that there is a change in Eu^{III} speciation over this

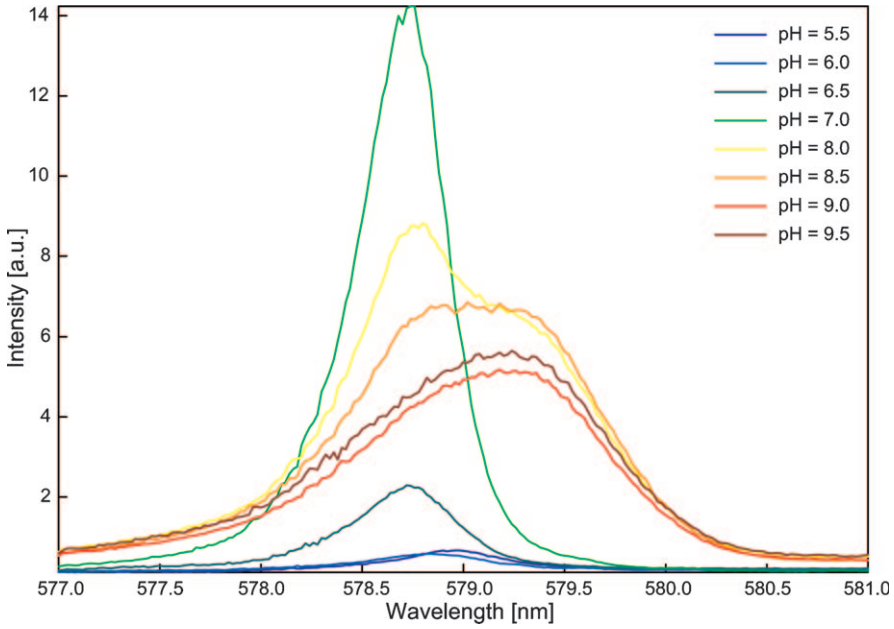

Fig. 1. *Excitation spectra of the luminescence 25* μM Eu^{III} $^7F_0 \rightarrow {}^5D_0$, *and dependence on pH. I* = 0.100M (NaCl), 20.0 mM buffer.

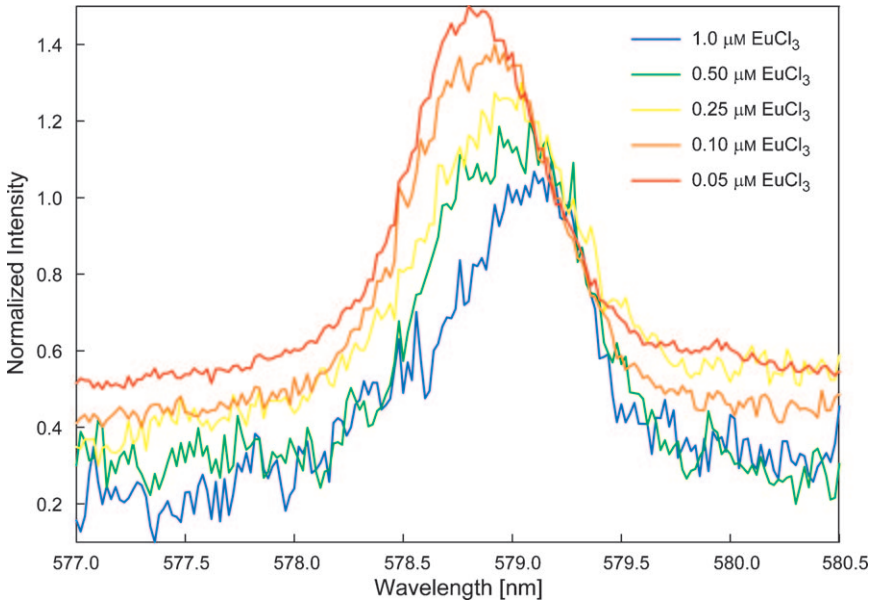

Fig. 2. *Excitation spectra of the* Eu^{III} $^7F_0 \rightarrow {}^5D_0$ *transition showing the luminescence dependence on the concentration of* Eu^{III} *for* I = *0.100*M *NaCl, 20.0 m*M *MES, pH 6.5*. Spectra are normalized and offset for clarity.

concentration range. One likely speciation change is the formation of a dinuclear or multinuclear complex from a mononuclear complex as the concentration of the complex is increased. Titration of $Eu^{3+}_{(aq)}$ with MES (2-morpholinoethanesulfonic acid) buffer shows that buffer complexation is weak under the conditions of these experiments, suggesting that a buffer complex is not a dominant species (*Fig. 3*). *Förster* resonance-energy transfer (FRET) studies by using Nd^{III} as a quencher were carried out to test for the presence of di- or multimeric complexes in solution as shown below.

Time-resolved photoluminescence intensity decays give important information about the lanthanide-ion coordination sphere because there is a large non-radiative quenching contribution by ligand groups, especially OH groups in H_2O. Replacement

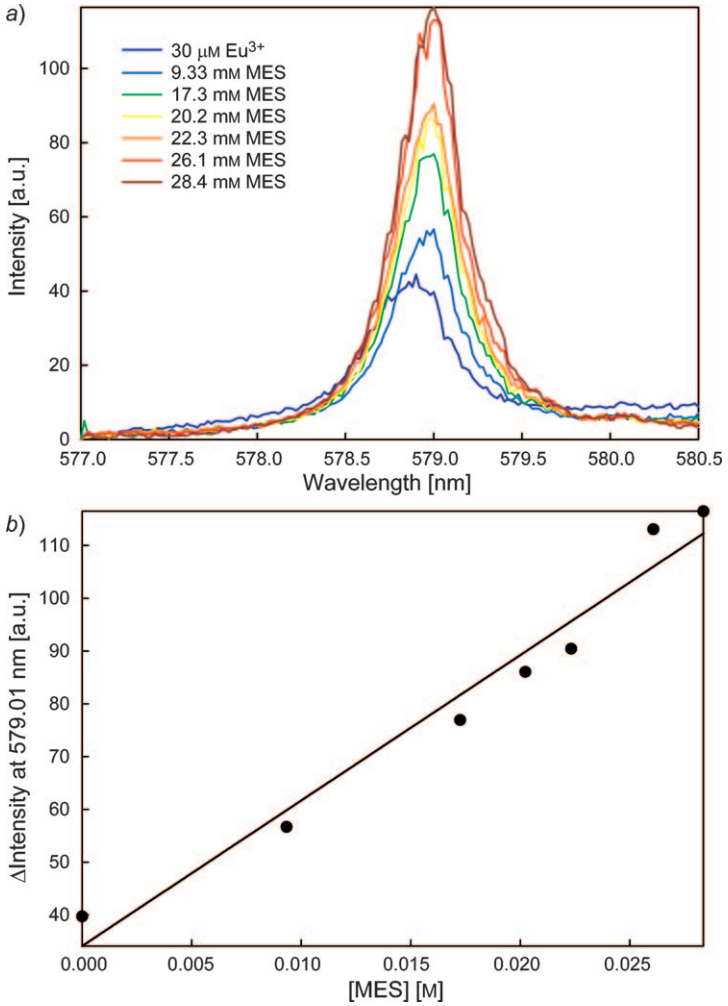

Fig. 3. *a*) *Excitation spectra for the titration of 25 μM Eu^{III}, I = 0.100M NaCl with MES buffered at pH = 6.5. b*) *Change of intensity at 579.00 nm as a function of the MES concentration.*

of bound OH with OD or other ligands typically leads to less efficient de-excitation, such that the photoluminescence lifetime experiments conducted alternately in H_2O and D_2O give information about the number of OH groups coordinated to the Ln^{III} [18–20]. The relationship between the number of bound H_2O molecules, q, and the difference in the rate constants for photoluminescence in H_2O compared to D_2O is given (*Eqn. 1*) for Eu^{III}. Recovered luminescence lifetimes and q numbers for solutions of 25 μM $Eu^{3+}_{(aq)}$ at pH 6.5 in various electrolytes are given in the *Table*. Similar values are obtained at Eu^{III} concentrations ranging from 1 mM to 1 μM [11]. Recovered luminescence lifetimes are not reliable at nanomolar concentrations of Eu^{III}, so there is no information on the number of bound H_2O ligands under these conditions.

$$q = A(k_{H_2O} - k_{D_2O} - \alpha) \quad (1)$$

Table. *Recovered Time-Resolved Luminescence Lifetimes for 25* μM *Eu^{III} in H_2O and D_2O, with 20.0 m*M *MES, and at a pH of 5.0 and 6.5*. Concentration of the salts corresponds to the last point in the titration of the Eu^{III} spectra. Apparent q numbers were calculated using *Eqn. 1* [15].

