Direct determination of stability constants of lanthanide ion chelates by laser-excited europium(III) luminescence spectroscopy: application to cyclic and acyclic aminocarboxylate complexes

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Excitation spectroscopy of the $^7F_0 \longrightarrow {}^5D_0$ transition of Eu^{3+} in the visible has been used to quantitate the complexation of this ion to various chelating carboxylate ligands including 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate (dota). Two methods were employed. The first involves the measurement of conditional stability constants at constant low pH values where hydrogen ions compete with Eu^{3+} for the ligand. The second method involves measurement of the concentration of the Eu^{3+}–ligand complex as a function of pH in the low pH region. The data are then fitted by an equation which accounts for the multiple equilibria involved (ligand and complex protonation) with the stability constant being a parameter to be fit. For $[Eu(dota)]^-$ a log K value of 26.21 ± 0.10 was obtained which falls among a wide range of reported values. The values for the remaining ligands are in excellent accord with literature values. The log K values for complexes of dota were obtained for the entire Ln^{3+} ion series by a metal ion competition method. This ligand discriminates effectively against the larger members of this series.

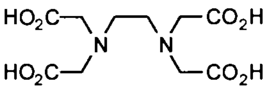
Prompted by the search for safe and efficient magnetic resonance imaging (MRI) contrast agents,[1–5] a variety of new polyaminopolycarboxylates and their lanthanide ion (Ln^{3+}) chelates ($Ln^{3+} = Gd^{3+}$, Dy^{3+}) have been synthesized in recent years. These chelate complexes often exhibit extremely high stability and frequently slow complexation rates. These factors sometimes lead to difficulty in measuring their stability constants using conventional methods, *e.g.* pH-potentiometry.[6–11] Since a knowledge of their stability constants and the selectivity of these ligands for various metal ions is important to their application in medicine and for the understanding of their coordination chemistry, development of reliable methods for making such measurements is of considerable importance. Efforts in this direction have included the use of chromophoric ligands as spectroscopic indicators,[12,13] proton relaxation measurements in cases where relaxivities are significantly different for the complex and the free Ln^{3+}(aq) ion,[14,15] methods based on kinetic measurements of formation and dissociation,[16] and a batch method for long equilibration in pH-potentiometric titrations.[10,11] Very recently Tóth and Brücher[17] have reported the determination of the stability constants of the tetraazatetracarboxylate macrocycle H_4dota (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), using UV absorption spectroscopy of the complexes of Ce^{3+} and Eu^{3+}, working in the millimolar concentration region.

Laser-excited europium(III) luminescence spectroscopy provides a unique and sensitive way to monitor directly the binding of this ion to ligands in solution.[18,19] Since the absorptive transition is between non-degenerate levels, the $^7F_0 \longrightarrow {}^5D_0$ transition of Eu^{3+} in the 577–581 nm range exhibits a single excitation band for each unique europium(III) environment, which is frequently characterized by a unique excited state lifetime as well. Development of this technique in this laboratory over the past decade has resulted in our ability to measure, accurately and routinely, complexed Eu^{3+} in the 10^{-6}–10^{-8} mol dm^{-3} range. Thus, the method presents an attractive analytical technique to apply to stability constant measurements. Recently we reported[20] a general method for determining stability constants for a given ligand toward Eu^{3+} relative to a reference ligand by means of ligand–ligand competition experiments. Since hydrogen ions, H^+, are able to compete with the metal ion for the ligand, the degree of metal ion complexation generally decreases as the pH is lowered. This is quantitatively described by a decrease in the effective or conditional stability constant, K_{cond}. In this reserch, competition by H^+ is also exploited to establish another general method for determining stability constants for complexes of Eu^{3+} from measurements in the low pH range. The stability constants of a number of complexes of aminopolycarboxylate ligands, including dota, were determined and compared to literature values. Based on the Eu^{3+}–dota results, the stability constants for dota with the entire Ln^{3+} ion series have been determined *via* Eu^{3+}–Ln^{3+} competition experiments.

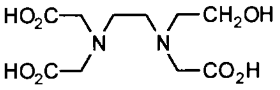
Experimental

Materials

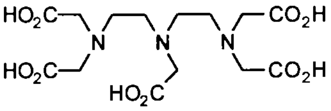
All lanthanide salts used were the purest commerically available from Alfa or Aldrich. Ethylenedinitrilotetraacetic acid (H_4edta) (98%), *N*-carboxymethyl-*N*′-(2-hydroxyethyl)ethylenediiminodiacetic acid (H_3hedta) (99%), [(carboxymethyl)imino]bisethylenenitrilotetraacetic acid (H_5dtpa) (97%) and 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (H_4teta) were from the Aldrich Chemical Company. 2-(Morpholino)ethanesulfuric acid (mes) was purchased from Sigma Chemical Co. The H_4dota was provided by Nycomed Salutar, Inc. 9,17-Dioxo-1,4,7,10,13,16-hexaazacyclooctadecane-1,4,7-triacetic acid (H_3L^1) was a gift from S. J. Franklin and K. N. Raymond of the University of California, Berkeley. 1,4,7-Triazacyclononane-1,4,7-triacetic acid (H_3nota) was synthesized by a method described in the literature.[21] The water used was deionized and doubly distilled and all remaining reagents were the purest commercially available. The metal ion standard solutions were prepared at ≈10 mmol dm^{-3} from the nitrate or chloride salts of the metals and were standardized with an edta titration by using an arsenazo indicator. The edta titrant was standardized using a terbium(III) atomic absorption standard solution (1005 μg cm^{-3} of Tb in 1% HNO_3) supplied by Aldrich Chemical Company. The concentrations of ligand stock solutions (2–4 mmol dm^{-3}) were determined *via* titrations with standardized Eu^{3+} at pH 6 on equilibrated samples using laser-excited europium(III) luminescence to monitor complexation, which is quantitative at this pH value.



