The measurement of the fluorescence detected circular dichroism (FDCD) from a chiral Eu(III) system

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The first measurement of the fluorescence detected circular dichroism (FDCD) from a chiral Eu(III) system, made possible by use of a variable liquid crystal circular polarizer, is reported.

The measurement of the differential absorption of circularly polarized light, *i.e.* circular dichroism (CD) through the measurement of the differential luminescence intensity resulting from the differential absorption, *i.e.* fluorescence detected circular dichroism (FDCD) has long been recognized as a way of probing the chirality of absorption chromophores that lead to luminescence.[1–4] This approach may also be a way to study local chirality of a specific chromophore in complex systems, since overlapping absorption chromophores that do not lead to luminescence will not contribute to the total signal.

Applications of FDCD have been limited due to complications associated with data analysis and various experimental problems. For strongly absorbing or concentrated solutions, the output differential fluorescence intensities must be corrected for differential absorption of the incident light as it passes from the front to back of the sample cell. More problematic are intrinsic polarization artifacts due to the non-isotropic orientational distribution of emitting molecules due to photoselection effects. Various experimental techniques have been proposed and used to deal with these experimental constraints,[5,6] but the potential artifacts have severely affected the general utility of this spectroscopic technique.

In this communication we report on the first measurement of FDCD from a chiral lanthanide(III) system. In particular, we have measured the CD of a perturbed chiral equilibrium involving the tris(terdentate) complex of Eu(III) with 2,6-pyridine-dicarboxylate (dipicolinate = DPA). This complex is known to form chiral racemic D_3 complexes in aqueous solution which can be made non-racemic through the addition of chiral non-coordinating environment compounds.[7–9] The perturbed racemic mixture used for the new measurements reported here has been chosen only because it is well characterized, and CPL and conventional CD results have previously been published. This lanthanide system is particularly suited to the FDCD technique, since the intraconfigurational f → f absorptions are generally very weak ($\varepsilon < 1$), eliminating the data analysis complication, and the Eu(III) excited state lifetime is very long (approximately 1 ms) allowing for complete scrambling of any photoselected orientational distribution in solutions. Furthermore, transitions of Eu(III) that obey magnetic-dipole selection rules ($\Delta J = 0, \pm 1$, except $0 \leftrightarrow 0$) are very often associated with large chiral discriminations with circularly polarized light.

An experimental consequence of the long excited state lifetime is that the conventional way of generating circularly polarized exciting light using high frequency (*e.g.* 50 kHz) photoelastic modulators may not be used since this is insufficient time for the excited molecules to relax to the ground state. To overcome this problem, as described below, we employ a liquid crystal quarter wave polarizer (LCP) with a variable cycle time which may be calibrated for the absorption wavelength selected.

$Eu(DPA)_3^{3-}$ solutions were prepared from stock solutions of Eu(III) ($EuCl_3$, Aldrich) and 2,6-pyridine dicarboxylic acid (Aldrich). The pH of the final solutions were adjusted to a final pH of approximately 5. The Eu(III) concentration was 0.02 M. The same quartz fluorescence cuvette was used for both CPL and FDCD measurements. The ratio of Eu(III) to ligand was 1 : 3.5 to ensure complete formation of the tris complex. (+)-Dimethyl-L-tartrate, (+)-DMT, was added to the solutions prepared above and stirred until clear. The ratio of Eu(III) to (+)-DMT in the solutions examined in this work was 1 : 275.

Total emission and CPL measurements were made on a system described previously.[10] FDCD measurements were made possible by a straightforward modification of the CPL spectrometer. The output of a Coherent CR-599 tunable dye laser was directed through a liquid crystal quarter-wave polarizer (Meadowlark Optics, LVR-100-VIS-IL) connected to a liquid crystal controller (Meadowlark Optics, B1020) oriented at 90° to the direction of emission detection. The total emission intensity was measured in phase with the incident polarization modulation and used to calculate the FDCD signal as described below. The pump laser was a Coherent Innova-70, and the dye used was rhodamine 6G. The voltages applied by the liquid crystal controller were selected to correspond to left and right circularly polarized light at 590 nm. Characterization of the instrument set-up including calibration procedures will be described in a forthcoming publication.[11]