Salt	pH	λ_{max} [nm]	Wavelength [nm]	Average lifetime in H_2O [μs]	Average lifetime in D_2O [μs]	*Horrocks* q (±0.1)
NaCl	5.0	579.04	578.74	108 ± 5	2945 ± 167	9.5
			579.10	109 ± 6	3113 ± 80	9.5
	6.5	578.74	578.74	107 ± 4	2643 ± 56	9.7
			579.10	109 ± 7	2689 ± 199	9.4
$NaClO_4$	5.0	579.02	578.74	101 ± 7	2834 ± 373	10.3
			579.10	107 ± 4	3055 ± 281	9.7
	6.5	578.74	578.72	114 ± 6	2435 ± 326	8.9
			579.10	118 ± 11	2308 ± 446	8.6
NaF	5.0	578.44	578.44	253 ± 15	296 ± 4	0.3
	6.5	578.44	578.44	214 ± 25	245 ± 12	0.3
NaI	5.0	579.03	578.74	106 ± 8	1423 ± 216	9.3
			579.10	108 ± 6	1350 ± 176	9.1
	6.5	578.92	578.74	111 ± 6	1437 ± 200	8.9
			579.10	116 ± 10	1413 ± 240	8.4
$NaNO_3$	5.0	579.10	578.74	110 ± 1	3093 ± 227	9.4
			579.10	111 ± 0	3239 ± 65	9.3
	6.5	578.74	578.74	117 ± 8	2937 ± 118	8.8
		579.01	579.10	116 ± 4	2870 ± 137	8.8
None	5.0	579.04	578.74	106 ± 5	2263 ± 199	9.6
			579.10	104 ± 4	2344 ± 107	9.8
	6.5	578.74	578.74	109 ± 4	2445 ± 115	9.4
			579.10	117 ± 7	3076 ± 244	8.8

Nd^{III} Quenching Experiments. Solutions containing 1.00 mM $Eu^{3+}_{(aq)}$ were titrated with increasing concentrations of $Nd^{3+}_{(aq)}$ in the presence of either NaCl or $NaNO_3$ (I = 100 mM) at pH 5.0 or 6.5. Nd^{III} is an efficient quencher for Eu^{III} through luminescence resonance energy transfer (LRET) [21]. Experiments carried out with either supporting salt show quenching of Eu^{III} luminescence by Nd^{III} at pH 6.5. Recovered lifetimes of Eu^{III} are shortened to 55 and 69 μs in NaCl and $NaNO_3$ when 10.0 mM of Nd^{III} is present, respectively, from an unquenched value of *ca.* 110 μs. Dissociation

constants were calculated from the decrease of intensity of the excitation peak (*Fig. 4*, *b* and *d*) to give a K_d for the quenched $Eu^{III}-Nd^{III}$ complex of 1.43 ± 0.07 and 1.6 ± 0.2 mM for NaCl and $NaNO_3$ supporting electrolytes, respectively.

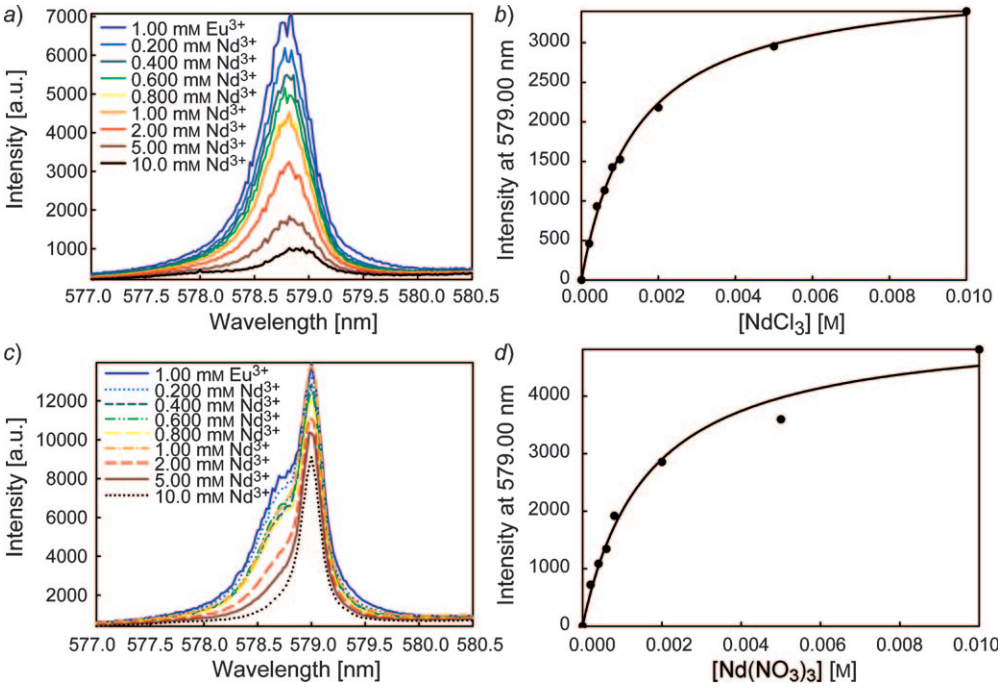

Fig. 4. *Eu^{III} $^7F_0 \rightarrow {}^5D_0$ Excitation spectra in 20.0 mM MES at pH 6.5, while quenching with various amounts of Nd^{III}. a*) Titration of 1.00 mM Eu^{III} with Nd^{III} $I = 0.100$M NaCl. *b*) Binding curve of the absolute change of intensity at 579.00 nm as a function of the Nd^{III} concentration, $I = 0.100$M NaCl, K_d 1.43 ± 0.07 mM. *c*) Titration of 1.00 mM Eu^{III} with Nd^{III}, $I = 0.100$M $NaNO_3$. *d*) Binding curve of the absolute change of intensity at 579.00 nm as a function of the Nd^{III} concentration, $I = 0.100$M NaCl, K_d 1.6 ± 0.2 mM.

The intensity of the Eu^{III} excitation peak decreases for solutions containing both nitrate and chloride. However, there are two peaks for solutions containing $NaNO_3$. One peak is centered at 578.74 (similar to NaCl) and another is centered at 579.00 nm (*Fig. 4*, *a* and *c*). Nd^{III} quenches the 578.74 nm peak, but does not markedly change the $NaNO_3$ specific excitation peak. This is more clearly observed in a plot of the area of the two peaks as a function of Nd^{III} concentration (data not shown). We tentatively assign the species at 578.74 nm to a multinuclear lanthanide species and the species at 579.00 nm to a mononuclear nitrate complex. In contrast, titrations at pH 5.0 with Nd^{III} showed little affect on the intensity of $Eu^{3+}_{(aq)}$ luminescence for solutions containing either NaCl or $NaNO_3$ (*Fig. 5*).