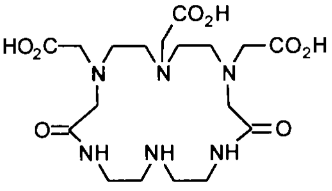
$\mathrm{H_4}$ edta



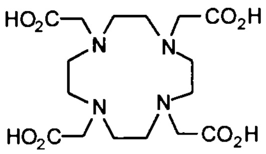
$\mathrm{H_3}$ hedta



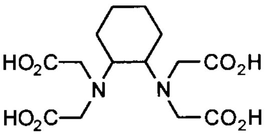
$\mathrm{H_5}$ dtpa



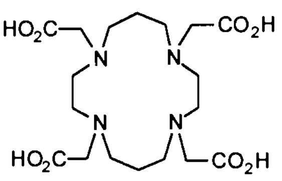
$\mathrm{H_3}$ $\mathrm{L^1}$



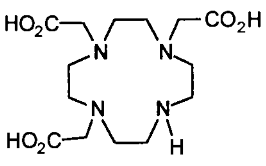
$\mathrm{H_4}$dota



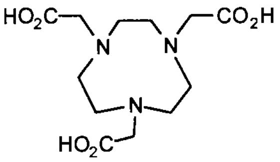
$\mathrm{H_4}$cdta



$\mathrm{H_4}$teta



$\mathrm{H_3}$dotra



$\mathrm{H_3}$nota

Methods

Europium(III) excitation spectra, excited state lifetimes and excitation intensities were measured using a Continuum YG-581C pulsed (10 Hz) Nd:YAG laser-pumped tunable TDL-50 dye laser described elsewhere.[22] The $^7F_0 \longrightarrow {}^5D_0$ transition of the Eu^{3+} ion (578–581 nm) was excited by using a mixture of Rhodamine 590 (Excition Co.) and 610 (Kodak Chemical Co.) resulting in 60–90 mJ per pulse. The $^5D_0 \longrightarrow {}^7F_2$ emission band at 614 nm was monitored in each case. Solution pH values were measured by using an Orion pH meter (model 720A) with an Orion glass electrode. The pH meter was calibrated using standard pH buffer solutions (VWR Scientific, West Chester, PA). The measured pH values are accurate to ±0.002 pH units.

Determination of stability constants of europium(III) complexes by measurement of the luminescence intensity, *I*, in the low-pH region. Except as indicated, for *I vs.* pH titrations a series of 2 cm^3 samples containing about 2 μmol dm^{-3} Eu^{3+}, 2 μmol dm^{-3} ligand and 0.1 mol dm^{-3} KCl with various pH values adjusted in the range 2–4 was prepared for each ligand. For *I vs.* added ligand titrations, samples were prepared containing 2 μmol dm^{-3} Eu^{3+} ion (or different concentration indicated in the text), 0.1 mol dm^{-3} KCl and various ligand concentrations ranging from 0 to 4 μmol dm^{-3} at a particular constant pH, as noted in the text. Since the low-pH solutions in the range 2–4 have some self-buffering effect and only small concentrations of H^+ ion are released in the reactions, no buffer is necessary in these measurements. Except for dota, all samples were kept at 25 °C for about 12 h of equilibration before their europium(III) luminescence intensities were recorded. For dota, since the formation reaction is slow, the samples were incubated at 70 °C for 24 h (or 3 d) followed by an additional 3 d (or 10 d) of room temperature (25 °C) equilibration. One set of samples was equilibrated for 891 d at 27 ± 1 °C.

Determination of stability constants for the $Ln(dota)^-$ complexes across the lanthanide series by Ln^{3+}–Eu^{3+} competition experiments. The samples were made up in 20 mmol dm^{-3} mes at pH 6.00, 80 mmol dm^{-3} KCl and 5 μmol dm^{-3} each in dota, Eu^{3+} and Ln^{3+}. Three samples were made up with each Ln^{3+} ion. All samples were maintained at 70 °C for 24 h followed by a 3 d room-temperature equilibration.

For each equilibrium sample described above, the excited state lifetime and associated amplitude were recorded at the peak maximum wavelength for carefully measured periods of between 2 and 20 min. After being corrected for any variations in the laser power and the time of data accumulation, the measured amplitude represents a quantity directly proportional to the concentration of the europium(III) complex. The commercially available PEAKFIT program (Jandel Scientific) was employed in the data analysis. Data in the form of amplitude, I_t, *vs.* pH were fitted by equation (10) using non-linear regression, where the parameters of the fit are defined in the following section. Many of the parameters are known from other experiments and were held fixed during the analysis which determines the thermodynamic stability constant of the complex, *K*. The protonation constants for the chelating ligands used in the computations are listed in Table 1.

