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Anion Effects on the Luminescence of Europium Complexes

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ABSTRACT

Anions such as fluoride and phosphate, both known to be able to coordinate to lanthanide ions, can compete with water in luminescent europium complexes, involving a sensitizer and shielding ligand, hence, in some cases, affecting the luminescent behaviour of these complexes in aqueous solution. © *1997 Published by Elsevier Science Ltd*

INTRODUCTION

Certain lanthanide ions, such as Eu^{3+}, can exhibit luminescence under defined conditions [1]. In aqueous solution the europium ions have little absorbance, since the excited states are formally forbidden; also, any excited-state species formed is readily quenched by vibronic coupling to solvated water molecules [2,3]. Luminescence may be observed when, a) a sensitizing ligand is employed and b) water is removed from the solvation shell by other ligands ('shielding' ligands). In recent work [4] it has been demonstrated that ligands like 1,10-phenanthroline-2,9-dicarboxylic acid (**1**) (PDCA) can act as a sensitizer and form either 1:1 or 2:1 complexes with the metal ion. The terpyridine dicarboxylic acid (**2**) can also act as a sensitizer.

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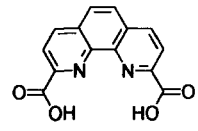

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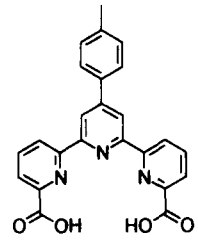

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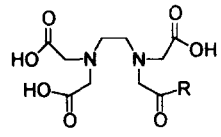
The europium ions can also be complexed to a non-sensitizing ligand—a shielding ligand—such as EDTA (**3**) or its monoamide derivatives, such as the butylamide (**4**).



3, R = OH

4, R = NHC_4H_9

Since europium ions can accommodate about nine coordinating sites, in such EDTA complexes sufficient sites are still available around the ion to allow the ingress of a second, sensitizing ligand, such as PDCA (**1**), thus forming 1:1:1 Eu^{3+}:shield:sensitizer complexes that exhibit luminescence [5]. Such complexes are of value in diagnostic systems [6].

Lifetime studies on these complexes show that, for the most part, some water remains coordinated to these ions [4,5]. Recently the superior affinity of fluoride anions for the lanthanides has been exploited to help displace these residual water molecules from the coordination sphere and hence to increase the luminescence efficiency of the complexes [7]. Furthermore, phosphate groups, which are ubiquitous in biological systems, also have an affinity for lanthanides; for example the redox properties of lanthanide

cryptates is affected by the presence of phosphate as well as fluoride ions, but are negligibly affected by anions such as chloride, sulphate and azide ions [8]. In this paper we report some results of the effect of fluoride and phosphate groups on the luminescence behaviour of these europium complexes.

EXPERIMENTAL

All glassware was washed with Caro's acid, distilled water and dilute HCl before further washing with distilled water and then methanol and then air drying. *N*-(2-hydroxyethyl)piperazine-*N'*-ethanesulphonic acid (HEPES, 0.01 mol dm^{-3}) was used throughout as buffer, adjusting the pH with NaOH or HCl as required. Europium (III) chloride hexahydrate was dissolved in distilled water containing one drop of conc. HCl per 100 cm^3 to produce a stock solution at 2×10^{-3} mol dm^{-3}. The acid prevents precipitation of hydroxides and oxides of the metal. Dilutions of stock solutions were made with the HEPES buffer. The ligands **1**, **2** and **4** were prepared as described previously [9] and stock solutions at 2×10^{-4} mol dm^{-3} were made up. The stock solution of potassium fluoride was made up to 2 mol dm^{-3} and the phosphate solution was made up from a 1:1 molar ratio of potassium dihydrogen phosphate and disodium hydrogen phosphate at a phosphate concentration of 2 mol dm^{-3}.

Working concentrations of the Eu^{3+} complexes were generally held around 1×10^{-5} mol dm^{-3}.