At pH 5.0, the excitation peak for the Eu^{III} species in $NaNO_3$ is more narrow and red-shifted than that of the NaCl species. The excitation wavelengths for both solutions at pH 5.0 were more red-shifted than at pH 6.5 (*Table*). Recovered lifetimes did not change at all in the presence of Nd^{III} at pH 5.0. As a control for speciation changes that

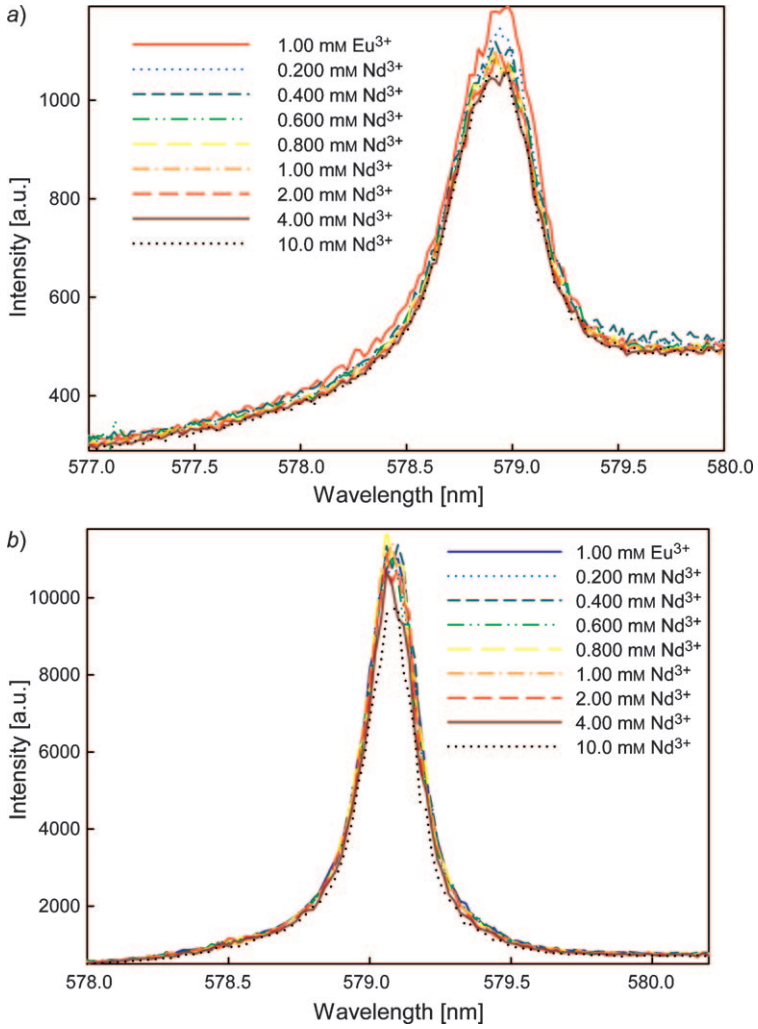

Fig. 5. *Addition of Nd^{III} to Eu^{III} showing the effect on the Eu^{III} $^7F_0 \rightarrow {}^5D_0$ excitation spectra; 20.0 mM MES, pH 5.0. a*) Titration of 1.00 mM Eu^{III} with Nd^{III}, $I = 0.100$M NaCl. *b*) Titration of 1.00 mM Eu^{III} with Nd^{III}, $I = 0.100$M $NaNO_3$.

might affect luminescence properties, La^{III}, which does not quench Eu^{III} luminescence by *Föster* and/or *Dexter* energy-transfer mechanisms, was titrated into similar samples containing either NaCl or $NaNO_3$ at the same ionic strength. No changes in recovered luminescence lifetimes were observed for the La^{III} samples. At pH 6.5, the luminescence intensity of Eu^{III} was slightly reduced as the concentration of La^{III} was increased for both NaCl and $NaNO_3$ experiments (data not shown). No changes in the excitation spectra were observed at pH 5.0 (data not shown) upon addition of La^{III} for either NO_3^- or Cl^- containing solutions.

Luminescence resonance energy transfer (LRET) experiments were carried out to determine inter-lanthanide distances between Nd^{III} and Eu^{III} in the presence of NaCl or $NaNO_3$ at pH 6.5. The quantum yields Φ of the Eu^{III} solutions containing NaCl or $NaNO_3$ were calculated to be 0.012 by using a method pioneered by *Werts* [22]. The overlap integral J was calculated to be 5.36×10^{-18} and 5.06×10^{-18} cm^6 mol^{-1} for NaCl and $NaNO_3$ systems, respectively. The distance for 50% energy transfer, R_0, was 4.78 and 4.76 Å for NaCl and $NaNO_3$, respectively. The distance r between Nd^{III} and Eu^{III} was determined to be 4.8 and 5.2 Å for NaCl and $NaNO_3$ solutions, respectively.

Effect of Added Salts on Eu^{III} Luminescence at pH 6.5. The effect of NaF, NaCl, $NaClO_4$, NaI, and $NaNO_3$ salts on the Eu^{III} $^7F_0 \rightarrow {}^5D_0$ excitation spectrum at pH 6.5 was examined. Samples containing NaCl, $NaClO_4$, or NaI all show a decrease in excitation peak intensity (*Fig. 6*). Solutions containing NaI show an excitation peak with a slight red-shift compared to the peak maximum for the solutions containing NaCl or $NaClO_4$ (*Table*). Plots of luminescence intensity as a function of salt concentration lead to a binding isotherm that was fit to a 1:1 binding equation to give apparent dissociation constants of 41, 45, and 20 mM for NaCl, $NaClO_4$, and NaI, respectively (*Fig. 7,a–c*). In contrast, excitation spectra of $NaNO_3$ and NaF show the formation of new Eu^{III} species by the shift of the excitation maximum from 578.74 nm to 579.04 nm and to 578.45 nm, respectively (*Fig. 8*). Dissociation constants, calculated from fitting of plots

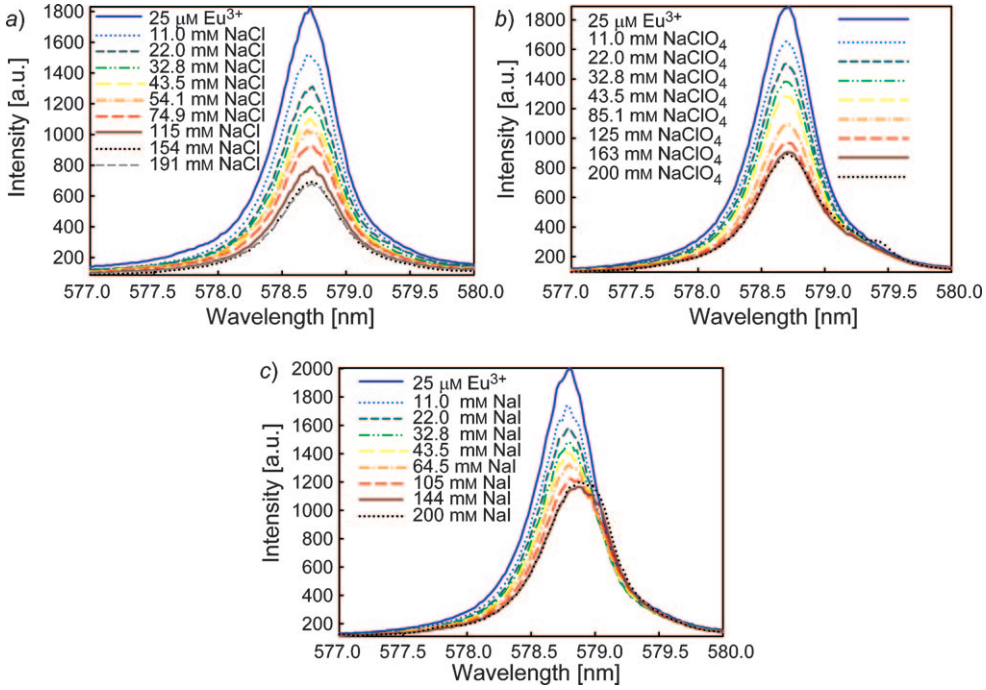

Fig. 6. *$^7F_0 \rightarrow {}^5D_0$ Excitation spectra of 25 μM Eu^{III}, 20.0 mM MES, pH 6.5. a*) Titration with 0 to 191 mM NaCl; K_d 41 mM. *b*) Titration with 0 to 200 mM $NaClO_4$; K_d 45 mM. *c*) Titration with 0 to 200 mM NaI; K_d 20 mM.

