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Luminescent lanthanide sensors for pH, pO_2 and selected anions

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Contents

Abstract

Stable lanthanide complexes have been devised in which the emission intensity or lifetime is a sensitive function of pH, pO_2 or halide concentration following excitation in the range 350–380 nm. The pH-dependent systems report the pK_a of the excited singlet or triplet of an integral phenanthridine chromophore. Modulation of the emission may occur via ligand or metal-centred processes: quenching of the singlet or triplet phenanthridine excited state occurs by halide and oxygen respectively; in complexes with chiral heptadentate ligands displacement of bound water molecules by lactate, phosphate and hydrogencarbonate leads

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to enhancements in the emission intensity and lifetime and pronounced changes in emission polarisation.

Keywords: Lanthanides; Luminescence; pH; Hydrogencarbonate; Sensors

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1. Introduction

The lifetime of emission from the excited state of the lanthanide ions falls in the range of microseconds (e.g. Yb, Nd) to milliseconds (e.g. Eu and Tb). Such relatively long-lived emission is an attractive feature from an analytical viewpoint, as it allows the implementation of time-gating procedures so that the lanthanide luminescence is readily distinguished from the shorter-lived (sub-microseconds) background present in most biological systems [1,2]. In addition, practical problems associated with autofluorescence and Rayleigh scattering are obviated. Lanthanide f–f transitions are Laporte-forbidden and very weak, with extinction coefficients of the order of only 0.5–3 $dm^3 mol^{-1} cm^{-1}$. This renders direct excitation of the lanthanide ion very inefficient, and only practicable with lasers. One solution to this sensitivity problem is to incorporate a sensitising chromophore (often termed an ‘antenna’) into the lanthanide-binding ligand, allowing indirect excitation of the complexed lanthanide ion, usually via intramolecular energy transfer [3]. A multitude of different ligands bearing aromatic sensitising chromophores has been devised including bipyridines [4], terpyridines [5], triphenylenes [6], substituted phenyl [7] and naphthyl groups [8] — all showing a common requirement: the energy of their triplet excited-state needs to lie at least 1700 cm^{-1} above the energy of an accepting lanthanide energy level (often the emissive state). If this energy gap is less than 1500 cm^{-1}, then thermally activated back-energy transfer competes re-populating the ‘fragile’ aryl triplet state. On the other hand, if the energy gap is too great, then the efficiency of the energy transfer step is compromised — so a balance needs to be struck between these opposing factors.

A further important boundary condition, this time on the singlet excited energy of the chromophore, is imposed by the need to avoid the excitation of common biomolecules — such as proteins and nucleic acids. Therefore the sensitising chromophore needs to be excited at wavelengths in excess of 350 nm (ca. 28 600 cm^{-1}) which also obviates the need for quartz optics. The two longest-lived-and most commonly studied-emissive lanthanide ions are Eu and Tb, which possess luminescent $^5D_0$ and $^5D_4$ excited states with an energy of 17 200 and 20 500 cm^{-1}, respectively. Therefore in seeking a suitable antenna for sensitising their emission, the chromophore must possess a relatively low singlet excited state energy, with a fairly small singlet-triplet energy gap.

A typical photochemical sequence that characterises sensitised lanthanide luminescence is given below (Fig. 1). The overall quantum yield for emission reflects the efficiency of each of the three main steps involved in this process. Firstly, the quantum yield of formation of the intermediate aryl triplet state, ϕ_A is important

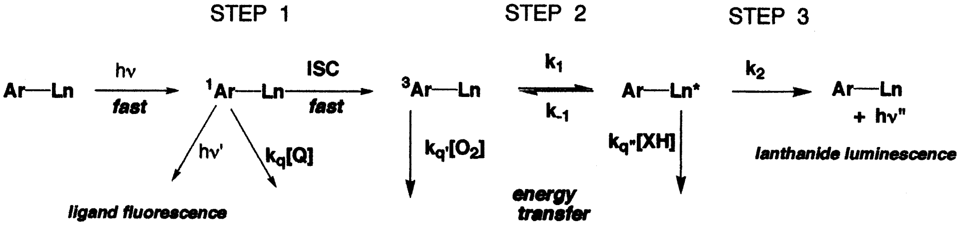


Fig. 1. Kinetic scheme showing the three key photophysical processes that occur during sensitised lanthanide luminescence.

and is primarily determined by the facility of inter-system crossing (ISC). Next the energy transfer step needs to be considered (step 2), and its rate and efficiency, η_{ET}, is maximised when there is a short distance between the chromophore and the lanthanide ion, a relatively small energy gap and no competing back-energy transfer. If the rate of energy transfer is sufficiently slow, then rate processes that lead to deactivation of the triplet state may compete: quenching by molecular oxygen often occurs in such cases due to the high second-order quenching rate constants (ca. 1×10^9 dm^3 mol^{-1} s^{-1}) typically found with aryl triplet states [8,9]. Finally, the emissive quantum yield of the

$$\phi_{\text{overall}} = \phi_A \eta_{ET} \phi_{em} \quad (1)$$

$$\phi_{em} = k_o \tau_{obs} \quad (2)$$

$$\phi_{\text{overall}} = \phi_A \eta_{ET} k_o \tau_{obs} \quad (3)$$

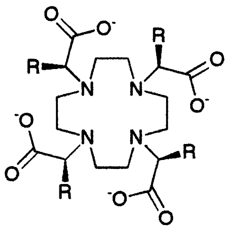
lanthanide ion, ϕ_{em}, needs to be considered (step 3). Given that the quantum yield of lanthanide emission is the product (Eq. (2)) of the observed luminescence lifetime, τ_{obs}, and the natural radiative rate constant (k_o, i.e. the rate constant in the absence of deactivating processes), then the overall efficiency for sensitised emission is given by Eq. (3). The main quenching process in this third step (Fig. 1) involves vibrational energy transfer to higher harmonics of solvent and ligand oscillators [10–15]. Quenching by OH and amine NH oscillators is particularly efficient with Eu, so that the ligand that binds the lanthanide ion should shield it effectively from proximate OH oscillators (i.e. water molecules).

To summarise, the main features that a lanthanide complex should possess in order to be suitable for use as a luminescent probe [16] in competitive biological media may be listed as follows:

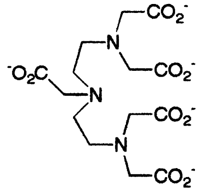
1. High kinetic stability in the pH range 3–10 of the 1:1, metal:ligand complex, i.e. a robust, single-component system that is stable in biological media.
2. An inbuilt chromophore, which is only a short distance from the lanthanide ion, which absorbs light efficiently (high ε) at wavelengths in excess of 350 nm, and possesses a triplet excited state that is efficiently populated and preferably lies in the energy range 22 000–24 000 cm^{-1} (allowing Tb and Eu excitation).

3. The lanthanide complex should be effectively shielded from the deactivating effect of energy-matched XH oscillators, especially OH and amine NH vibrations, suggesting that octa- and/or nonadentate ligands be used.