Luminescence data were collected on a Perkin-Elmer LS50-B spectrofluorimeter, using a 350 nm emission filter and 1 cm silica cuvettes. The data was processed using the Perkin-Elmer FLDM software package, using the following parameters: phosphorescence decay; delay time, 0.1 ms; gate time, 10 ms; slit widths, 10 nm; flash count, 1; cycle time, 100 ms; excitation, 290 nm; emission, 615 nm; emission range, 500–750 nm. Mixtures were made up to 4 cm^3 and runs were generally carried out in triplicate to ensure reproducibility.

RESULTS AND DISCUSSION

Fluoride

1:1 Complexes

Results from addition of KF to a 1:1 complex of Eu^{3+}:PDCA at 1×10^{-5} mol dm^{-3} are given in Table 1. These show that added fluoride initially enhances luminescence, a three-fold increase being observed at a 100-fold level of fluoride. Further addition of fluoride leads to a decrease in

TABLE 1
Effect of Added KF to Luminescence Emission From Eu^{3+} Complexes

[KF], mol dm^{-3}	*1:1 Eu^{3+} : **1**[a]*		*1:1 Eu^{3+} : **2**[b]*		*1:2 Eu^{3+} : **1**[c]*		*1:1:1 Eu^{3+} :**4**:**1**[d]*	
	[KF]:[Eu^{3+}]	I_{max}	*[KF]:[Eu^{3+}]*	I_{max}	*[KF]:[EU^{3+}]*	I_{max}	*[KF]:[Eu^{3+}]*	I_{max}
Control	—	6	—	210	—	651	—	184
1×10^{-5}	1	14	1	227	1	642	1	220
1×10^{-4}	10	17	10	235	10	632	10	224
1×10^{-3}	100	18	100	217	100	615	100	257
1×10^{-2}	1000	19	1000	185	1000	606	1000	235
1×10^{-1}	10 000	2	10 000	125	10 000	597	10 000	93
1	100 000	0	100 000	14.4	100 000	380	100 000	31

[a] $[Eu^{3+}]$, 1×10^{-5} mol dm^{-3}; **[1]**, 1×10^{-6} mol dm^{-3}; pH 6.5 in HEPES buffer.
[b] $[Eu^{3+}]$, 1×10^{-5} mol dm^{-3}; **[2]**, 1×10^{-5} mol dm^{-3}; pH 6.5 in HEPES buffer.
[c] $[Eu^{3+}]$, 1×10^{-5} mol dm^{-3}; **[1]**, 2×10^{-5} mol dm^{-3}; pH 7.5 in HEPES buffer.
[d] $[Eu^{3+}]$, 1×10^{-5} mol dm^{-3}; **[1]**, 1×10^{-6} mol dm^{-3}; **[4]**, 1.5×10^{-5} mol dm^{-3}; pH 7.5 in HEPES buffer.

luminescence; at 1 mol dm^{-3} virtually all of the luminescence is gone, reflecting a displacement of the PDCA sensitizer by the fluoride ions. An apparently similar trend was observed with the 1:1 complex between Eu^{3+} and the terpyridine (**2**) (see Table 1), although in this case the relative enhancement of luminescence was much less (maximum of a 10% signal increase) with a quenching of luminescence occurring at high fluoride excesses.

Further information on what is happening in the absence or presence of fluoride ions can be obtained from the lifetime decays of the luminescent signals. Amongst other factors, the lifetimes are known to be dependent on the degree of hydration around the cation. Thus for the Eu^{3+}:PDCA complex the luminescence decays exponentially, with a lifetime of half-life of 0.22 ms, whereas in the presence of 100-fold F^- ions this increases to 0.27 ms (Fig. 1), indicating some direct chelation of the fluoride species to the metal ion at this concentration. Such direct involvement of the fluoride ion with the metal is also reflected in the shape of the emission spectrum; in the presence of the fluoride ion an enhancement of the emission peak at 580 nm is observed. This peak is produced by a non-symmetrically coordinated species and is commonly found when F^- ions are associated around the Eu^{3+} ion [10,11]. For the 1:1 teryridyl:Eu^{3+} complex no change in the lifetime is observed in the presence of 100-fold fluoride concentration, indicating that, under these conditions, the fluoride ion is not directly involved in coordination to the cation (Fig. 2). Only at concentrations of fluoride ion $> 10^{-2}$ mol dm^{-3} does interference occur.