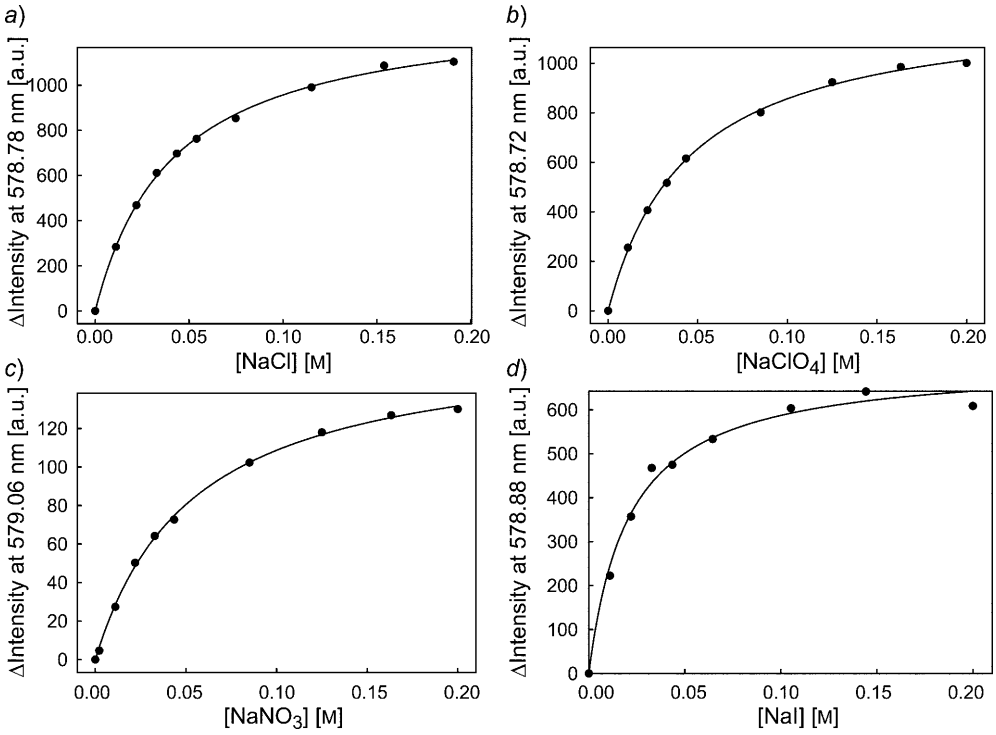

Fig. 7. *Binding curves plotted to a simple 1:1 isotherm at pH 6.5, 20.0 mM MES, 25 μM Eu^{III}. a*) NaCl sample; K_d 41 mM. *b*) $NaClO_4$ sample; K_d 45 mM. *c*) NaI sample; K_d 20 mM. *d*) $NaNO_3$ sample; K_d 54 mM.

of the luminescence intensity as a function of salt concentration to a 1:1 binding curve, were 54 mM for $NaNO_3$ (*Fig. 7,d*) binding. The dissociation curve for NaF with no supporting electrolyte was sigmodal when the intensity of the 578.44 nm peak was plotted *vs.* concentration, therefore, the change in the peak area of the aqueous Eu^{III} was used as a function of concentration giving a K_d of 144 μM (*Fig. 9,a*). When 0.100 mM $NaNO_3$ was used, the K_d was calculated to be 420 μM for the addition of NaF to solutions of Eu^{III} (*Fig. 9,b*).

Effect of Added Salts on Eu^{III} Excitation Luminescence at pH 5.0. We examined the effect of NaF, NaCl, $NaClO_4$, NaI, and $NaNO_3$ concentration on the Eu^{III} $^7F_0 \rightarrow {}^5D_0$ excitation peak at pH 5.0. NaCl and $NaClO_4$ show a slight intensity decrease in the excitation luminescence spectrum as the concentration of salt increases (*Fig. 10*) with no change in the excitation maximum at 579.05 nm. In contrast, titrations with NaI show an increase in the intensity of the Eu^{III} excitation peak and no shift in the peak excitation maximum (*Fig. 11,a*). Addition of $NaNO_3$ shows an increase in excitation luminescence and a shift in excitation wavelength to 579.12 nm. Titration with NaF gave identical results with those at pH 5.0 (data not shown). All excitation spectra at pH 6.5 and 5.0 are summarized in *Fig. 12* to clearly show changes in peak widths and excitation maximums.

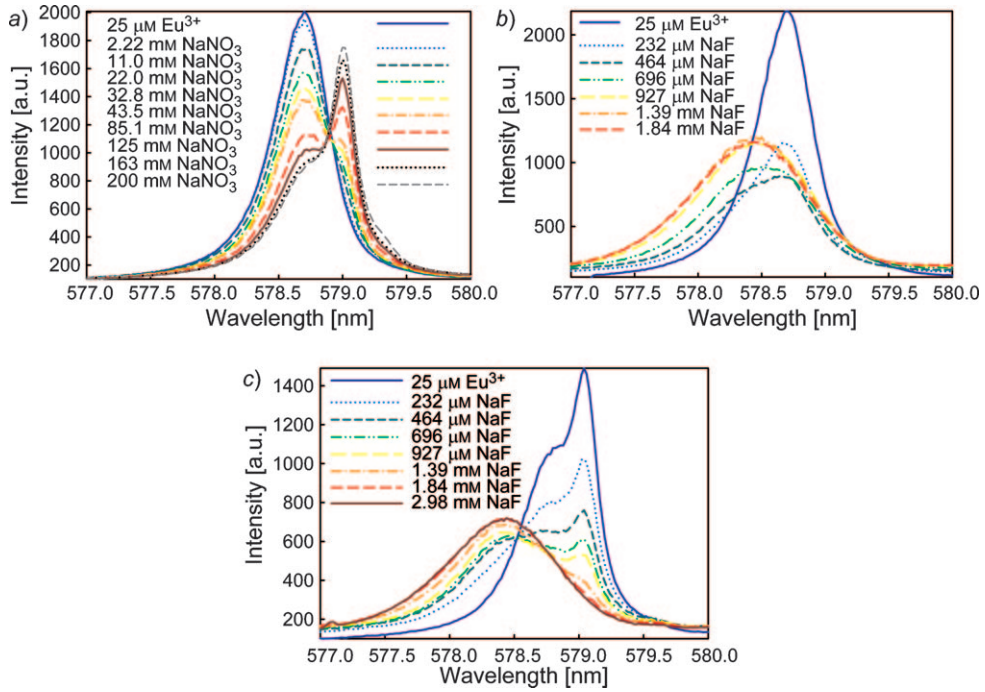

Fig. 8. *$^7F_0 \rightarrow {}^5D_0$ Excitation spectra of 25* μM *Eu^{III}, 20.0 m*M *MES, pH 6.5. a*) Titration with 0 to 200 mM $NaNO_3$; K_d 53.7 mM. *b*) Titration with 0 to 1.84 mM NaF; K_d 144 μM. *c*) Titration with 0 to 2.98 mM NaF, with 0.100M $NaNO_3$; K_d 420 μM.

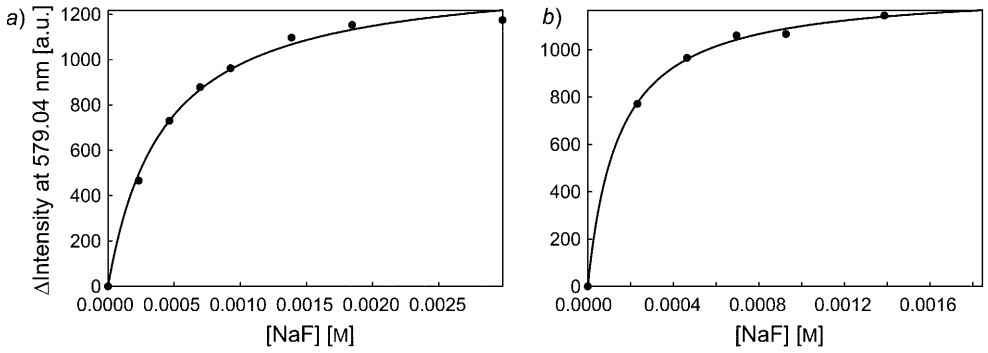

Fig. 9. *Binding curves plotted to a simple 1:1 isotherm at pH 6.5, 20.0 m*M *MES, 25* μM *Eu^{III}. a*) NaF sample; no salt; K_d 144 μM. *b*) NaF sample; $I = 0.100$M $NaNO_3$; K_d 420 μM.

Time-Resolved Luminescence Studies. Time-resolved luminescence of the Eu^{III} excited state lifetime yields additional information on the environment of the Eu^{III} such as the number of innersphere H_2O molecules around the cation. All of the recovered lifetimes in H_2O and D_2O are summarized in the *Table.* Standard deviations

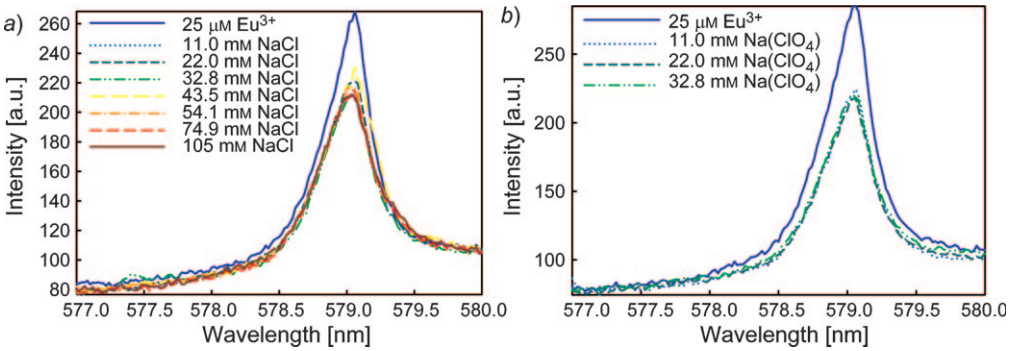

Fig. 10. *$^7F_0 \rightarrow {}^5D_0$ Excitation spectra of 25 μM Eu^{III}, 20.0 mM MES, pH 5.0. a*) Titration with 0 to 105 mM NaCl. *b*) Titration with 0 to 32.8 mM $NaClO_4$.