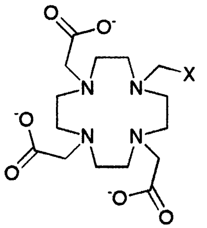
Some of these constraints are relaxed in complexes of Yb — where the mechanism of excitation may involve either triplet energy transfer [17] or electron transfer with electron-rich aromatic donors [18], as the ytterbium $^2F_{5/2}$ excited state lies at 10 200 cm^{-1} ($\lambda_{em} = 980$ nm), allowing a much wider range of sensitising chromophores to be used. Considerations of points 1 and 2 suggests that the range of ligands that have been used to good effect for targeted ^{90}Y-radiotherapy [19] and in paramagnetic contrast agents for MRI (Gd) [16,20] are entirely appropriate for this purpose. Examples of such ligands include: firstly the poly-aminocarboxylates, dota **1** and dtpa **2** [16], their mono- or di-amides, **3** and **4** [21] or related mono- and di-substituted derivatives, e.g. **5** [22]; the tetra-phosphinates **6** [23] and their mono-substituted derivatives, e.g. **7** (for each of which NMR studies have revealed that one predominant stereoisomer exists in solution [24]), the tetra-amides **8** and their mono-substituted derivatives [25]. The chiral tetra-amides, e.g. **8a**, are particularly attractive as a single enantiomeric complex exists in solution [7].



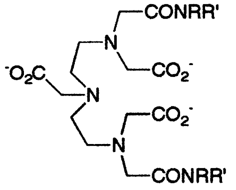
1 a) R = H (dota)
b) $R = CH_2CH_2CO_2^-$



2 (dtpa)



3 a) X = CONRR'
b) X = CH(OH)R



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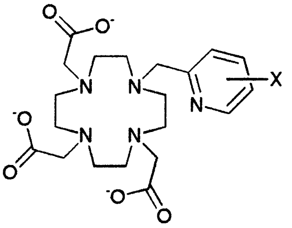

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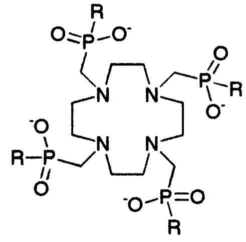
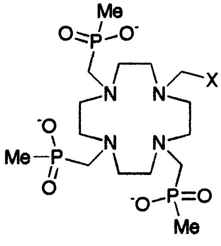
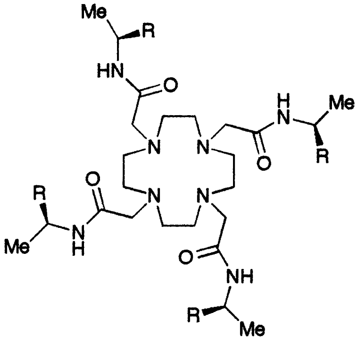


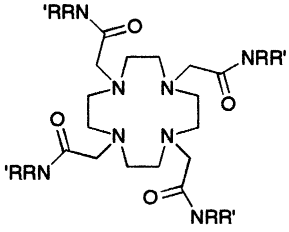
6 a) $R = CH_2Ph$
b) R = Bu
c) R = Me



7 e.g. X = CONRR'



8a R = Ar or CO_2^-



8b R, R' = H or Me

2. Mechanisms of action of responsive luminescent lanthanide complexes

Examination of the photophysical scheme in Fig. 1 suggests that there are three main opportunities to allow modulation of the final lanthanide emission. The first requires perturbation of the singlet excited state either through inter- or intramolecular quenching usually involving electron or charge transfer (including the Ln^{3+} or the ligand), or by a reversible binding process that changes the singlet excited state energy. This latter process may allow selective excitation of a bound complex, or at least may perturb the efficiency of inter-system crossing to the triplet. The second possibility involves the triplet excited state itself. In this case, its energy may be altered by reversible binding (e.g. protonation of the chromophore) affecting the rate of forward (or reverse) energy transfer. In addition, the triplet state may be quenched itself: molecular oxygen is the most common quencher in this respect. Finally, the excited lanthanide ion is also sensitive to quenching by vibrational energy transfer [15]. For lanthanide complexes of heptadentate ligands in aqueous media, up to two water molecules are bound. When these quenching water molecules are displaced — for example by the reversible binding of an anion such as hydrogencarbonate — then the intensity, lifetime and (for chiral complexes) polarisation of emission is markedly changed, signalling the different nature of the ternary complex, [26].

2.1. Modulation of luminescence via the sensitising chromophore

The sensitising chromophore serves a dual function when its singlet or triplet excited states are reversibly perturbed by the local ionic or molecular environment (Fig. 2) [27,28]. The depicted binding interaction may involve reversible protonation, metal ion, anion or molecule binding to a receptor site integrated into the chromophore sub-unit. Alternatively, 'collisional' deactivation of the singlet and triplet excited states — for example by halide anions and triplet O_2 — may be involved. Such processes have frequently been harnessed in the design of classical fluorescent sensors and are used in the measurement of intracellular chloride

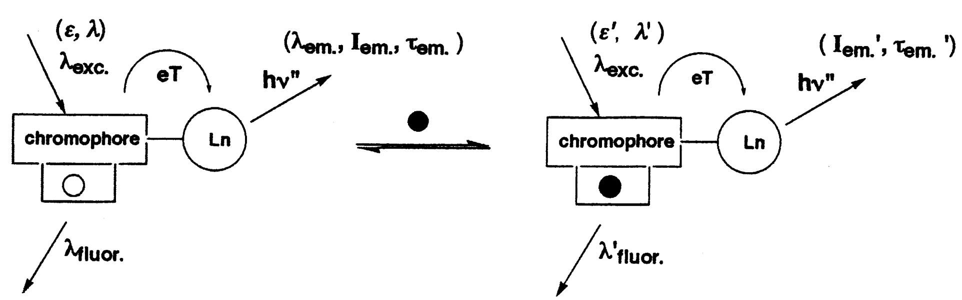


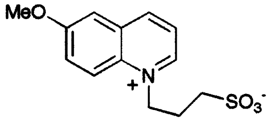
Fig. 2. Schematic representation of the modulation of lanthanide emission by reversible binding of an analyte at (or near to) the sensitising chromophore.

concentrations with substituted quinolines [29] and of pO_2 (e.g. using $Ru(bpy)_3^{2+}$ [30] or Pd/Pt porphyrins [31]) in a variety of thin film applications. Overall, both the ligand-based fluorescence and the delayed lanthanide luminescence may be modulated by the presence of selected ions or molecules, allowing concentration changes to be signalled by emission intensity (or intensity ratio), lifetime, or polarisation measurements.