Data analysis

Since the multidentate ligands of interest here form 1:1 complexes with lanthanide ions, the general complexation equilibria (1)–(5) apply, where $K_1, K_2 \ldots K_n$ are the protonation constants

$$Eu^{3+} + L^{n-} \rightleftharpoons [EuL]^{(n-3)-},\ K = [EuL]/[Eu^{3+}][L] \quad (1)$$

$$[EuL]^{(n-3)-} + H^+ \rightleftharpoons [Eu(HL)]^{(n-2)-},\ K_H = [Eu(HL)]/[EuL][H] \quad (2)$$

$$L^{n-} + H^+ \rightleftharpoons HL^{(n-1)-},\ K_1 = [HL]/[H][L] \quad (3)$$

$$HL^{(n-1)-} + H^+ \rightleftharpoons H_2L^{(n-2)-},\ K_2 = [H_2L]/[HL][H] \quad (4)$$

$$H_{n-1}L^- + H^+ \rightleftharpoons H_nL,\ K_n = [H_nL]/[H_{n-1}L][H] \quad (5)$$

of the ligand, *K* is the stability constant for the complex of the fully deprotonated carboxylate ligand called the thermodynamic stability constant and K_H is the protonation constant

Table 1 Protonation constants of carboxylates used in this study

Carboxylate	log K_1	log K_2	log K_3	log K_4	log K_5	log K_6
edta[a]	10.19	6.13	2.69	2.00	1.5	0.0
hedta[a]	9.84	5.39	2.67	1.6		
cdta[a]	12.3	6.12	3.49	2.40	1.6	
dtpa[a,b]	10.48	8.60	4.28	2.6	2.0	1.6
L^1[c]	10.02	8.87	4.10	2.62	1.80	
dota[d]	11.14	9.69	4.84	3.95		
nota[a]	11.7	5.7	3.17	1.7		
dotra[e]	11.59	9.24	4.43	3.48		
teta[a]	10.75	10.13	4.11	3.27	2.17	1.4

[a] Ref. 23, 25 °C, 0.1 mol dm^{-3} KCl. [b] log K_7 0.7, log K_8 −0.1. [c] Ref. 24, 25 °C, 0.1 mol dm^{-3} KCl. [d] Ref. 10, 25 °C, 0.1 mol dm^{-3} KCl. [e] Ref. 25.

of the complex. For simplicity, the charges on the ionic species in the equilibrium expressions have been omitted, *i.e.* $[H^+] = [H]$; C_1 and C_2 denote the total concentrations of Eu^{3+} $\{[EuL] + [Eu(HL)] + [Eu^{3+}]\}$ and ligand $\{[EuL] + [Eu(HL)] + [L] + [HL] + \ldots + [H_nL]\}$, respectively, α is the fraction of uncomplexed carboxylate present as the fully deprotonated species, L. Based on the above equilibrium equations one obtains expressions (6)–(8).

$$[EuL] = [(C_1 - C_2)(1 + K_H[H]) + (K_\alpha)^{-1} - \{[(C_1 + C_2) \times (1 + K_H[H]) + (K_\alpha)^{-1}]^2 - 4C_1C_2(1 + K_H[H])^2\}^{\frac{1}{2}}]/2(1 + K_H[H])^2 \quad (6)$$

$$[Eu(HL)] = K_H[H][EuL] \quad (7)$$

$$\alpha = (1 + K_1[H] + K_1K_2[H]^2 + \ldots K_1K_2 \ldots + K_n[H]^n)^{-1} \quad (8)$$

At very low pH values it is possible that some of the EuL complexes become protonated. Our spectroscopic results show that such protonation has a minor effect on the environment of the complexed Eu^{3+} ion as evidence by the fact that the position of the ${}^7F_0 \longrightarrow {}^5D_0$ excitation maximum does not change upon protonation; nor is there any observed effect on the ${}^5D_0$ excited state lifetime. We have, however, in the case of $[Eu(dota)]^-$, observed a 41% increase in the luminescence intensity upon protonation. This is not unexpected since addition of a proton, most likely at a non-co-ordinated oxygen of one of the four co-ordinated carboxylate groups, would lower the overall symmetry of the complex and make the transition somewhat more allowed. Thus the measured intensity, I_t, may involve contributions from both EuL and Eu(HL). Therefore, we obtain equation (9) where k' is the proportionality constant between I_{EuL}

$$I_t = I_{EuL} + I_{Eu(HL)} = k'[EuL] + k''[Eu(HL)] \quad (9)$$

and [EuL], which can be directly determined at neutral pH where the Eu(HL) concentration is vanishingly small; k'' is the proportionality constant between $I_{Eu(HL)}$ and [Eu(HL)]. Combination of equations (6), (7) and (9) gives (10). Equation (10)

$$I_t = (k' + k''K_H[H])[(C_1 + C_2)(1 + K_H[H]) + (K\alpha)^{-1} - \{[(C_1 + C_2)(1 + K_H[H]) + (K\alpha)^{-1}]^2 - 4C_1C_2(1 + K_H[H])^2\}^{\frac{1}{2}}]/2(1 + K_H[H])^2\} \quad (10)$$