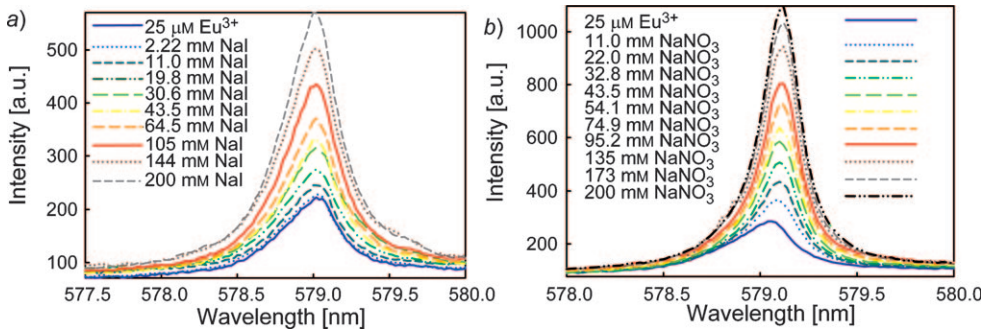

Fig. 11. *$^7F_0 \rightarrow {}^5D_0$ Excitation spectra of 25 μM Eu^{III}, 20.0 mM MES, pH 5.0. a*) Titration with 0 to 200 mM NaI. *b*) Titration with 0 to 200 mM $NaNO_3$.

in luminescence lifetimes for Eu^{III} complexes are generally $< 8\%$ for measurement of multiple samples prepared on different days [11]. Apparent q numbers were calculated based on *Eqn. 1*. Note that the parameters in *Eqn. 1* were largely determined for Eu^{III} complexes with multidentate ligands and are not necessarily accurate for highly hydrated Eu^{III} [13–15]. Poor parameters, and in some cases, additional excited state quenching contributions from anions may lead to q numbers that are higher than the expected value of nine.

The q numbers do not vary more than *ca.* 1 with respect to different supporting electrolytes, excluding NaF. The NaF solutions gave Eu^{III} species with an apparent q number of 0.3. This normally would indicate that all H_2O molecules have been displaced and the identical q number at pH 5.0 and 6.5 would suggest that the NaF species was insensitive to pH. However, the exceptionally short lifetime of the Eu^{III} complex in D_2O (250–300 μs) suggests that an additional quenching mechanism is operative, making it difficult to determine the number of bound H_2O molecules. The general trend in the *Table* shows that, with the exception of solutions containing NaCl and NaF, Eu^{III} species have slightly larger apparent q numbers in solutions at pH 5.0

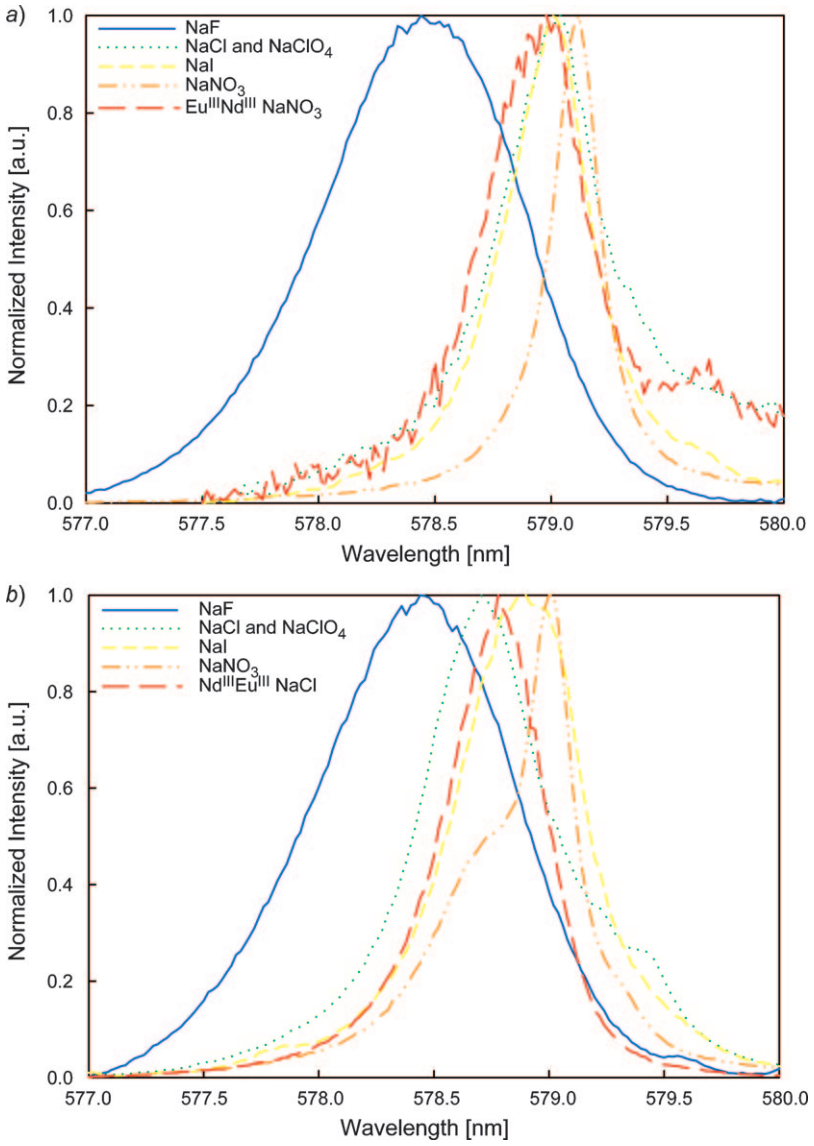

Fig. 12. *Normalized* $^7F_0 \rightarrow {}^5D_0$ *excitation spectra of 25* μM Eu^{III} *in solution with various salts at concentrations of the last point of the titration curve, 20.0* mM *MES, a) pH 5.0, and b) pH 6.5.*

than at pH 6.5. This is consistent with the expectation that the Eu^{III} species at pH 6.5 is a OH complex and thus has fewer OH groups for quenching than the species at pH 5.0.

Steady State Eu^{III} *Emission Spectra.* Emission spectra were collected on 25 μM Eu^{III} salt solutions by exciting the $^7F_0 \rightarrow {}^5D_2$ transition (*ca.* 465 nm). At pH 5.0, samples containing no salt, NaCl, $NaClO_4$, and NaI had similar emission spectra (*Fig. 13*). The NaI sample had decreased emission intensity relative to no salt at the $^5D_0 \rightarrow {}^7F_4$

emission. The $NaNO_3$ and NaF samples had emission spectra with distinctly different intensities compared to each other or spectra for the other salts. The $NaNO_3$ sample showed an enhancement of all the detectable emission bands $^5D_0 \rightarrow {}^7F_1$, $^5D_0 \rightarrow {}^7F_2$, and $^5D_0 \rightarrow {}^7F_4$. The NaF showed a relative decrease in emission of all the bands and a slight blue-shift in the emission intensity of the $^5D_0 \rightarrow {}^7F_2$ band from *ca.* 618 to 616 nm.