Two different cases can be defined for the quenching of the singlet excited state of the sensitising chromophore: the first involves *intermolecular* quenching and is exemplified by halide quenching of the excited state of quinine and related *N*-alkyl acridinium and quinolinium salts, e.g. **9** [32]. In this case, at relatively high concentrations of added X^- anions, encounter of the anion with the excited state results in electron transfer from the halide anion to the excited aromatic group. In the second case, *intramolecular* electron transfer is involved — in which the donor may be any electron occupying a relatively high energy molecular orbital or may be the excited singlet itself. The likelihood of this event can be predicted, using the approach adopted by Weller for photo-induced electron transfer processes [33].

$$\Delta G_{\mathrm{ET}} = nF([E_{\mathrm{ox}} - E_{\mathrm{red}}] - E_{\mathrm{s}} - e^2/\varepsilon r)\ \mathrm{J\ mol^{-1}} \tag{4}$$

where E_{ox} is the oxidation potential of a donor (e.g. amine lone pair, electron rich aromatic group), E_{red} is the reduction potential of the acceptor (e.g. aryl group or Ln^{3+} centre), E_{s} is the singlet excited state energy in eV and $e^2/\varepsilon r$ is the attractive energy term related to formation of a radical ion pair (this term is usually less than ca. 0.2 eV)).



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The Weller equation (Eq. (4)) describes the free energy of activation for the electron transfer process. Relevant values for selected donors and acceptors are collated in Table 1. Two examples of the application of the Weller equation,

Table 1
Details of the excited state energies and redox potentials of selected chromophores as electron donors and acceptors[a]

Compound	E_{singlet} (eV)	E_{triplet} (cm^{-1})	$E_{1/2}^{\text{ox}}$/V (DMF or MeCN)	$E_{1/2}^{\text{red}}/V$ (MeCN)
Anthracene	3.30 (319)	14 900 (178)	+1.09	−1.95
Phenanthridine[c]	3.54 (342)	22 100 (264)	+1.1[b]	−2.12
Phenanthrene	3.57 (345)	21 500 (257)	+1.50	−2.44
Triphenylene	3.65 (352)	23 400 (280)	+1.55	−2.46
1-*O*-Me-naphthalene	3.88 (374)	20 900 (250)	+1.38	−2.65
O-Me-benzene	4.46(431)	28 200 (338)	+1.76	> −2.7

[a] Values in parentheses refer to energies in kJ mol^{-1}. Data is taken from reference [50]. The energies of the emissive states of Eu (^5D_o), Tb ($^5D_4$) and Yb ($^2F_{5/2}$) lie at 17 200, 20 500 and 10 200 cm^{-1}, respectively; the reduction potential for Eu^{III} and Yb^{III} complexes of octadentate polyaza–carboxylate and tetra–aza–amide complexes (which stabilise the tripositive complex) lie in the region −0.9 to −1.3 V and −1.7 to −2.0 V, respectively, compared to −0.35 (Eu) and −1.05 V (Yb) for the free aqua ions.

[b] On protonation; $E_{\text{singlet}} = -3.30$ (319 kJ mol^{-1}, E_{triplet} ca. 21 300 cm^{-1} (254.5 kJ mol^{-1}) and the oxidation potential is greater that +2.6 V.

illustrate its utility. Firstly, consider the photoinduced electron transfer from a tertiary amine nitrogen to an excited singlet excited state. Triethylamine ($E_{1/2}^{\text{ox}} = +1.15$ V in MeCN) and *N*,*N*-dimethylaniline ($E_{1/2}^{\text{ox}} = +0.53$ V) may be considered as donors, to the phenanthridine excited singlet state. The free energy for the electron transfer process with Et_3N as the donor is given by Eq. (5), and is energetically favourable. Protonation of the tertiary amine increases the oxidation potential by at least 1.5 V, so that electron transfer only occurs if its rate is faster than proton transfer. Protonation therefore suppresses photo-induced electron-transfer to the singlet, leading to an enhancement in ligand-based fluorescence, which may be echoed by increases in the intensity of lanthanide luminescence (Fig. 1).

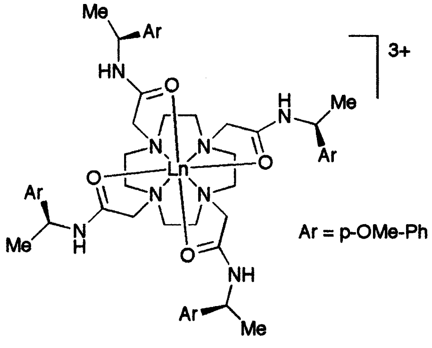
$$\Delta G_{\text{ET}} = F[(1.15 + 2.12) - 3.54 - 0.15] \tag{5}$$

hence $\Delta G_{\text{ET}} = -30.9$ kJ mol^{-1}

A second example concerns electron transfer from the excited singlet state of a sensitising chromophore to the complexed lanthanide ion. This process is not so important with complexes of Tb^{3+} as this ion is very difficult to reduce ($E_{1/2}^{\text{red}} < -3.5$ V). However in complexes of Eu and Yb with polar octadentate ligands (e.g. polyamino–carboxylates/amides), the reduction potential for each complex falls in the range −0.8 to −1.2 (Eu) and −1.4 to −2.0 V for Yb. Thus while methoxybenzene is a superb sensitiser for Tb^{3+}, in complexes containing this chromophore [7,23] (e.g. **10a** in which $\phi_{\text{overall}}^{\text{H}_2\text{O}} = 0.49$ and $\phi_{\text{overall}}^{\text{D}_2\text{O}} = 0.81$), the quantum yield for Eu emission in **10b** is at least 10 000 times lower [23]. Application of the Weller equation highlights the facility of photoinduced electron transfer to the Eu^{3+} centre in the latter case (Eq. (6)). This process may not occur to a significant extent with the

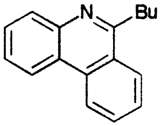
$$\Delta G_{ET} = F[(1.76 + 1.1) - 4.46 - 0.15] = -1.75F = -169 \text{ kJ mol}^{-1} \tag{6}$$

para-carboxymethyl analogue **10c**, as the electron-withdrawing CO_2Me group raises the oxidation potential of the potential aryl donor to $> +3.3$ V (cf. $+1.76$ V for MeO–Ph): the overall quantum yield for sensitised emission now is a respectable 12%.



10 a) Ln = Tb
b) Ln = Eu
c) Ar = p-CO_2Me-Ph

A further important mechanism may be considered for cases where reversible binding to a receptor site in the chromophore changes the energy of the excited singlet (and/or triplet) state. A simple example is provided by the phenanthridines which may be conjugated to lanthanide complexes[34]: protonation of 6-butylphenanthridine itself, **11**, lowers the singlet excited state by about 27 kJ mol^{-1} (2250 cm^{-1}) and the absorption maximum shifts to longer wavelength by about 30 nm. Thus highly selective excitation of the protonated ligand is possible at wavelengths above, say, 365 nm (Fig. 3). The energy of the triplet state also lowers following protonation (or *N*-alkylation) — see Section 3. Thus by selecting an appropriate excitation wavelength, pH-dependent luminescence may be envisaged. This process is also favoured by the accompanying inhibition of photoinduced electron transfer that may limit the lifetime of the S_1 state: formation of a radical dication is energetically disfavoured so further increases in emission intensity arise.