In Fig. 1A we show a schematic partial energy level diagram for Eu(III). Only the Eu(III) states involved in the measurements reported here are given in this figure. In D_3 symmetry there are two possible states for a $J = 3$ term. It should be noted that the $^7F_1$ set of crystal field states is situated only 320 cm^{-1} above the ground state, so that at room temperature it has a significant thermal population (~15%). In Fig. 1B we plot the total emission spectrum for an aqueous solution of $Eu(DPA)_3^{3-}$ in the region of the $^5D_0 \rightarrow {}^7F_2$ transition. In Fig. 2 we plot the luminescence spectrum of an aqueous solution of $Eu(DPA)_3^{3-}$ for

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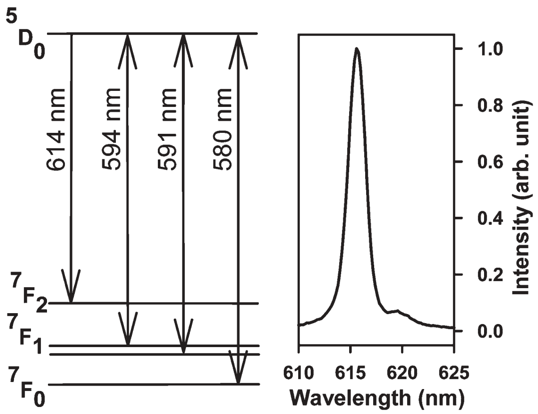

Fig. 1 (A) Approximate partial energy level diagram for Eu(III). (B) Total luminescence spectrum of $Eu(DPA)_3^{3-}$ in the spectral region corresponding to the $^5D_0 \rightarrow {}^7F_2$ transition. λ_{exc} = 285 nm.

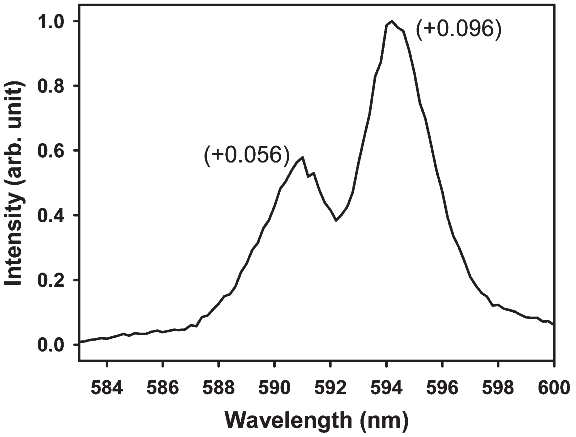

Fig. 2 Total luminescence spectrum for the $^5D_0 \rightarrow {}^7F_1$ transition of a 0.02 M aqueous solution of $Eu(DPA)_3^{3-}$ containing 5.5 M (+)-DMT. Numbers in parentheses correspond to the experimental g_{lum} values measured at the peak wavelengths.

the $^5D_0 \rightarrow {}^7F_1$ transition into which the (+)-DMT has been added. It has been shown previously that addition of (+)-DMT has the effect of perturbing the racemic equilibrium between the Δ and Λ forms of this complex without significantly affecting the molecular structure.[7] This has the result of making the initially racemic complex net optically active. In CPL spectroscopy it is common to report the net chirality of a transition in terms of the luminescence dissymmetry factor, g_{lum}, which is defined as follows

$$g_{\rm lum} = \frac{I_{\rm L} - I_{\rm R}}{\frac{1}{2}(I_{\rm L} + I_{\rm R})} \tag{1}$$

I_L and I_R refer, respectively, to left and right circularly polarized emitted intensity. In Fig. 2 we give the measured g_{lum} values for the two crystal field peaks of the $^5D_0 \rightarrow {}^7F_1$ transition.

In Fig. 3 we plot the $^5D_0 \leftarrow {}^7F_1$ excitation spectrum for the solution of $Eu(DPA)_3^{3-}$ and (+)-DMT. This spectrum was measured by monitoring the intensity of the emission centered at 614 nm while scanning the dye laser through the spectral region corresponding to the $^5D_0 \leftarrow {}^7F_1$ absorption. For this solution, the CD at the peak absorption wavelengths was determined by passing the dye laser through the LCP and measuring the intensity while the retardation of the LCP was slowly varied (5 s per cycle) from left to right circular polarization. In CD spectroscopy the differential absorption is often reported in terms of the absorption dissymmetry factor, g_{abs}, defined as