shows that I_t is the function of the concentrations (C_1, C_2 and [H]). Further analysis of equation (10) indicates that the intensity, I_t (or the concentration), of a given complex becomes sensitive to the three concentrations (C_1, C_2 and [H]) only when the value of $(K\alpha)^{-1}$ is large enough not to be neglected in the term $(C_1 + C_2)(1 + K_H[H]) + (K\alpha)^{-1}$. For the lanthanoid(III) complexes of the common polyaminocarboxylate ligands under neutral pH conditions the $(K\alpha)^{-1}$ value is usually much lower than the working concentration of the present technique (10^{-6}–10^{-8} mol dm^{-3}). However by lowering the pH, the conditional stability constants of the complexes rapidly drop. It is always possible to find a pH value below which the condition $(K\alpha)^{-1} \geqslant (C_1 + C_2)(1 + K_H[H])$ will be satisfied for a given ligand–Eu^{3+} system. Thus determinations of stability constant may be carried out based on equation (10) either by titrations of excitation intensity *vs.* pH or excitation intensity *vs.* concentration (C_1 or C_2) in the low pH range (2–4).

Results and Discussion

Protonation of the Eu^{3+}–dota complex

The ${}^5D_0 \longrightarrow {}^7F_0$ excitation spectra of Eu^{3+} are highly sensitive to minor changes in the environment of Eu^{3+}. Nevertheless at low pH values where $[Eu(dota)]^-$ would be expected to be protonated to form [Eu(Hdota)] no change in excitation band position or excited state lifetime are observed. As noted earlier there is an instantaneous increase in excitation band intensity when the pH is suddenly lowered. Since equilibria involving protonation are very rapidly established and the metal ion dissociates only slowly for dota complexes, it is possible to quantitate the protonation equilibria for $[Eu(dota)]^-$ using luminescence spectroscopy.

An instantaneous increase in the excitation intensity of the ${}^7F_0 \longrightarrow {}^5D_0$ band of $[Eu(dota)]^-$ at 579.77 nm is observed upon suddenly dropping the pH of 10 μmol dm^{-3} solutions of the complex from 5 to values less than 2.5 by adding aliquots of 12 mol dm^{-3} HCl. The augmentation in the excitation amplitudes increases with increasing amounts of added HCl. Following this instantaneous luminescence intensity increase, this quantity slowly decreases as the metal ion dissociates from the complex. Fig. 1 shows a plot of the instantaneous intensity increases, Δ*I*, *vs.* the final pH for solutions prepared by adding various quantities of concentrated HCl; Δ*I* is defined as $I_t - I_t^0$, where I_t^0 and I_t are the total intensities measured before and immediately after adding HCl, respectively. For the protonation equilibrium (11) the relationship (12) between Δ*I* and the

$$[Eu(dota)]^- + H^+ \rightleftharpoons [Eu(Hdota)] \quad (11)$$

$$\Delta I = (k'' - k')[K_H][H][Eu(dota)^-]_t/(K_H[H] + 1) \quad (12)$$

protonation constant, $K_H/dm^3\ mol^{-1}$, where k' and k'' have the same meaning as in equation (9) and $[Eu(dota)^-]_t$ is the total concentration of both protonated and unprotonated complexes is obtained. Fitting the data of Fig. 1 by equation (12) yields log $K_H = 1.46 \pm 0.07$. This value is quite comparable to the values log $K_H = 1.8$ for $[Eu(dota)]^-$ and log $K_H = 1.35$ for $[Gd(dota)]^-$ obtained by direct pH-potentiometric titrations of the corresponding complexes.[18,19] It is, however, significantly smaller than the value $K_H = 2.76$ for $[Gd(dota)]^-$ estimated from the instantaneous decrease in luminescence of that complex as a function of added acid.[13]

Determination of stability constants of europium(III) complexes

Titration of Eu^{3+} with ligand at constant pH. For metal ion chelates such as the ones of interest here which have thermodynamic stability constants log $K > 14$, conditional stability constants ($K_{cond} = K\alpha$) in the neutral pH region are such that K_{cond}^{-1} values are much smaller than the working sensitivity limit of our instrumentation (≈10^{-8} mol dm^{-3}). Under such neutral pH conditions a titration of metal ion with ligand would reveal quantitative binding with a sharp break in the titration curve at a 1:1 stoichiometry. Such a titration curve does not yield a stability constant (except perhaps a lower limit). By lowering the pH to a value such that $(K\alpha)^{-1} \geqslant (C_1 + C_2)(1 + K_H) \approx C_1 + C_2$ [see equation (10)] the titration curve (I_t *vs.* [L]) will show some curvature. Curves of this type for Eu^{3+} and dtpa, edta, L^1 and hedta are shown in Fig. 2. These data, and others not shown, were analysed using equa-