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2.2. Modulation of luminescence via binding to the lanthanide centre

The excited state of the lanthanide ion itself offers a short-lived window of opportunity for modulation of emission (step 3 in Fig. 1). Two mechanisms exist, the first involves a change in the extent of vibrational quenching by energy-matched

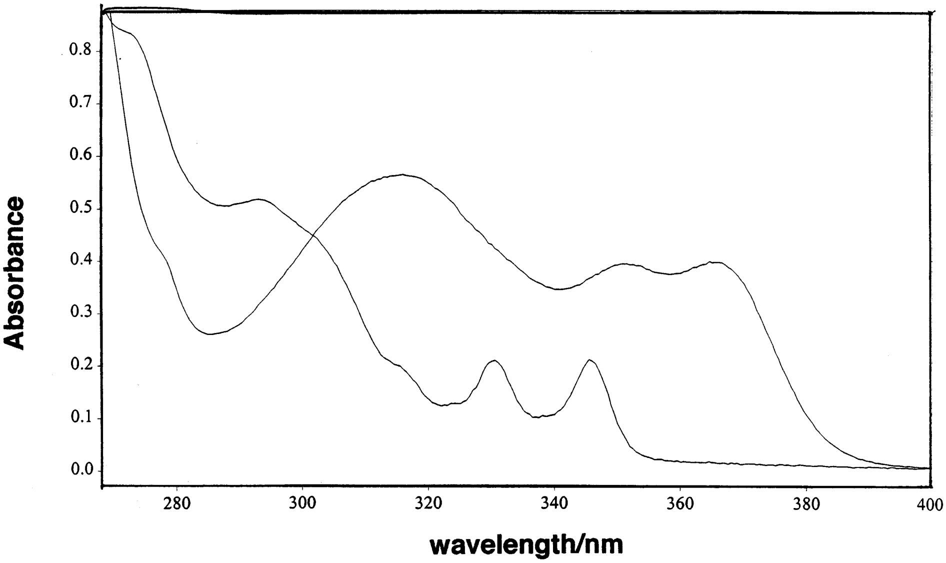


Fig. 3. Absorption spectra for 6-butylphenanthridine in the neutral and protonated forms showing the absorption at longer wavelength for the protonated ligand (298 K, MeOH).

XH oscillators upon binding to the lanthanide complex and the second involves perturbation of the rate of back-energy transfer from the excited ion to the triplet chromophore. Deactivation of excited lanthanide ions may occur in solution by means of vibrational energy transfer to high energy vibrations (OH, NH, CH, C=O) of the bound ligand or of the solvent [10–15]. Therefore in complexes where one or two solvent (usually H_2O) molecules are displaced by binding of an anion or a molecule to the metal centre, then the removal of the quenching OH oscillators will be signalled by an increase in the lifetime and intensity of the metal-based emission [26]. Given that displacement of a bound water in nine-coordinate complexes with octadentate ligands in aqueous solution (55.6 M) is a very rare event (and only likely with *small* nucleophiles), the systems which merit attention possess two bound waters and will usually require a complex of a heptadentate ligand. A typical scheme that highlights the putative equilibria in such a process (Fig. 4) involves labilisation of one of the waters via deprotonation, followed by loss of one or-in the case of chelating species — two water molecules. Given that the lanthanide centre will possess a residual positive charge — even in complexes with polyanionic ligands — then the most likely species to bind in aqueous solution are anions.

It is important to recall that the loss of electronic energy from an excited lanthanide ion to vibrational energy of solvent/ligand is likely [35] to occur in accordance with the general energy transfer formula, developed by Förster [36] wherein a dipolar interaction predominates (Eqs. (7) and (8)). The rate of energy transfer, k_{12}, between the two centres varies with the

$$k_{12} = cr^{-6} \tag{7}$$

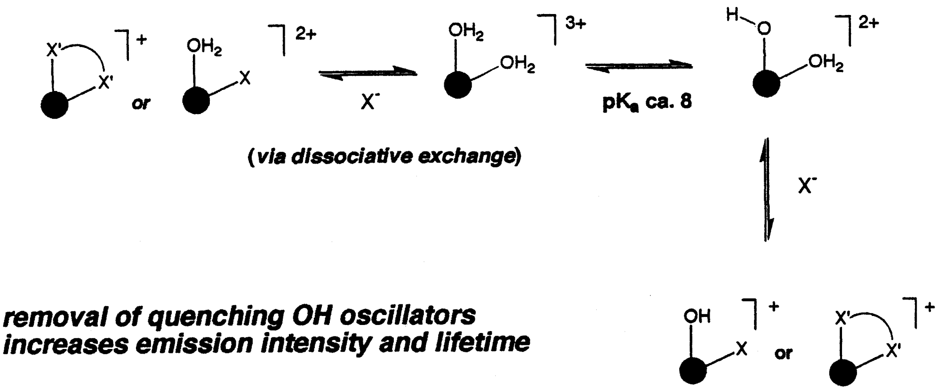


Fig. 4. Reversible anion binding at a di-aqua lanthanide centre, involving labilisation of one of the bound water molecules following deprotonation.

$$\text{in which} \quad c'_{\text{d-d}} = \frac{[(3e^4 f_1 f_2)]}{[8\pi^2 m^2 c^3 \eta^4 \bar{v}^2]} \int g_1(\bar{v}) g_2(\bar{v}) \mathrm{d}\bar{v} \qquad (8)$$

inverse sixth power of the distance between them (Eq. (8)), in which $\bar{v}$ is the energy of the resonant transition, $g_1(\bar{v})$ and $g_2(\bar{v})$ are the normalised lineshapes of the emission and absorption transitions, respectively and f_1 and f_2 are the corresponding oscillator strengths. In complexes of the central lanthanides, Ln–O and Ln–N bond lengths are typically 2.4 and 2.6 Å, so that the distance between the Ln ion and a *bound* OH or NH oscillator (maximum motion for the lighter H atom) will be close to 2.9 and 3.1 Å. Using this value as representing a 100% efficiency of vibrational energy transfer, then the effect of increasing the distance on the normalised rate of energy transfer may be calculated, in each case. The results show (Figs. 5 and 6), that for OH oscillators at 3.5–3.6 Å from the Ln centre, the rate will be 25% of the bound water case [15]. Such a distance corresponds to that associated with the 'second sphere of hydration', and changes to it may also be significant [37].

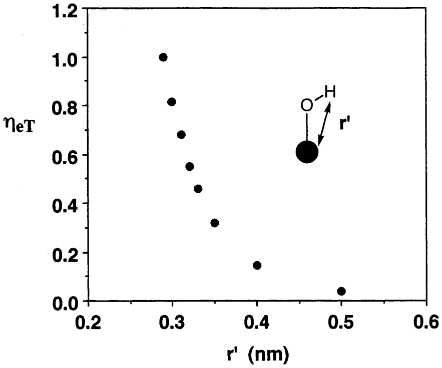


Fig. 5. Effect of distance on the efficiency of vibrational energy transfer from an excited lanthanide ion to proximate OH oscillators.