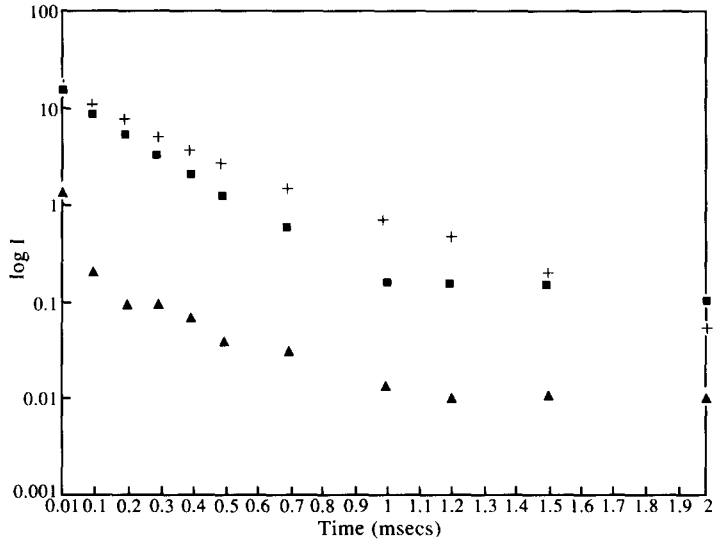


Fig. 1. Lifetime curves for 1:1 Eu^{3+}:**1** complex in the presence of F^-; see Table 1 for details. ■, Control; +, 1×10^{-2} mol dm^{-3} F^-; ▲, 1 mol dm^{-3} F^-.

1:2 Complexes

PDCA (**1**) forms a stable 2:1 complex with Eu^{3+} ions [4]. Lifetime studies show that an average of one water molecule can also complex with the cation in these complexes. Addition of fluoride ions to this complex showed no effect on the luminescence behaviour up to concentrations of greater than 10000:1 (0.1 mol dm^{-3}) (Table 1). Lifetime measurements gave the same value (τ_m 0.75 ms) throughout the fluoride concentration range indicating no interaction of this anion with the (formally negatively charged) 2:1 PDCA:Eu^{3+} complex. Only at extremely high fluoride ion concentrations, as luminescence intensity decreases, does one observe the increase in the 580 nm emission.

1:1:1 Complexes

The EDTA monobutylamide (**4**) complex of Eu^{3+} was studied using PDCA (**1**) as the sensitizer. These are known to exist as a dynamically equilibrating set of species in solution [5], in which the EDTA monoamide ligands are continuously associating and dissociating from the cation. The results of the addition of fluoride ions are correspondingly complex (Table 1). After an initial decrease in luminescence, this increases to a maximum before slowly decreasing as the fluoride concentration is increased to a vast excess. The behaviour is similar to that observed with the 1:1 species, which may reflect the fact that the fluoride can compete with the EDTA ligands for sites around the cation. These must be mainly displacing the EDTA ligands rather