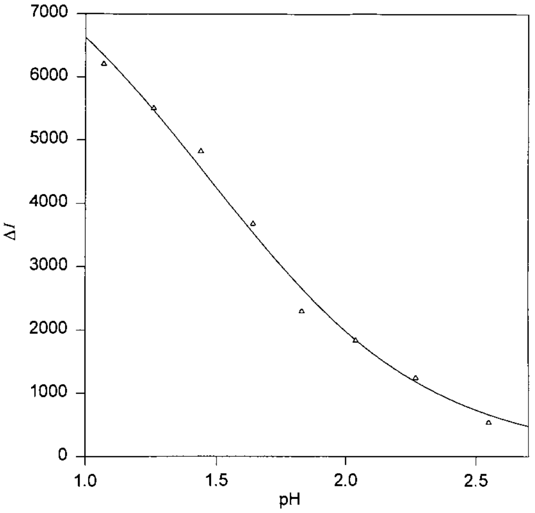


Fig. 1 Instantaneous luminescence intensity increase (Δ*I*) of $[Eu(dota)]^-$ *vs.* final pH caused by dropping the pH of 10 μmol dm^{-3} $[Eu(dota)]^-$ solutions from pH 5 by addition of aliquots of 12 mol dm^{-3} HCl. The solid curve represents the non-linear regression fit of the data by equation (12) as described in the text. The fitting yields log K_H of $[Eu(dota)]^- = 1.46 \pm 0.07$

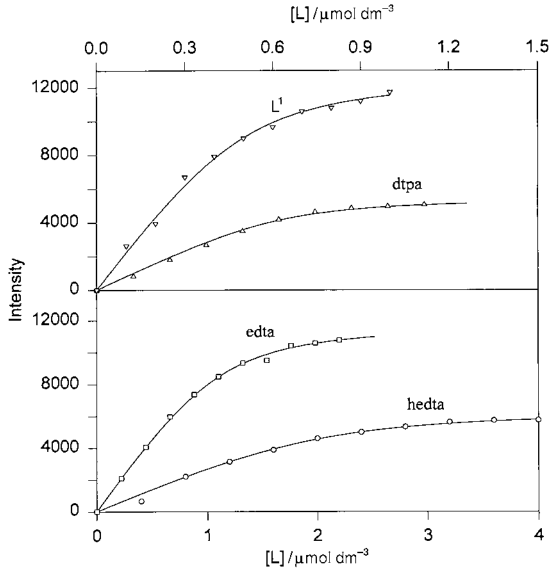


Fig. 2 Binding curves of Eu^{3+} titrated with varying amounts of each ligand: (▽) L^1, $[Eu^{3+}] = 0.5$ μmol dm^{-3} and pH 3.50; (Δ), dtpa, $[Eu^{3+}] = 0.62$ μmol dm^{-3} and pH 2.75; (□), edta, $[Eu^{3+}] = 1.1$ μmol dm^{-3} and pH 3.00; (○), hedta, $[Eu^{3+}] = 2$ μmol dm^{-3} and pH 2.75. The solid lines are the theoretical fits for each set of data, with the parameters indicated in Table 2

tion (10) and non-linear regression methods. The reciprocal conditional stability constants $(K\alpha)^{-1}$ and log *K* values calculated from them are set out for the above ligands and H_4cdta (cyclohexane-1,2-diyldinitrilotetraacetic acid) in Table 2.

Complex formation as a function of pH at low pH. The fact that the value of the conditional stability constant, K_{cond} (= $K\alpha$), drops rapidly as the pH is lowered guarantees that there is a pH range where the concentration of EuL goes from its maximum value to zero. In this work a series of samples containing equimolar quantities of Eu^{3+} and carboxylate (≈2 μmol dm^{-3}) was prepared, each at a different pH. After suitable equilibration the $^7F_0 \longrightarrow {}^5D_0$ excitation intensity of each sample was measured at the wavelength of the peak maximum. The curves of intensity *vs.* pH value so obtained for the Eu^{3+}–hedta, –edta, –dtpa, –dota, –dotra (1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid), –nota and –teta systems are shown in Fig. 3. The solid curves represent non-linear regression fittings of the data points by equation (10). In the fits only *k*′, *k*″ and *K* were treated as adjustable parameters. The remaining parameters were held constant; protonation constants for each carboxylate were taken from the literature (see Table 1), as were most of the protonation constants (K_H) of the complexes. The log K_H values used for $[Eu(edta)]^-$, $[Eu(dtpa)]^{2-}$ and $[Eu(dota)]^-$ were 1.37,[26] 2.15[23] and 1.46 (this work), respectively. For [Eu(hedta)] the protonation constant was taken as 0 since none is reported in the literature and its electroneutrality would tend to make it less basic than its negatively charged counterparts. This assumption was also made for [Eu(dotra)], [Eu(nota)] and $[Eu(teta)]^-$ since their complexation data were obtained in a higher pH range. The log *K* results are collected in Table 2. With the possible exception of $[Eu(dota)]^-$, where there is a wide discrepancy in the reported literature values, the agreement with literature values is excellent.