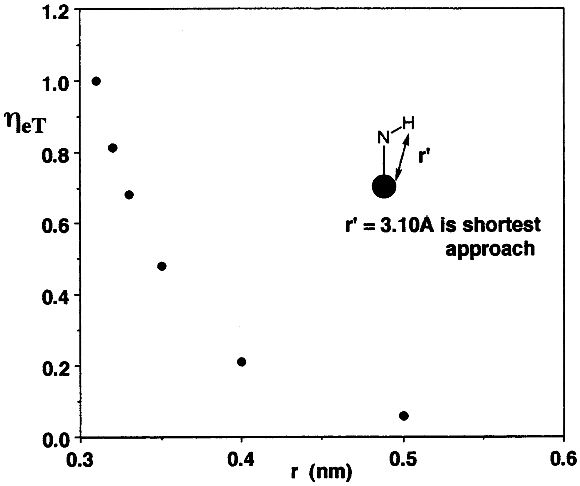


Fig. 6. Effect of distance on the efficiency of vibrational energy transfer from an excited lanthanide ion to a proximate NH oscillator, showing how a 2° amide oscillator can still exert a quenching effect over 4.5 Å from the lanthanide centre.

Two further changes may also signal the change in the coordination environment of the lanthanide ion. Firstly, slight changes in the ligand-field at the lanthanide ion can lead to small shifts in the 'ionic' transitions of the lanthanide ion (Fig. 7) [38]. Examples include the ^5D_o–^7F_o ($\Delta J = 0$) transition for Eu (ca. 580 nm), and the $^5D_2$–$^7F_0$ and $^7F_1$ transitions at 464 and 473 nm, respectively. Thus direct laser excitation (e.g. using the weaker lines of an Ar-ion laser at 465.8 and 427.7 nm [38]) or application of powerful modern light emitting diodes (e.g. 465 nm), may lead to differential efficiencies of lanthanide emission from a bound and an unbound complex. Given that ligand field splittings are unlikely to exceed 100 cm^{-1} in most lanthanide complexes (equivalent to a change of ± 1 nm at 465 nm!), then laser excitation is the likely way forward here. Secondly, changes in the coordination environment at the lanthanide centre can lead to marked changes in the *intensity* of hypersensitive bands (e.g. the $\Delta J = 2$ manifold at ca. 616 nm for Eu^{3+}). This is particularly useful analytically, as it allows the *ratio* of the $\Delta J = 2$ bands to $\Delta J = 1$ or 4 (for Eu^{3+}) to be measured, giving a parameter which is independent of sample concentration and absorbance.

Finally, for chiral lanthanide complexes, the local helicity at the lanthanide centre may change on formation of the ternary complex when an anion binds [26]. In the complexes of dota **1** and the tetra-amides **8**, the two major diastereoisomers in solution possess a mono-capped square-antiprismatic geometry (with a twist angle of the N_4/O_4 planes of ca. 40°) and a twisted square-antiprismatic geometry (twist angle 29°) [39,40]. The relative proportion of these two isomers varies as a function of the nature of the substituents in the ligand [41], and of lanthanide size. Analysis of the solution ^1H-NMR spectra of europium complexes, such as **12**, shows how binding of HCO_3^- leads to a change in this isomeric ratio (Fig. 8) from ca. 2:1 (D_2O, 293 K) to $< 1{:}10$ (in 30 mM HCO_3^- at pH 7.5). The *circular polarisation* of

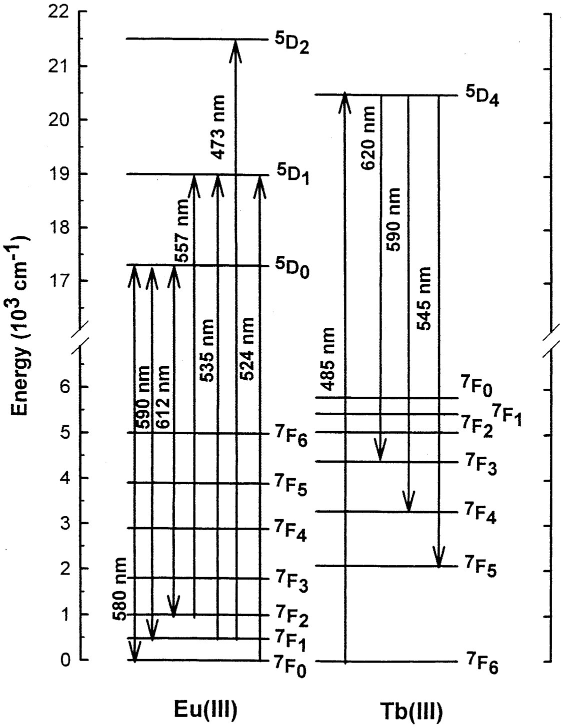
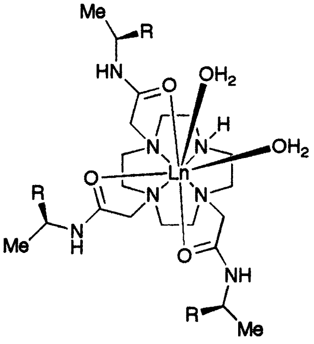


Fig. 7. Schematic energy level diagram for Eu(III) and Tb(III) [38].

the emission in the magnetic-dipole allowed transitions (e.g. $\Delta J = \pm 1$) is sensitive to the helicity at the metal centre as this determines the degree of mixing of the magnetic and electric dipole transition moments. Therefore the change in twist angle upon anion binding is also signalled by changes in the emission polarisation, [26,42].



12 R = Ar or CO_2^-

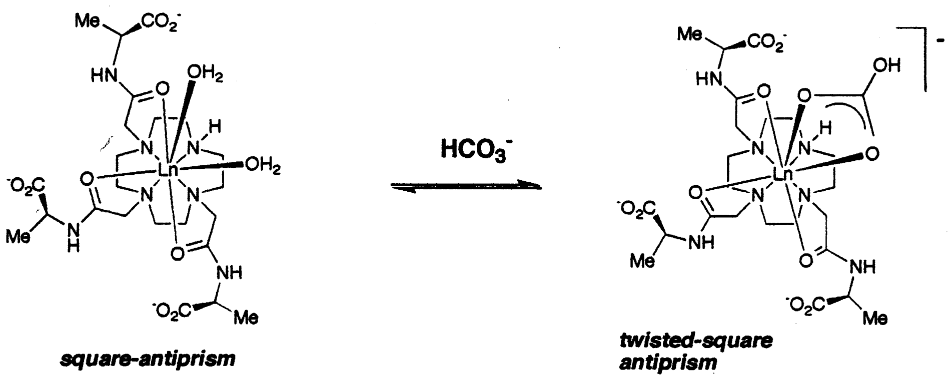
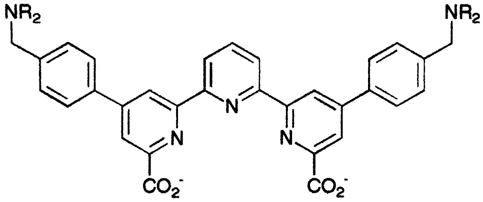


Fig. 8. Interconversion between square antiprismatic (twist angle 40°) and 'twisted' square-antiprismatic coordination geometries caused by chelation of HCO_3^- at the lanthanide centre.