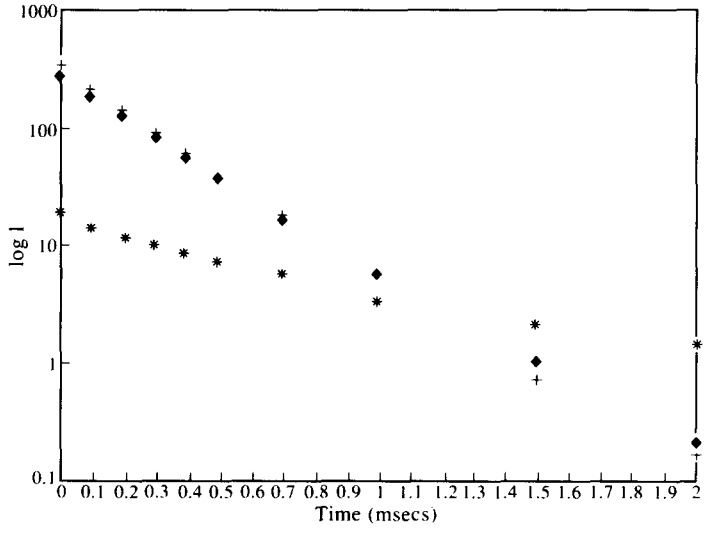


Fig. 2. Lifetime curves for 1:1 Eu^{3+}:**2** complex in the presence of F^-; see Table 1 for details. ◆, Control; +, 1×10^{-2} mol dm^{-3} F^-; *, 1 mol dm^{-3} F^-.

than water, as no large increases in luminescence behaviour are observed. The lifetimes of luminescence species were largely unaffected by the presence of the fluoride ions (Fig. 3).

Phosphate

1:1 Complexes

The addition of one equivalent of phosphate to the PDCA 1:1 complex showed only a little enhancement in the luminescence intensity (*ca* 30%) (Table 2) but, as for the effect of fluoride ions, there was a large increase in the lifetime of the emission from 0.23 ms to *ca* 0.4 ms. Inspection of the lifetime curves (Fig. 4) shows that it is not linear, indicating the presence of mixtures of species. On further increasing the phosphate concentration, luminescence intensity decreases although the lifetimes for these reduced emissions are similar. In contrast, the 1:1 Eu^{3+}:terpyridyl complex is little affected by the presence of phosphate until large excesses (> 1000:1 ratios) are present.

2:1 Complex

As observed with the fluoride ion, the presence of phosphate ions has little effect on the 2:1 PDCA:Eu^{3+} complex until in very large concentrations (Table 2). Thus the 2:1 PDCA complex appears to be kinetically very stable. No changes in the lifetimes of the emission were observed (Fig. 5).

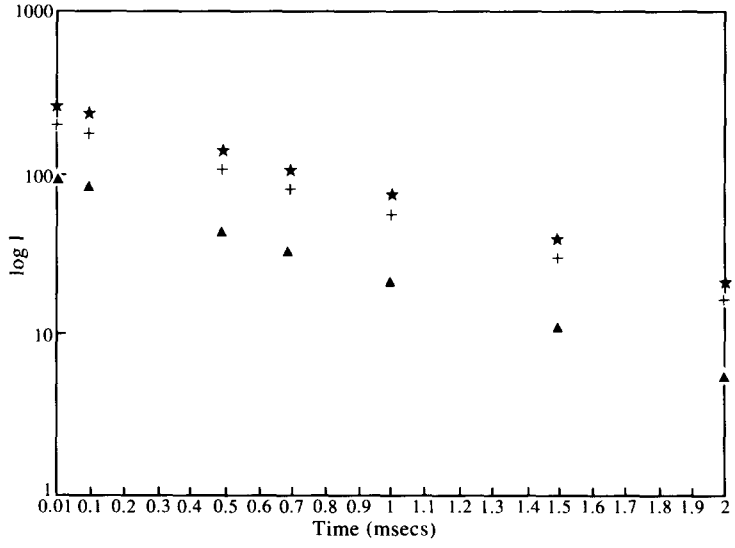


Fig. 3. Lifetime curves for 1:1:1 Eu^{3+}:**4:1** complex in the presence of F^-; see Table 1 for details. +, Control; ★, 1×10^{-4} mol dm^{-3} F^-; ▲, 1×10^{-1} mol dm^{-3} F^-.