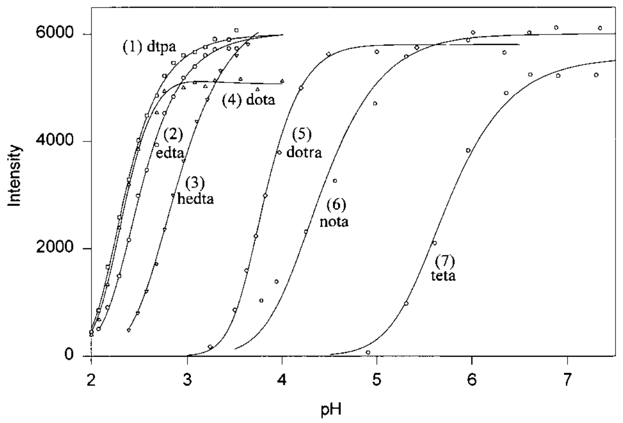


Fig. 3 Excitation intensities of the $^7F_0 \longrightarrow {}^5D_0$ transition of the complexes: (1) $[Eu(dtpa)]^{2-}$ (□); (2) $[Eu(edta)]^-$ (○); (3) [Eu(hedta)] (▽); (4) $[Eu(dota)]^-$ (Δ); (5) [Eu(dotra)] (◇); (6) [Eu(nota)] (○); and (7) $[Eu(teta)]^-$ (○) as a function of pH. Each set of data was fitted by equation (10) (solid lines). For systems 1–5, $[Eu^{3+}]$ = [ligand] = 2 μmol dm^{-3}; for 6, $[Eu^{3+}]$ = [nota] = 10 μmol dm^{-3}; and for 7, $[Eu^{3+}]$ = [teta] = 50 μmol dm^{-3}

Effect of protonation of complexes on the stability constant determination. Since the method described in the previous section necessarily involves working at low pH it is likely that some fraction of the complexes formed will be protonated. As mentioned earlier, such protonation modifies the environment of the Eu^{3+} only slightly and does not affect the $^7F_0 \longrightarrow {}^5D_0$ excitation peak position but may affect its intensity to some extent. When applying the present methods to new ligands where the protonation constants of the formed complexes are not known it is important to have knowledge of how the stability constants so determined will be affected. First, it should be noted that the protonation of the complexes is largely controlled by electrostatics. Thus the mononegative complexes $[Eu(edta)]^-$ and $[Eu(dota)]^-$ have similar log K_H values, 1.37[26] and 1.46, respectively, while the dinegative $[Eu(dtpa)]^{2-}$ has a higher value (2.15).[23] It can be expected that other europium(III)–polyaminocarboxylate complexes will have similar K_H values and that protonation will be significant only below pH 2.5. Two extreme cases may be considered. At very low pH values where Eu(HL) is the sole complex species in solution, equation (10) reduces to (13). Since *K* and K_H appear as a product in equation (13) they are not individually determinable.

$$I_t = 0.5k''\{C_1 + C_2 + (KK_H[H]\alpha)^{-1} - (\{C_1 + C_2 + (KK_H[H]\alpha)^{-1}\}^2 - 4C_1C_2)^{\frac{1}{2}}\} \quad (13)$$

On the other hand at higher pH values where the concentration of Eu(HL) is negligible equation (10) reduces to (14).

Table 2 Formation constants for the europium(III) complexes studied at 25 °C, 0.1 mol dm^{-3} KCl

		Method					
Complex	Excitation wavelength/nm	Intensity *vs.* [L]				Intensity *vs.* pH log *K*	Literature value
		pH	α	$(K\alpha)^{-1}$/mol dm^{-3}	log *K*		
$[Eu(dtpa)]^{2-}$	579.89	2.25	5.12×10^{-18}	3.26×10^{-6}	22.78 ± 0.07[a]	22.77 ± 0.04	22.39[b]
		2.50	5.07×10^{-17}	2.99×10^{-7}	22.82 ± 0.2		
		2.75	4.15×10^{-16}	5.06×10^{-8}	22.68 ± 0.16		
		3.00	2.92×10^{-15}	7.29×10^{-9}	22.67 ± 0.19		
$[Eu(edta)]^-$	579.60	3.00	3.11×10^{-11}	1.30×10^{-7}	17.39 ± 0.07	17.52 ± 0.16	17.29[b]
[Eu(hedta)]	579.50	3.50	4.51×10^{-9}	6.34×10^{-8}	15.54 ± 0.1	15.62 ± 0.02	15.45[b]
$[Eu(cdta)]^-$	579.60	3.50	1.85×10^{-12}	4.03×10^{-8}	19.13 ± 0.19		19.49[b]
$[Eu(HL^1)]^+$	579.87	2.75	1.81×10^{-8}	2.46×10^{-7}	14.35 ± 0.19		14.2 ± 0.3,[c] 14.11 ± 0.05[d]
$[Eu(dota)]^-$	579.77					26.21 ± 0.10[e]	23.7,[f] 23.5,[g] 28.2,[h] 22.1,[i] 24.0,[j] 25.3[k]
[Eu(dotra)]	579.71					20.05 ± 0.07	21.0[k]
$[Eu(teta)]^-$	579.53					14.02 ± 0.08	13.77[j]
[Eu(nota)]	579.51					13.9 ± 0.12	13.7[k]

[a] The uncertainties listed are the standard errors provided by the statistics of the non-linear regression analysis of individual data sets as carried out by the program PEAKFIT. [b] Ref. 23, 25 °C, 0.1 mol dm^{-3} KCl. [c] Ref. 20, 25 °C, 0.1 mol dm^{-3} KCl. [d] Ref. 24. [e] Average value from data in Figs. 3 and 4. [f] Ref. 17, 37 °C, 1 mol dm^{-3} NaCl. [g] Ref. 12, 25 °C, 0.1 mol dm^{-3} KCl. [h] Ref. 7, 20 °C, 1.0 mol dm^{-3} NaCl. [i] Ref. 16, 25 °C, 1.0 mol dm^{-3} NaCl, for $[Gd(dota)]^-$. [j] Ref. 10, 25 °C, 0.1 mol dm^{-3} KCl for $[Gd(dota)]^-$. [k] Ref. 25.