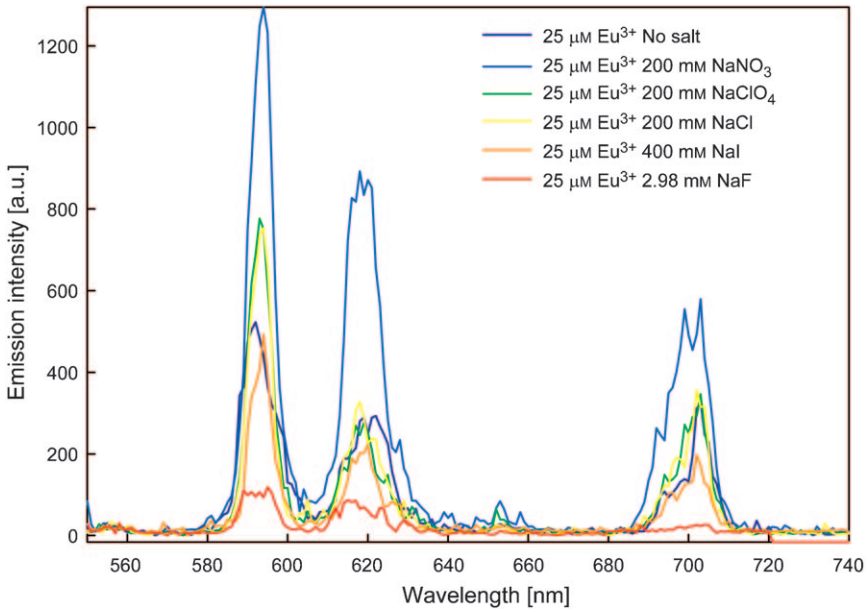

Fig. 13. *Emission spectra of 25.0 μM Eu^{III} solutions containing various salts at mM concentrations, pH 5.0, and with 20.0 mM MES.* Excitation at the $^5D_2 \rightarrow {}^7F_0$ transition (*ca.* 465 nm).

At pH 6.5, similar trends to pH 5.0 are observed for the sample containing no salt, NaCl, $NaClO_4$, or NaI (*Fig. 14*). The NaI sample had decreased emission intensity relative to no salt at the $^5D_0 \rightarrow {}^7F_4$ emission. The $NaNO_3$ and NaF samples had emission spectra significantly different from each other and spectra for solutions with other salts. The $NaNO_3$ sample showed an enhancement of all the detectable emission bands $^5D_0 \rightarrow {}^7F_1$, $^5D_0 \rightarrow {}^7F_2$, and $^5D_0 \rightarrow {}^7F_4$. The NaF showed a relative decrease in emission of all the bands and a blue-shift in the emission intensity of the $^5D_0 \rightarrow {}^7F_2$ band from *ca.* 618 to 616 nm.

Discussion. – The data presented here show the utility of excitation luminescence spectroscopy for studying Eu^{III} aqueous solution chemistry. Excitation spectra collected at different pH values clearly indicate changes in the speciation of $Eu^{3+}_{(aq)}$ by showing shifts in excitation maxima and changes in the luminescence intensity. Changes in excitation spectra over the pH range of 5.0 to 6.5 are consistent with ionization of a bound H_2O molecule of $Eu^{3+}_{(aq)}$ to give a hydroxide complex which is substantially more luminescent than the complex at acidic pH. The observed pH dependence of the excitation spectra between pH 6 and 7 shows that the the Eu^{III} H_2O ligand deprotonates

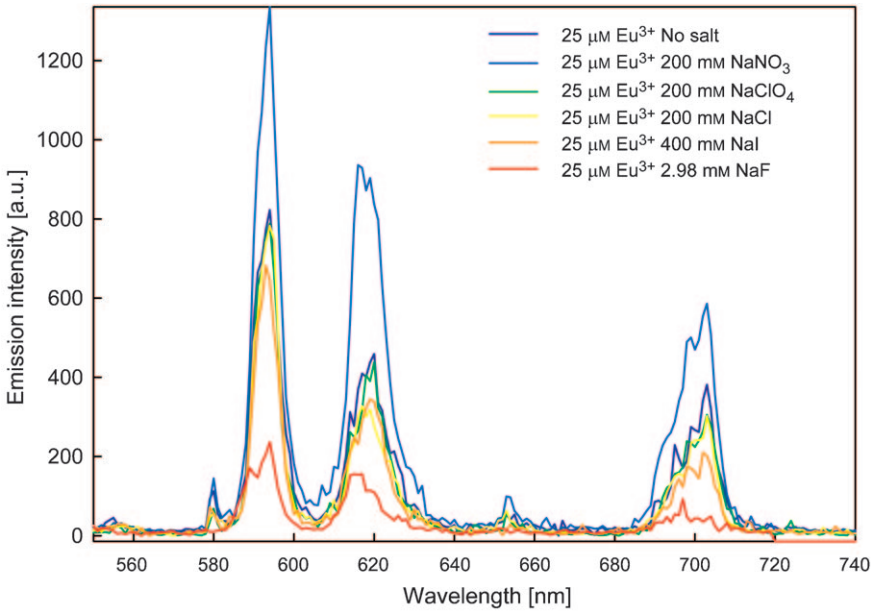

Fig. 14. *Emission spectra of 25.0* μM *Eu*^{III} *samples containing various salts at m*M *concentrations, pH 6.5, and with 20.0 m*M *MES.* Excitation of $^5D_2 \rightarrow {}^7F_0$ transition (*ca.* 465 nm).

over this range. By comparison, the H_2O ligand pK_a obtained from pH potentiometric titrations is close to 8 [1][23].

The form of the hydroxide complex is, however, not simple. Our data suggest that the major Eu^{III} species at pH 6.5 and millimolar concentrations is a dinuclear (or multinuclear complex), in contrast to the species at pH 5.0 which is present predominantly as the mononuclear species. This is supported by both concentration dependent excitation spectra and by the effect of Nd^{III} ions on Eu^{III} luminescence intensity. The plot of Eu^{III} luminescence intensity of the 578.74 nm excitation peak as a function of $Nd^{3+}_{(aq)}$ shows a decrease in the intensity with increasing Nd^{III} concentration. The intensity change is curved, not linear, consistent with the formation of a $Nd^{III}-Eu^{III}$ complex at pH 6.5. This heterodinuclear $Nd^{III}-Eu^{III}$ complex presumably contains bridging OH ligands that maintain the dinuclear complex framework [24]. No such changes are observed at pH 5.0, conditions where hydrolysis and dimerization are not favorable. Changes in luminescence lifetimes of the proposed $Nd^{III}-Eu^{III}$ complex at pH 6.5 are consistent with Ln–Ln distances of 4.8 to 5.2 Å. This distance is larger than that reported for crystal structures of Tm^{III} dimers, *ca.* 4 Å with two bridging OH molecules [25]. However, the nature of the multinuclear complex and the number of bridging OH molecules is unknown. The longer distance measured by LRET is consistent with the larger ionic radii for the early to mid lanthanide^{III} ions used here compared to Tm^{III}.

Our data shows that there are multiple Eu^{III} species present at pH 6.5 that may include $[Eu(H_2O)_9]^{3+}$, $[Eu(OH)(H_2O)_8]^{2+}$, and $[(Eu_2(\mu\text{-}OH)_2(H_2O)_{16}]^{4+}$. Other

possible species include an eight coordinate complex, $[Eu(H_2O)_8]^{3+}$, which is in equilibrium with the nine coordinate Eu^{III} complex [26]. The q number for $Eu^{3+}_{(aq)}$ at pH 5.0 and 6.5 (*Table*) is most consistent with nine coordinate Eu^{III} complexes. The slightly lower q value at pH 6.5 compared to pH 5.0 under most conditions is consistent with expectations for a OH complex if a OH ligand is considered to contribute one half the quenching contribution of a H_2O ligand. However, OH quenching parameters for terminal and bridging OH ligands have not been previously determined, so this assumption is not tested. In any case, the experimental uncertainties of the recovered luminescence lifetimes (*Table*) show that the q numbers of the Eu^{III} complexes at pH 5.0 and 6.5 are generally within experimental error of each other. This attests to the difficulty of sorting out speciation changes by using luminescence lifetimes alone.

Our excitation luminescence spectra are consistent with the formation of additional species above pH 7.0, most likely due to multinuclear complexes of Eu^{III} that are extensively hydrolyzed. Previous studies using pH-potentiometry show that basic solutions of Eu^{III} contain both monomeric and multimeric Eu^{III} hydroxide complexes with one, two or three OH molecules [1], consistent with the multiple excitation peaks observed here.