TABLE 2
Effect of addition of phosphate to Eu^{3+} complexes

$[PO_4^{3-}]$, mol dm^{-3}	1:1 Eu^{3+} : **1**[a]		1:1 Eu^{3+} : **2**[b]		1:2 Eu^{3+} : **1**[c]		1:1:1 Eu^{3+} : **4**:**1**[d]	
	$[PO_4^{3-}]:[Eu^{3+}]$	I_{max}	$[PO_4^{3-}]:[Eu^{3+}]$	I_{max}	$[PO_4^{3-}]:[Eu^{3+}]$	I_{max}	$[PO_4^{3-}]:[Eu^{3+}]$	I_{max}
Control	—	9	—	242	—	695	—	225
1×10^{-5}	1	14	1	236	1	700	1	270
1×10^{-4}	10	16	10	246	10	695	10	261
1×10^{-3}	100	0.6	100	255	100	700	100	252
1×10^{-2}	1000	0.12	1000	261	1000	699	1000	132
1×10^{-1}	10 000	0.1	10 000	21	10 000	450	10 000	6.4

[a] $[Eu^{3+}]$, 1×10^{-5} mol dm^{-3}; [**1**], 1×10^{-6} mol dm^{-3}; pH 6.5 in HEPES buffer.
[b] $[Eu^{3+}]$, 1×10^{-5} mol dm^{-3}; [**2**], 1×10^{-5} mol dm^{-3}; pH 6.5 in HEPES buffer.
[c] $[Eu^{3+}]$, 1×10^{-5} mol dm^{-3}; [**1**], 2×10^{-5} mol dm^{-3}; pH 7.5 in HEPES buffer.
[d] $[Eu^{3+}]$, 1×10^{-5} mol dm^{-3}; [**1**], 1×10^{-6} mol dm^{-3}; [**4**], 1.5×10^{-5} mol dm^{-3}; pH 7.5 in HEPES buffer.

1:1:1 Complex

The presence of phosphate does not interfere with the luminescence behaviour of this complex at ratios up to 100:1. However, at higher phosphate concentrations (Table 2) the luminescence efficiency of the system falls away, although the lifetime for luminescence (τ_m 0.78 ms) remains constant (Fig. 6).

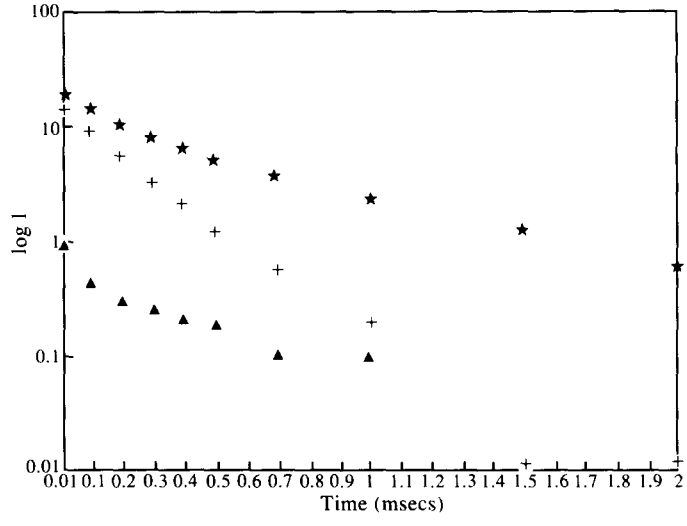


Fig. 4. Lifetime curves for 1:1 Eu^{3+}:**1** complex in the presence of PO_4^{3-}; see Table 2 for details. +, Control; ★, 1×10^{-5} mol dm^{-3} phosphate; ▲, 1×10^{-3} mol dm^{-3} phosphate.