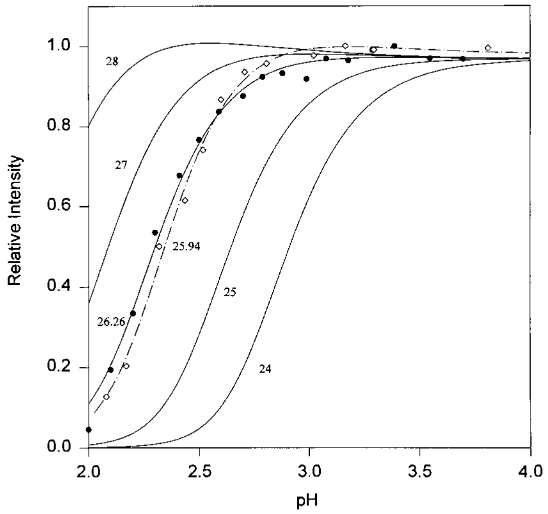


Fig. 4 Excitation intensity of $[Eu(dota)]^-$ as a function of pH for the samples incubated at 70 °C for 3 d followed by 10 d of 25 °C equilibration (●) or for 891 additional days at 27 ± 1 °C (◇). Included also are the simulated curves for different values of the thermodynamic stability constant, with the $k'':k'$ ratio [equation (9)] set at 1.41:1, its experimental value

$$I_t = 0.5k'(C_1 + C_2 + (K\alpha)^{-1} - \{[C_1 + C_2 + (K\alpha)^{-1}]^2 - 4C_1C_2\}^{\frac{1}{2}}) \quad (14)$$

This equation does not, of course, involve K_H, and K can be determined directly.

In reality the situation will often be intermediate between the above extremes and equation (10) will then be needed. Three approaches to fitting data by equation (10) are considered. First, as described earlier, the log K_H values can be held constant at their known values; the results of this approach are listed in Table 2. Secondly, they could all be set equal to zero as an approximation. This yields log K values: $[Eu(edta)]^-$ 17.78 ± 0.03, $[Eu(dtpa)]^{2-}$ 22.93 ± 0.04, [Eu(hedta)] 15.62 ± 0.02 and $[Eu(dota)]^-$ 26.29 ± 0.02. Lastly the K_H values can be allowed to vary in the non-linear regression fit. The results here for log K are: $[Eu(edta)]^-$ 17.52 ± 0.3, $[Eu(dtpa)]^{2-}$ 22.39 ± 0.04, [Eu(hedta)] 15.62 ± 2.0 and $[Eu(dota)]^-$ 26.24 ± 0.12. The respective log K_H values are 1.77, 2.65, −5.93 and 1.06. Comparison of the results from the three fitting methods suggests that log K values are not very sensitive to the K_H values and that reasonable estimates of the latter can be obtained using the third fitting procedure.

Sensitivity of the excitation intensity *vs.* pH method in stability constant determination. Owing to the considerable interest in dota as a ligand, particularly from the MRI point of view, its stability constant toward Ln^{3+} ions has been determined a number of times by different research groups, not necessarily under exactly identical conditions. Six reported values, ranging from log K = 22.1 to 28.2, for complexes of Eu^{3+} or Gd^{3+} with dota are listed in Table 2. With the exception of the 23.7 value,[17] which is for 37 °C, the experiments carried out did not directly measure the concentration of $[Eu(dota)]^-$. The present method, which directly monitors an electronic transition in the complex of interest, eliminates all ambiguity from the data analysis. Fig. 4 shows intensity data for the Eu^{3+}–dota system as a function of pH and the non-linear regression fit yielding $K = 1.82 \times 10^{26}$. Also shown are predicted curves for K values ranging from 10^{24} to 10^{28} dm^3 mol^{-1}. It is clear that our data are inconsistent with K values which differ markedly from the one determined by us. The discrepancies between the present value and those in the literature may be partly due to problems with attainment of true equilibrium. Tóth and Brücher[17] and Clarke and Martell[10] incubated samples at their temperature of measurement for 10 and 20 d, respectively. Cacheris *et al.*[12] prepared their samples by heating them at 60 °C for 12–18 h followed by an additional 6–10 h room temperature equilibration. The heating procedure is favourable since the $\Delta H°$ of the complexation reaction is most probably negative (it is negative for complexation of dota with Ca^{2+} and the heavier alkaline-earth-metal cations),[27] and the formation constant will be smaller at higher temperatures.[17] Thus when cooling occurs it is the formation reaction which proceeds and this occurs at a much faster rate than dissociation,[28] allowing equilibrium to be approached readily. The samples used to obtain the data shown in Fig. 4 were prepared by incubating them at 70 °C for 3 d followed by 10 d at 25 °C. These data yield log K = 26.26 ± 0.09. The data shown in Fig. 3 on the other hand correspond to samples incubated for 1 d at 70 °C followed by 3 d at 25 °C. The log K value obtained from the latter data (26.15 ± 0.10) is in excellent agreement with that obtained in the other experiment. The average of these two values is reported in Table 2. In order to prove that equilibrium has been reached in the Eu^{3+}–dota system using the above described incubation procedures, the samples of Fig. 4 were kept at 27 ± 1 °C for 891 additional days, at which time the pH values and luminescence excitation intensities were re-