The low Eu^{III} and salt concentrations and the near neutral pH values used here are different from those of previous studies which featured high Eu^{III} (200 mM) concentrations at uncontrolled pH or at acidic pH [7][9][10]. However, our data are comparable to many previous observations. Monoanions such as Cl^- and ClO_4^- have been suggested to form outersphere complexes with Eu^{III} in the work of *Choppin* [10], *Horrocks* [7], and others [5][27]. In contrast, other anions such as F^- and NO_3^- [7][28][29] are thought to form inner-sphere complexes with Eu^{III}. In our studies, both NO_3^- and F^- influence the hypersensitivity of the $^5D_0 \rightarrow {}^7F_2$ emission, the excitation maximum of the $^7F_0 \rightarrow {}^5D_0$ transition, and the recovered time-resolved luminescence lifetime, characteristic of innersphere complexes. The excitation maximum of the $^7F_0 \rightarrow {}^5D_0$ peak blue-shifts for F^- and red-shifts for NO_3^-, compared to $Eu^{3+}_{(aq)}$ in the absence of a salt or in the presence of NaCl or $NaClO_4$ at pH 5.0 and 6.5. Notably, the excitation maxima of the $^7F_0 \rightarrow {}^5D_0$ Eu^{III} peaks are similar for $NaNO_3$ at both pH 5.0 and 6.5, as well as for NaF at both pH 5.0 and 6.5. The difference in the appearance of the excitation spectra at the two pH values results from the different speciation of $Eu^{3+}_{(aq)}$ at pH 5.0 compared to 6.5. For example, addition of either NO_3^- or F^- to a solution containing $Eu^{3+}_{(aq)}$ at pH 5.0 leads to a new peak of different luminescence intensity, frequency, and peak width, consistent with the formation of an innersphere complex. At pH 6.5, however, the new excitation peaks of the F^- or NO_3^- complexes are shifted from that of the $Eu^{3+}_{(aq)}$ complex but are of similar intensity because the predominant $Eu^{3+}_{(aq)}$ species at pH 6.5 is a more highly emissive complex. The distinct emission spectral changes are supportive of the formation of innersphere complexes by NO_3^- and F^- at both pH values.

Our emission and excitation data for the F^- complex are consistent with an innersphere complex, however, luminescence lifetime data is inconclusive for the number of bound H_2O molecules. The extremely short recovered luminescence lifetimes in D_2O at both pH values show that the F^- ions themselves are very effective quenchers. In addition, the luminescence lifetime of the F^- complex in H_2O is shorter than anticipated for a complex with no bound H_2O molecules according to equations

that enable the calculation of q based on luminescence lifetimes in H_2O alone [13]. In contrast, the q number of the NO_3^- complex supports the displacement of a single bound H_2O molecule in comparison to solutions containing NaCl. The dissociation constant for NO_3^- bound to Eu^{III} here is 54 mM. The K_d of the Eu^{III} complex with NO_3^- has been previously reported to be 0.709 ± 0.101M [7], however, direct comparison is not possible because the pH for these experiments was not reported. No binding constants for F^- complexes have been reported previously, to the best of our knowledge. In the absence of $NaNO_3$, the K_d is 144 μM based on the decrease of the aqueous Eu^{III} peak area. In the presence of $NaNO_3$, the K_d for the formation of a 1:1 complex with fluoride is 420 μM.

The analysis of the data of the other anions investigated in this study is not as clear. At pH 6.5, NaCl, $NaClO_4$, and NaI reduce the intensity of the Eu^{III} aqueous species excitation spectra from that of the initial low salt condition. Yet, emission spectra for $Eu^{3+}_{(aq)}$ are similar for all of these different salts, arguing against innersphere coordination. The recovered time-resolved lifetime decays show that the apparent q numbers do not change markedly under these conditions. *Choppin et al.* speculated that changes in the inner hydration sphere due to anions in the secondary solvation sphere could increase the quenching of O–H oscillators and increase the observed q numbers [4][8][10]. Such outersphere interactions might change the strength of interaction of the innersphere H_2O molecules and their orientation about the Eu^{III}. Increasing the symmetry around the Eu^{III} cation, specifically an inversion center, makes the electronic transitions *Laporte* forbidden and less allowed. Similarly, *Billard* notes that the addition of various salts changes the bulk properties of H_2O and that the change in these bulk properties is responsible for differences in H_2O interactions with $Eu^{3+}_{(aq)}$ that give rise to changes in luminescence properties [12]. A third possibility is that the addition of a supporting electrolyte changes the speciation of $Eu^{3+}_{(aq)}$ at pH 6.5. A shift in the mononuclear to dinuclear equilibrium or in the pK_a of the Eu^{III} H_2O ligands would give rise to a change in the excitation luminescence spectrum. The larger effects of NaCl, $NaClO_4$, and NaI on the luminescence spectrum of $Eu^{3+}_{(aq)}$ at pH 6.5 compared to pH 5.0 support this latter hypothesis. The formation of different Eu^{III} hydroxide species is underway at pH 6.5, and a slight change in their equilibrium could account for the changes in luminescence intensity.

Lastly, the speciation at pH 5.0 for no salt, NaCl, $NaClO_4$, and NaI is apparently less complex than at 6.5, but just as intriguing. The largest apparent q number is observed for $NaClO_4$ at 10.3 H_2O molecules. For the $NaClO_4$ system, an apparent increase of the Eu^{III} innersphere H_2O molecules has been observed previously in the literature [4][7][8][10], but at much greater concentrations of Eu^{III} (*ca.* 20 mM) and at molar concentrations of $NaClO_4$. Neither $NaClO_4$ nor NaCl show any shifting of the Eu^{III} excitation peak maximum under our conditions, but a slight decrease of the excitation luminescence occurs for both systems. This decrease in luminescence intensity is less pronounced than observed at pH 5.0, and possibly results from a shift in speciation equilibrium between the fully hydrated Eu^{III} and the more emissive OH complex which is present in low concentrations under these conditions. The NaI system is by far the oddest. The excitation maximum does not shift in the presence of NaI, but substantial increases in the excitation peak intensity are observed. This could be due to the large asymmetric electric field of the solvated I^- species [30] affecting the hypersensitivity of

the $^5D_0 \rightarrow {}^7F_2$ transition. Notably, unlike previous reports on Eu^{III} complexes [31], I^- does not markedly quench Eu^{III} luminescence.

Conclusions. – Eu^{III} $^7F_0 \rightarrow {}^5D_0$ excitation luminescence spectroscopy is a useful tool to probe the solution chemistry of Eu^{III} solutions at micromolar concentrations and near neutral pH. Our results show surprisingly complex Eu^{III} speciation. At pH 5.0, the Eu^{III} aqua complex is predominantly a monomer but at pH 6.5, hydroxide species that include a multinuclear Eu^{III} complex are formed. Monoanions such as F^- and NO_3^- form innersphere complexes. The most curious effects are for weak monoanions such as Cl^- and ClO_4^- that quench luminescence at pH 5.0 and 6.5 at millimolar concentrations of anion, but do not appear to form innersphere complexes. Changes in speciation for micromolar concentrations of Eu^{III} are more readily documented by $^7F_0 \rightarrow {}^5D_0$ excitation spectroscopy than by time-resolved luminescence spectroscopy alone. In addition, Eu^{III} excitation spectroscopy in combination with luminescence resonance energy transfer studies to other lanthanide ions such as Nd^{III} are useful for probing lanthanide ion speciation.

J. R. M. thanks the *National Science Foundation for a Major Instrumentation Award* (CHE-0321058) to build the MOPO/laser system and *NSF* grants (CHE-0415356 and -0911375) for support of this work.

Experimental Part

General. Europium(III) and Neodymium(III) salts were purchased from *Sigma–Aldrich*, and 2-(*N*-morpholino)ethanesulfonic acid (MES) was from *Fisher Scientific*. The Eu^{III} stock solns. (20.0 mM or greater) were standardized by an EDTA titration with an arsenazo(III) indicator [32]. Aq. solns. were prepared using triply dist. H_2O or in D_2O purchased from *Cambridge Isotope Labs, Inc.*

Soln. Preparation for Nd^{III}/Eu^{III} LRET Experiments. For Nd^{III} ($NdCl_3$ or $Nd(NO_3)_3$) quenching studies 1.00 mM Eu^{III} ($EuCl_3$ or $Eu(NO_3)_3$) were prepared with 20.0 mM MES (pH 5.0 or 6.5) and an ionic strength of 0.100M (either NaCl or $NaNO_3$). Solns. with increasing concentrations of Nd^{III} from 0 to 10.0 mM were prepared while keeping the concentration of Eu^{III} constant at 1.00 mM.