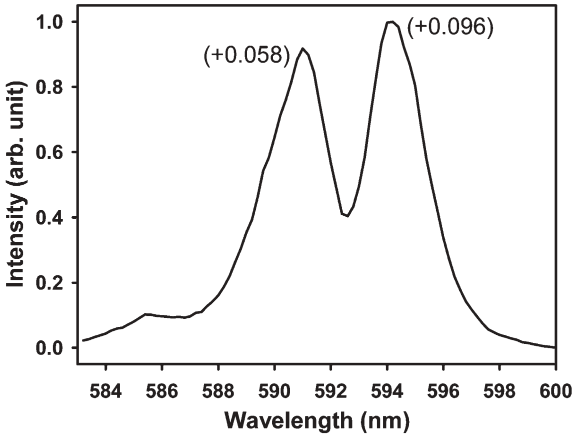

Fig. 3 Excitation spectrum for the $^5D_0 \leftarrow {}^7F_1$ transition of a 0.02 M aqueous solution of $Eu(DPA)_3^{3-}$ containing 5.5 M (+)-DMT. The emission was monitored at 614 nm. Numbers in parentheses correspond to the experimental g_{abs} values measured at the peak wavelengths.

$$g_{\rm abs}({\rm CD}) = \frac{\varepsilon_{\rm L} - \varepsilon_{\rm R}}{\frac{1}{2}(\varepsilon_L + \varepsilon_{\rm R})} \tag{2}$$

where ε_L and ε_R refer, respectively, to the extinction coefficient for a left and right circularly polarized absorption beam. In FDCD the absorption is related to the total emission intensity, which for the case here of weakly absorbing dilute solutions with no artifacts associated with photoselection effects may be simply expressed as

$$g_{\rm abs}({\rm FDCD}) = \frac{F_{\rm L} - F_{\rm R}}{\frac{1}{2}(F_{\rm L} + F_{\rm R})} \tag{3}$$

where we have used the symbol F_L and F_R to denote the intensity of the emission detected at 614 nm under conditions of left and right circularly polarized excitation. The resultant g_{abs} values are given on this figure, and the various results are also displayed in Table 1 along with the results for the measured CD of these transitions reported previously.[7] Note that the CD results were obtained by employing a 5 cm cell. All the measurements reported here were performed at room temperature, and the differences in the relative intensities of the two crystal field peaks displayed in Figs 2 and 3 reflect ground state population differences due to the Boltzmann distribution of initial crystal field states in absorption.

Examination of Table 1 shows that, as expected, the sign of the absorption and luminescence dissymmetry factors for the individual crystal field transitions are the same. This is a good example

Table 1 Absorption and luminescence dissymmetry values for the $^5D_0 \leftrightarrow {}^7F_1$ transition of 0.02 M $Eu(DPA)_3^{3-}$ containing 5.5 M (+)-DMT

	591 nm	594 nm
CPL	+0.056	+0.096
FDCD	+0.058	+0.096
CD[a]	+0.065	+0.103

[a] Calculated from the digitized spectrum given in ref. 7. Slight differences in wavelength have been ignored.

of the principle that if a certain transition in a chiral molecule absorbs more left circularly polarized light than right circularly polarized light, then in emission the transition will have the identical circular polarization preference. For the specific case of the chiral mixture studied here, the fact that the magnitudes are also identical indicates that the non-racemic population of excited complexes is the same as in the ground state. This implies that there has not been significant racemization of the set of excited complexes during the long lifetime of Eu(III). This result is in agreement with previous measurements where the temperature dependence of g_{lum} for a racemic solution of $\mathrm{Eu(DPA)_3}^{3-}$ was reported.[12]

It should be noted that lanthanide(III) complexes are known to be quite labile, especially on the long lifetime of Eu(III), and this technique offers the ability to study the chirality of these types of systems that could not be measured in CPL due to competing racemization. Furthermore, it is very difficult to measure the CD from chiral Eu(III) complexes using conventional CD instrumentation, due to the fact that CD measurements are only possible for these systems if relatively high concentrations and long cell pathlengths are used.

Finally, although in this communication we have used the conventional name of Fluorescence Detected Circular Dichroism for this measurement, it would be more correct to use a name such as *Emission* Detected CD or *Phosphorescence* Detected CD since the lifetime of Eu(III) is on the order of ms, and therefore would not be classified as fluorescence. Applications of this technique to other chiral Eu(III) systems including Eu(III) as a subsitutional replacement for Ca(II) in calcium binding proteins are underway.

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