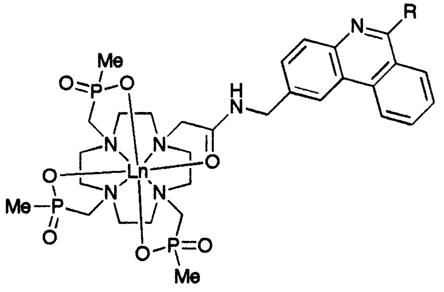
3. pH and pO_2 dependent luminescence

There have been reports of the suppression of photoinduced electron transfer, following protonation of a basic site, involving systems containing a spaced chromophore and a proximate luminescent metal centre. Thus protonation of a phenolate anion, in a calixarene derivative that has been conjugated to a $Ru(bpy)_3^{2+}$ reporter, leads to an enhancement of the metal-based emission [43]. In the Eu and Tb complexes of the pentadentate terpyridyl ligand **13**, protonation of the aminoalkyl substituents suppresses electron transfer to the singlet excited state giving enhancements of up to 16 in Ln^{3+} emission intensity [5]. However these complexes are not very stable in aqueous media (β_{ML} is ca. 10^6 and a 100-fold excess of Ln^{3+} ions was used) are strongly quenched by OH oscillators ($q = 4$) and the excitation wavelength used was < 350 nm.

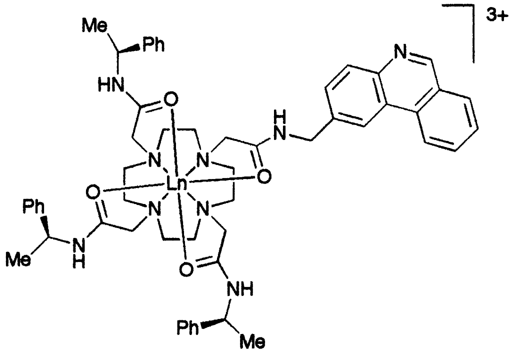


13

Stimulated by the need for kinetically stable, single-component, luminescent lanthanide complexes, the Durham group has based the design of their systems on the robust, structurally versatile series of complexes of substituted cyclen (12-N_4)-including tri-phosphinates, tetra-amides and mono-amide tricarboxylates, e.g. **5** and **10**. A phenanthridine chromophore has been incorporated into a range of complexes that includes **14** and **15** [27,34]. As discussed in Section 2.1, *N*-protonation or alkylation of the sensitising phenanthridine allows selective excitation at wavelengths in excess of 365 nm. Excitation at 370 nm of **14a**, **14b** and **15** gave rise to



14 a) R = H
b) R = Bu



15

both fluorescence from the excited chromophore and sensitised emission from the Eu ion that increased in intensity as the pH was lowered. The excited state pK_a values, reported both by changes in fluorescence intensity at 405 nm and luminescence increases at 616 (and 700) nm, were measured to be 4.4, 5.35 and 3.3 (298 K, $I = 0.1$ NMe_4ClO_4) for **14a**, **14b** and **15**, respectively (Fig. 9). This range of values reflects the weak electron releasing effect of the alkyl substituents and inhibition of protonation associated with charge repulsion. The lifetime and emission intensity was independent of dissolved oxygen concentration and the Eu lifetime was also pH independent [34].

The terbium complexes behaved in a very different manner. Whilst the phenanthridyl-based fluorescence intensity varied with pH in an identical manner, the metal-based emission intensity and lifetime *decreased* with increasing acidity [28]. For **14a** ($\tau_{H_2O} = 0.98$ ms, $\phi = 2.5\%$; [HTb **14a**]$^+$: ($\tau_{H_2O} = 0.1$ ms, $\phi = 0.09\%$), an observed pK_a of 5.75 was determined-which corresponds to the pK_a of the intermediate phenanthridine triplet excited state. Measurements of the triplet absorption spectra of the corresponding Gd complexes at 77 K revealed that the energy gap between the phenanthridine moiety and the Tb $^5D_4$ level (20 500 cm^{-1}), fell from 1500 to 800 cm^{1-} upon protonation. Therefore in the protonated complex only, there was a significant rate of back-energy transfer, repopulating the phenanthridyl triplet. A photo-equilibrium was established and the rate of decay of the terbium emission reported the pK_a of the phenanthridyl triplet state [34]. An added consequence of

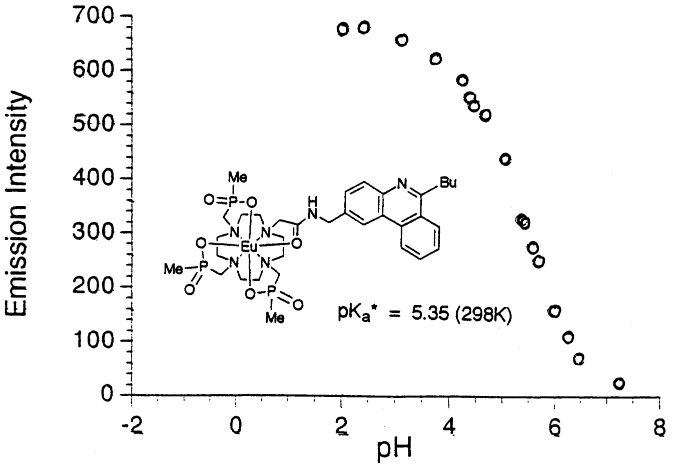


Fig. 9. Modulation of the europium emission intensity (616 nm) following protonation of the phenanthridine nitrogen in [Eu **14b**] (298 K, I = 0.1 M NMe_4NO_3).

this behaviour was that the rate of decay of the terbium emission was also sensitive to dissolved oxygen concentration, i.e. $k_q[O_2]$ and k_{-1} in Fig. 1, are competitive. Significant increases in overall quantum yield and emission lifetime were achieved by sample deaeration, e.g. with [Tb **14a**], deoxygenation gave $\tau_{H_2O} = 1.82$ ms ($\phi = 12\%$, λ_{exc} 350 nm) for the unprotonated complex and $\tau_{H_2O} = 0.83$ ms ($\phi = 5\%$) for the protonated form. The dual sensitivity of the terbium emission and lifetime to pH and pO_2 complements the pH-dependence observed with the Eu analogue.

The *N*-methylated Tb complexes **16a** and **16b**, retained their pO_2 sensitivity, but showed pH independent emission over the range 1–10. Classical Stern–Volmer quenching behaviour was observed with K_{sv}^{-1} values of 45 and 58 Torr for **16a** and **16b**, associated with a change in terbium emission lifetime of 0.8 ms, for the dissolved oxygen concentration range 0–0.3 mM. Such triplet excited state quenching is well known in a plethora of polyazaruthenium [31] and Pd/Pt porphyrin complexes [30], as well as in some more recently described terbium complexes [8,9].