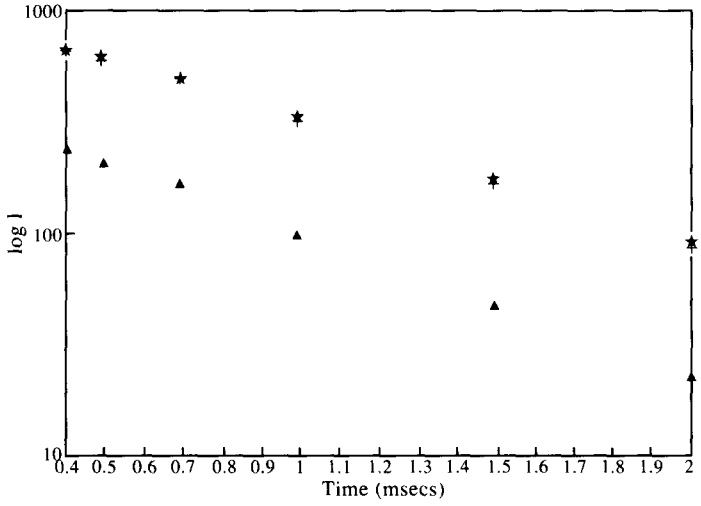


Fig. 5. Lifetime curves for 2:1 Eu^{3+}:**1** complex in the presence of PO_4^{3-}; see Table 2 for details. +, Control; ★, 1×10^{-5} mol dm^{-3} phosphate; ▲, 1×10^{-1} mol dm^{-3} phosphate.

It can deduced, as in the case for fluoride ions, that the EDTA monoamide shield may be being replaced with phosphate ions at very high concentrations, with little effect at lower concentrations.

CONCLUSIONS

The results presented demonstrate that for the ionic complexes described only the coordinatively highly unsaturated complexes (the 1:1 species) are greatly affected by the presence of coordinating anions such as fluoride and phosphate ions. Only modest enhancements in luminescence are observed, in contrast to the findings reported by Mathius *et al.* [7] with the cryptates. With the more saturated complexes, such as the 2:1 species, or the shielded 1:1:1 species no luminescence enhancement is observed except in the presence of high concentrations of these anions. In these cases an overall decrease in luminescence is observed although without any significant change in the lifetime of luminescence, indicating no increased displacement of water from the coordination sphere of the metal in these species. The lack of interference in the luminescence behaviour of the 1:1:1 complex at concentrations up to millimolar is of importance in the application of these to bioassays [6]. The sensitivity of the luminescence signal to the higher phosphate concentrations suggests, however, that the use of phosphate buffers should be avoided.

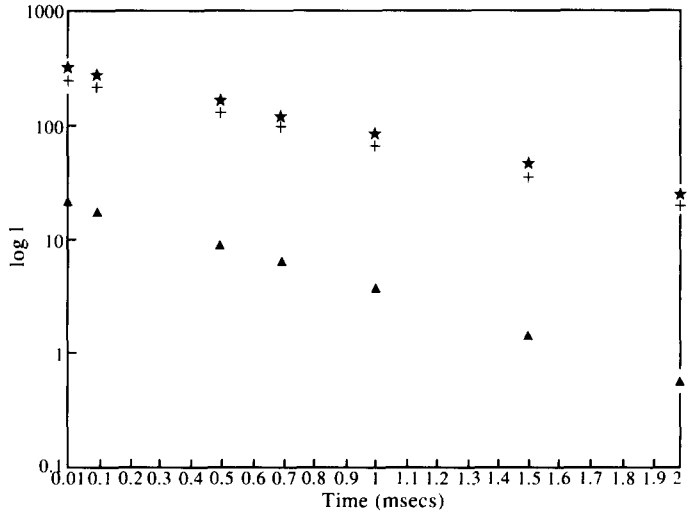


Fig. 6. Lifetime curves for 1:1:1 Eu3+:**1:4** complex in the presence of PO_4^{3-}; see Table 2 for details. +, Control; ⋆, 1×10^{-4} mol dm^{-3} phosphate; ▲, 1×10^{-1} mol dm^{-3} phosphate.

ACKNOWLEDGEMENT

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