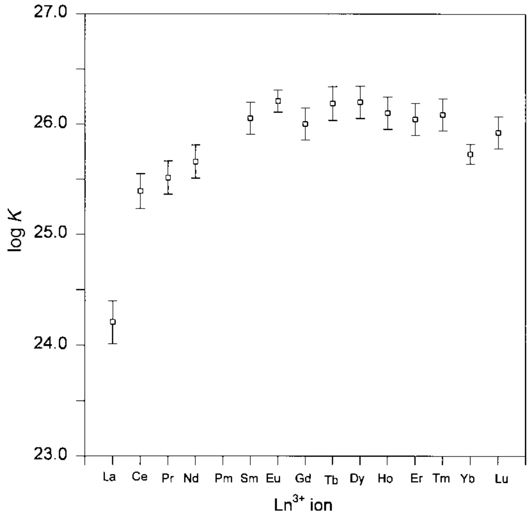


Fig. 5 Formation constants for $[Ln(dota)]^-$ complexes across the lanthanide series as determined from metal ion competition experiments carried out at pH 7. The log K values corresponding to the points plotted are: La^{3+} 24.25, Ce^{3+} 25.34, Pr^{3+} 25.54, Nd^{3+} 25.69, Sm^{3+} 26.08, Eu^{3+} 26.21, Gd^{3+} 26.03, Tb^{3+} 26.21, Dy^{3+} 26.22, Ho^{3+} 26.13, Er^{3+} 26.07, Tm^{3+} 26.11, Yb^{3+} 25.81 and Lu^{3+} 25.95. The error bars represent standard deviations based on triplicate measurements and the standard deviation of the europium(III) determination

measured. A log K value of 25.94 was obtained which is very close to the 25 °C result. The data are shown in Fig. 4. The degree of agreement among all three sets of experiments demonstrates that true equilibrium has been reached in each case.

Stability constants for $[Ln(dota)]^-$ complexes across the lanthanide ion series. Having established a reliable value for the stability constant of the complex of a given ligand with Eu^{3+}, the luminescence excitation experiment can be used to determine the stability constants of the ligand with other metal ions by means of competition experiments.[29,30] The method is illustrated here for the lanthanide series, although it is applicable to other ions as well. The ligand dota is mixed with equimolar quantities of a competing Ln^{3+} ion at neutral pH and allowed to equilibrate until the complexation equilibria are complete. Equilibrium (15) is established. It is easily shown

$$Ln^{3+} + [Eu(dota)]^- \rightleftharpoons [Ln(dota)]^- + Eu^{3+} \qquad (15)$$

that for this system the ratio of stability constants is given by equation (16) where I and I_0 are the excitation intensities arising

$$K_{Ln^{3+}}/K_{Eu^{3+}} = [(I_0 - I)/I]^2 \qquad (16)$$

from $[Eu(dota)]^-$ in the presence and absence of a competing Ln^{3+} ion, respectively. Equation (16) is a simplification of a more general equation[30] which applies when the concentrations of the two metal ions are not exactly equal. With a knowledge of $K_{Eu^{3+}}$, the formation constants for the other Ln^{3+} ions may be obtained directly from the measured ratios [equation (16)]. The results (a plot of log $K_{Ln^{3+}}$ *vs.* atomic number of the Ln^{3+} ion) are shown in Fig. 5. There is a dramatic increase in the log K values on going from the largest ion La^{3+} to its slightly smaller neighbour Ce^{3+}, followed by a more gradual linear increase to Eu^{3+}. The stability constants then remain roughly constant for the latter half of the series. This trend is not in good accord with the 25 °C data reported by Cacheris *et al.*[12] and quoted by Tóth and Brücher,[17] although the 37 °C data of the latter authors do follow this trend. The absolute magnitudes of the measured stability constants in either case[12,17] are not in good agreement with ours. The relatively constrained macrocyclic dota ligand is apparently able to accommodate the smaller members of the series with ease, but discriminates markedly against larger ions.

Conclusion

Laser excitation of the $^7F_0 \longrightarrow {}^5D_0$ transition of Eu^{3+} provides an accurate and sensitive means of monitoring complexation of this ion in solution at micromolar concentrations and below. This technique provides the basis for the reliable measurement of extremely high stability constants on samples which may require extensive equilibration time. Conditional stability constants can be obtained at constant low pH values where hydrogen ions compete with the metal ion for the ligand. A new method of measurement of complex concentration as a function of pH at low pH values with stability constant and complex protonation constants obtained by non-linear regression fitting of the results by an equation which takes account of all the equilibrium involved yields results in good accord with literature values obtained by other means. Metal ion competition experiments are shown to yield stability constant values for other metal ions as well.

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