Eu^{III} Steady State Excitation Luminescence. Luminescence experiments were conducted using a *Spectra-Physic Nd : YAG* pump laser (*Spectra-Physics*, model *Quanta Ray PRO-270-10*) operating at the third harmonic (355 nm). The *Nd : YAG* laser output pumps a master oscillator power oscillator, MOPO, optical parametric device (*Spectra-Physics*, model *Quanta Ray MOPO-SL*) is described in detail elsewhere [11]. Excitation scans were collected between 577–581 nm ($^7F_0 \rightarrow {}^5D_0$) in 0.02 nm increments while monitoring the emission of the $^5D_0 \rightarrow {}^7F_2$ band at *ca.* 617 nm using a bandpass filter and a gated photomultipler tube (*Hamamatsu*, model *H7680-01MOD*) with a 2 μs delay. Ten shots were averaged per increment and the average power output of the MOPO was *ca.* 55 mJ. Dissociation constants, K_d, were determined using Sigma Plot 10 (*Systat Software, Inc.*) and a simple one-to-one ligand binding curve.

Eu^{III} Time-Resolved Luminescence. Using the same laser system, five time-resolved luminescence decays of the $^5D_0 \rightarrow {}^7F_2$ emission band were collected while exciting the $^7F_0 \rightarrow {}^5D_0$ transition. These decays consisted of 512 laser pulses at three different wavelengths and were averaged for three different samples. Intensity decay measurements were collected and fit to exponential decay models using GraphPad Prism 4 v4.03 (*GraphPad Software, Inc.*). In all cases, the best fit model for the time-resolved intensity decay traces was a single exponential decay as determined by the linearity of log intensity *vs.* time plots and a symmetrical distribution of the residuals about zero. In some cases, the first 20 μs of the decay were not fit due to asymmetric distributions in the residual plots. This short component is sometimes observed in the time-resolved luminescence lifetimes and ranges from 1 to 30 μs. We have been unable to correlate this to sample effect and believe it to be an instrument artifact.

Eu^{III} Steady State Emission Luminescence. Samples were excited at the $^7F_0 \rightarrow {}^5D_2$ excitation transition (464–467 nm). Emission scans were collected in 1.0 nm increments using a monochromator

(*CVI Laser Corporation CM110*) and detected by a thermoelectrically-cooled photomultiplier tube (*Hamamatsu*, model *R928* with a gated socket *C1392–57*). Entrance and exit slits of the monochromator were 0.125 mm giving a bandpass of *ca.* 1 nm. Emission spectra were blank corrected. Quantum yields were calculated from the area of the Eu^{III} emission peaks using PeakFit v4.12 (*SeaSolve Software, Inc.*); a *Voigt* function was chosen to fit the peaks in order to determine peak center maximum, full width at half-maximum (FWHM or peak width) and peak area.

Absorbance Spectra. UV/VIS Absorbance spectra were measured by using a *Beckman DU 800* UV/VIS spectrophotometer for luminescence resonance energy transfer experiments. Spectra were collected with a resolution of *ca.* 2 nm from 200–900 nm. A 5 cm pathlength quartz cell (*Starna Cells Inc.*) was used with a custom built cell holder. Samples were prepared at concentrations of 1.00 mM for Nd^{III} under conditions similar to the samples used for steady state and time-resolved photoluminescence spectroscopy experiments.

REFERENCES

[1] C. F. Baes, R. E. Mesmer, 'The Hydrolysis of Cations', 2nd edn., Robert E. Krieger Publishing Company, Inc., Malabar FL, 1976.
[2] W. D. Horrocks, in 'Methods in Enzymology', Eds. J. F. Riordan, B. L. Vallee, Academic Press Inc., San Diego, CA, 1993, Vol. 226, p. 495.
[3] D. Parker, R. S. Dickins, H. Puschmann, C. Crossland, J. A. K. Howard, *Chem. Rev.* **2002**, *102*, 1977.
[4] G. R. Choppin, D. R. Peterman, *Coord. Chem. Rev.* **1998**, *174*, 283.
[5] J. A. Rard, *Chem. Rev.* **1985**, *85*, 555.
[6] J.-C. G. Bünzli, A. Milicic-Tang, in 'Handbook of the Physics and Chemistry of Rare Earths', Eds. K. A. Gschneidner, L. Eyring, Elsvier Science B. V., Amsterdam, Netherlands, 1995, Vol. 21, pp. 306–362.
[7] P. J. Breen, W. D. Horrocks Jr., *Inorg. Chem.* **1983**, *22*, 536.
[8] G. R. Choppin, *J. Alloys Compd.* **1997**, *249*, 9.
[9] A. Nehlig, M. Elhabiri, I. Billard, A.-M. Albrecht-Gary, K. Lützenkirchen, *Radiochim. Acta* **2003**, *91*, 37.
[10] S. Lis, G. R. Choppin, *Mater. Chem. Phys.* **1992**, *31*, 159.
[11] C. M. Andolina, W. G. Holthoff, P. M. Page, R. A. Mathews, J. R. Morrow, F. V. Bright, *Appl. Spectrosc.* **2009**, *63*, 483.
[12] I. Billard, in 'Handbook on the Physics and Chemistry of Rare Earths', Eds. K. A. Gschneider, J.-C. G. Bünzli, V. K. Pecharsky, Elsevier, Amsterdam, Netherlands, 2003, Vol. 33, pp. 465–514.
[13] T. Kimura, Y. Kato, *J. Alloys Compd.* **1995**, *225*, 284.
[14] A. Beeby, I. M. Clarkson, R. S. Dickins, S. Faulkner, D. Parker, L. Royle, A. S. de Sousa, J. A. G. Williams, M. Woods, *J. Chem. Soc., Perkin Trans. 2* **1999**, 493.
[15] R. M. Supkowski, W. D. Horrocks Jr., *Inorg. Chim. Acta* **2002**, *340*, 44.
[16] W. G. Holthoff, Ph.D. Thesis, University at Buffalo, The State University of New York, 2007.
[17] R. A. Mathews, Ph.D. Thesis, University at Buffalo, The State University of New York, 2008.
[18] Y. Haas, G. Stein, J. Würzberg, *J. Chem. Phys.* **1974**, *60*, 258.
[19] G. Stein, E. Würzberg, *J. Chem. Phys.* **1975**, *62*, 208.
[20] J. L. Kropp, M. W. Windsor, *J. Chem. Phys.* **1965**, *42*, 1599.
[21] W. D. Horrocks Jr., D. R. Sudnick, *Acc. Chem. Res.* **1981**, *14*, 384.
[22] M. H. V. Werts, R. T. F. Jukes, J. W. Verhoeven, *Phys. Chem. Chem. Phys.* **2002**, *4*, 1542.
[23] E. N. Rizkalla, G. R. Choppin, in 'Handbook on the Physics and Chemistry of Rare Earths', Eds. K. A. Gschneidner, L. Eyring, Elsevier Science Publishers, Amsterdam, Netherlands, 1991, Vol. 15, pp. 393–442.
[24] H. G. Brittain, *Inorg. Chem.* **1979**, *18*, 1740.
[25] R. E. Wilson, S. Skanthakumar, G. Sigmon, P. C. Burns, L. Soderholm, *Inorg. Chem.* **2007**, *46*, 2368.
[26] L. Tilkens, K. Randall, J. Sun, M. T. Berry, P. S. May, *J. Phys. Chem. A* **2004**, *108*, 6624.
[27] S. Chaussedent, A. Monteil, *J. Chem. Phys.* **1996**, *105*, 6532.

[28] M. Albin, W. D. Horrocks Jr., *Inorg. Chem.* **1985**, *24*, 895.
[29] S. T. Frey, W. D. Horrocks, *Inorg. Chim. Acta* **1995**, *229*, 383.
[30] J. D. Smith, R. J. Saykally, P. L. Geissler, *J. Am. Chem. Soc.* **2007**, *129*, 13847.
[31] F. Kielar, C. P. Montgomery, E. J. New, D. Parker, R. A. Poole, S. L. Richardson, P. A. Stenson, *Org. Biomol. Chem.* **2007**, *5*, 2975.
[32] J. S. Fritz, R. T. Oliver, D. J. Pietrzyk, *Anal. Chem.* **1958**, *30*, 1111.

Received May 5, 2009