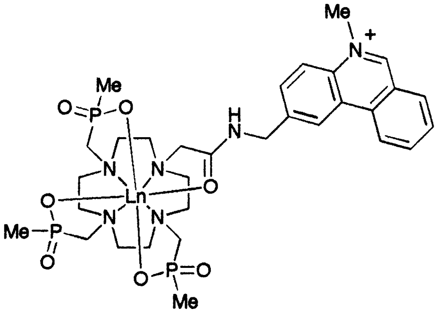
4. Anion dependent luminescence

In principle, anions may perturb either the excited singlet or triplet state of the chromophore, or may affect the rate of quenching of the lanthanide excited state. In the former case, examples reported so far are restricted to quenching of the singlet state [34], whereas in the latter, reversible anion binding to the lanthanide ion itself, leads to large changes in emission lifetime, polarisation and intensity as bound water molecules are displaced [26].

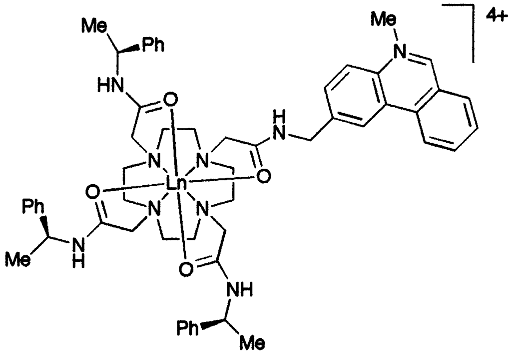
4.1. Quenching of the singlet excited state by anions

The fluorescence of *N*-alkylated quinolinium, acridinium and phenanthridinium ions is quenched by halide anions in aqueous media [29]. This phenomenon has been applied to the development of assays for the chloride ion in biological media [32]. There is a need to measure Cl^- concentrations (ca. 100 mM extracellular, and 10–75 mM intracellular) in the physiological range due its important role in controlling endosomal acidification [44]. Moreover, chloride ion channels are involved in the facilitated exchange of Cl^- for HCO_3^- in erythrocytes and the failure of this system has been demonstrated in cystic fibrosis patients [45]. The quenching effect follows the order $I^- > Br^- > Cl^-$, consistent with the ease of anion oxidation, and is most likely to involve charge transfer from the halide ion to the excited singlet of the cationic chromophore. In the europium complexes **16a** and **16b**, addition of halide ions leads to a decrease in the intensity of the phenanthridinium fluorescence (405 nm) and an accompanying diminution in europium luminescence (e.g. 594 and 616 nm), over the halide concentration range 0–250 mM. A reduction in emission intensity of a factor of 4 was observed over this range, with no change in Eu emission lifetime. The Stern–Volmer quenching constant of 40–50 mM falls in the expected range for this process (Table 2) and the quenching effect was independent of added sodium bicarbonate, dihydrogen phosphate, lactate or citrate, for concentrations of up to 30 mM [34]. Encounter of the quenching ion-pair was favoured for the more highly charged tetracationic complexes of **16b** (Table 2).

It has been established that *N*-methylphenanthridinium ions are attacked at C-6 by the hydroxide ion, in a reversible reaction [46] that leads to the disappearance of the longest wavelength absorption band as the conjugation length is reduced, (Fig.



16a



16b

Table 2
Stern–Volmer quenching constants (K_{sv}^{-1}, mM)[a] for halide ion quenching with the cationic europium-phenanthridinium complexes **16a** and **16b**

Anion/complex	K_{sv}^{-1}/mM phenanthridinium fluorescence (405 nm)	K_{sv}^{-1}/mM europium luminescence (616 nm)
Cl^-/[**16a** Eu]$^+$	40	50
Br^-/[**16a** Eu]$^+$	8.0	16
I^-/[**16a** Eu]$^+$	6.4	4.9
I^-/[**16b** Eu]$^{4+}$	1.9	1.2
Br^-/[**16b** Eu]$^{4+}$	2.5	12
Cl^-/[**16b** Eu]$^{4+}$	6.0	26

[a] K_{sv}^{-1} values have a 10% error; 293 K, $I = 0.1$ M NMe_4ClO_4. [**16a**/**16b**] $= 5 \times 10^{-5}$ M, with $\tau_{H_2O} =$ 0.73 ms. Corresponding Tb complexes showed similar reductions in phenanthridinium fluorescence, but only a small change in the pO_2 sensitive Tb emission intensity, e.g. for [**16b** Tb]$^{4+}$, at 30 mM added NaCl, the weak intensity of emission reduced by 14%.

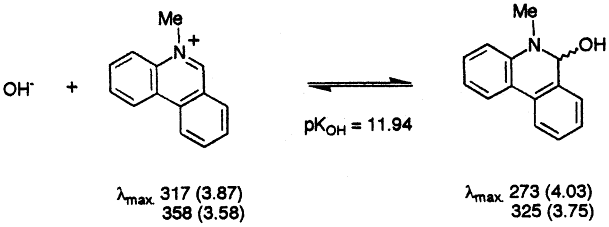


Fig. 10. Reversible pseudo-base formation, involving attack at C-6.

10). With [Eu **16a**]$^+$, addition of KOH over the pH range 10–13.5, led to a decrease in the europium emission intensity (λ_{exc} 380 nm, λ_{em} 594 nm) as reversible C–O bond formation occurred. The pseudo-base adduct does not absorb at the chosen excitation wavelength and the observed emission intensity was zero above pH 13.5. An effective dissociation constant of 16 mM (± 2), equivalent to $pK_{OH} = 12.2$ [34] is in good agreement with related literature values for ground-state ions, [46]. Thus the red europium emission is independent of pH over the range 2–9 but falls in intensity by a factor of > 200 as the pH rises to over 13.

4.2. Modulation of lanthanide luminescence following anion binding to the metal

Lanthanide complexes of heptadentate ligands, such as **17a**, possess two bound water molecules, which are most probably oriented at an angle of 70° to each other in a 9-coordinate environment. The successive displacement of these water molecules by coordinating anions gives rise to an increase in the emission lifetime and intensity as the primary quenching mechanism, involving vibrational energy transfer to bound OH oscillators (Section 2.2), is removed. The number of bound water molecules can be evaluated quite accurately by measuring the rate constant for depopulation of the excited state in H_2O and D_2O [15]. Provided that a correction is made for the effect of unbound (closely diffusing) H_2O molecules (see Fig. 5), and for other *exchangeable* XH oscillators (e.g. amide NH, amine NH —

see Fig. 6), then the hydration state may be estimated. By measuring the decay rates in H_2O and D_2O, and applying the empirically-derived relationships in Eqs. (9)–(11), wherein

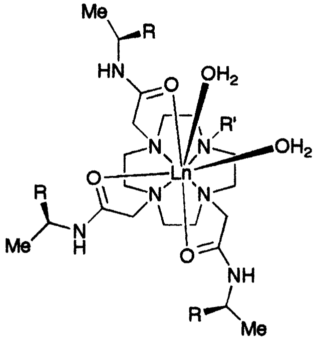
$$q_{\mathrm{Eu}} = 1.2[(\Delta k_{\mathrm{corr}}) - 0.25] \tag{9}$$

$$q_{\mathrm{Tb}} = 5[(\Delta k_{\mathrm{corr}}) - 0.06] \tag{10}$$

$$q_{\mathrm{Yb}} = 1[(\Delta k_{\mathrm{corr}}) - 0.25] \tag{11}$$

Δk_{corr} is the difference between k_{H_2O} and k_{D_2O} — corrected by 0.085 ms^{-1} per amide NH with Eu (zero for Tb, 0.06 $\mu\mathrm{s}^{-1}$ for Yb) — the q value for Eu, Tb or Yb may be calculated.

Whilst isolated reports have appeared on anion effects in $q = 2$ or 3 luminescent lanthanide complexes [47,48,15], a more systematic study has been made with [Eu **17a**]$^{3+}$ and [Tb **17a**]$^{3+}$, [26]. Anions such as NO_{3-}, Cl^- and I^- appeared not to bind at the di-aqua metal centre in the pH range 5–7.5, although deprotonation of one of the water molecules may labilise the second water molecule (Fig. 11). Another group of anions displaced one water molecule at ambient pH (HPO_4^{2-}, F^-, SO_4^{2-}) with differing affinities and chelating anions such as HCO_3^-, lactate and citrate displaced *both* water molecules, giving the largest enhancements in emission lifetime and intensity. The case of HCO_3^- binding is particularly interesting. Significant changes in the Eu $\Delta J = 2$ band (**17a–17c**) occur on adding $NaHCO_3$, and over the range 0–40 mM (pH 7.5) a 5- or 6-fold intensity change was measured. This behaviour is also pH-dependent (pK_a $HCO_3^-/H_2CO_3 = 6.38$, $I = 0.1$, 298 K) so that for a given starting bicarbonate concentration, a pH dependent lifetime and emission intensity was observed [49] (Fig. 12; 30 mM HCO_3^-, 0.1 M Cl^-, 2.3 mM lactate, 0.9 mM $H_2PO_4^-$, 0.13 mM citrate). Given that hydrogencarbonate (and carbonate) form a chelated adduct, whereas acetate binds only very weakly to [Eu **17a–17c**], the complexation mechanism may involve a stepwise process involving attack of a coordinated hydroxy group at the sp^2 centre, followed by loss of OH^- (Fig. 13).



17 a) R' = H, R = Ph
b) R' = H, R = CO_2^-
c) R' = Me, R = Ph

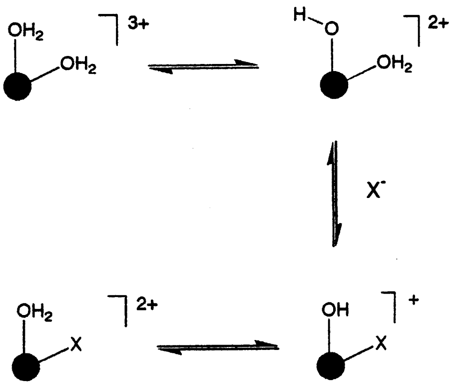


Fig. 11. Labilisation of a bound water following deprotonation.

Proton NMR studies with [Eu **17a**]$^{3+}$ and [Eu **17b**] have revealed that the di-aqua complex adopts a square-antiprismatic structure while the chelated adduct with HCO_3^- takes up a *twisted* square-antiprismatic geometry. Such a process — consistent with the pronounced changes reported by the hypersensitive $\Delta J = 2$ Eu emission band — also changes the helicity at the metal centre. The *circular polarisation* of the emission is a sensitive function of this helicity and pronounced changes in the circularly polarised luminescence from the excited lanthanide have been noted in the presence of different anions (e.g. phosphate, HCO_3^- and lactate) [26]. Thus, using chiral complexes such as [Ln **17a–17c**], the emission polarisation (in the $\Delta J = 1$ or 2 bands) may be used to signal the concentration of HCO_3^- (and thereby pH) in solution, for the first time.

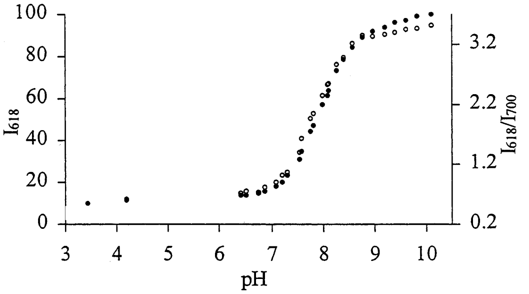


Fig. 12. Emission intensity ($\Delta J = 2$, 618 nm; ●) and ratio of emission intensities ($\Delta J = 2/\Delta J = 2$; ○) accompanying pH changes for [Eu **17b**] (298 K, 30 mM $NaHCO_3$, 0.9 mM Na_2HPO_4, 2.3 mM sodium lactate, 0.1 M NaCl, 0.13 mM sodium citrate).

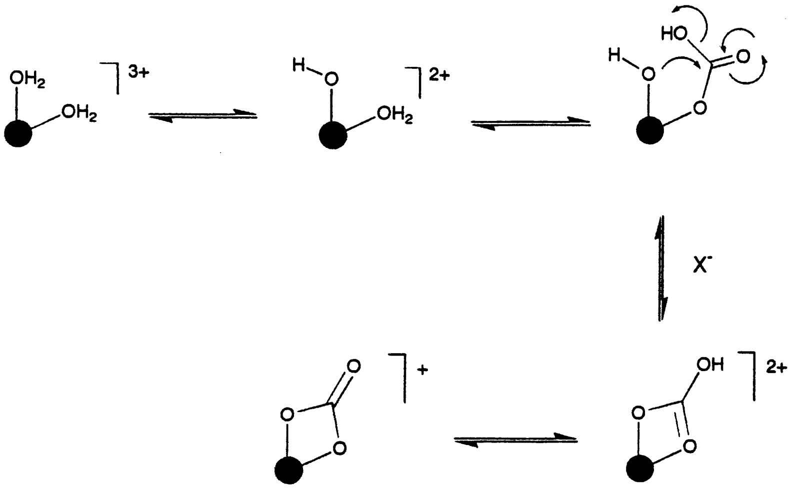


Fig. 13. Probable mechanism of chelation of hydrogencarbonate at a diaqua lanthanide centre.

5. Summary and prospects

The development of fibre-optic technology, the miniaturisation of solid-state photonic devices and the availability of inexpensive lasers and light-emitting diodes offer great scope for the development of luminescent sensors that operate either in vivo or in 'aggressive' environments. The measurement of the time-resolved luminescence from carefully designed, robust lanthanide complexes offers particular promise, in this respect. Systems in which the emission lifetime, intensity or polarisation varies as a function of pH, pO_2 or anion concentration have already been outlined. Improvements are needed in the overall efficiency of these systems, (e.g. suppression of competitive electron transfer, the need for longer wavelength excitation) but our understanding of the factors that determine this efficiency is now sufficiently advanced that it is only a question of time before practicable devices are developed.

Acknowledgements

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