

# Capillary electrophoresis, potentiometric and laser excited luminescence studies of lanthanide(III) complexes of 1,7-dicarboxymethyl-1,4,7,10-tetraazacyclododecane (DO2A)<sup>†</sup>

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The protonation constants of the ligand 1,7-dicarboxymethyl-1,4,7,10-tetraazacyclododecane (DO2A) were re-determined by the potentiometric pH titration method ( $\log K$  10.94, 9.55, 3.85, 2.55) and the macrocycle ring protonation sites have also been confirmed by NMR techniques to be the secondary amine nitrogen atoms. The stability constants of calcium(II) and all trivalent lanthanide ( $\text{Ln}^{3+}$ ) metal complexes of DO2A ( $\text{Ca}^{2+}$ ,  $\log K_{\text{ML}}$  7.16;  $\text{Ln}^{3+}$ ,  $\log K_{\text{ML}}$  10.94–13.31) were determined by the potentiometric pH titration and capillary electrophoresis methods, respectively. In general, the stabilities of the  $\text{Ln}(\text{DO2A})^+$  complexes increase with increasing atomic number for the lighter lanthanides ( $\text{La}^{3+}$ – $\text{Sm}^{3+}$ ) and remain relatively unchanged for the heavier lanthanides ( $\text{Eu}^{3+}$ – $\text{Lu}^{3+}$ ). Laser-excited spectroscopy of the  ${}^7\text{F}_0 \rightarrow {}^5\text{D}_0$  transition of  $\text{Eu}^{3+}$  is used to study the aqueous  $\text{Eu}^{3+}$ –DO2A complex system. At low pH (e.g. pH 5–6)  $\text{Eu}^{3+}$  forms a 1 : 1 species with the ligand DO2A, presumably  $\text{Eu}(\text{DO2A})(\text{H}_2\text{O})_q^+$ , where  $q$  is the number of inner-sphere coordinated water molecules. As the solution pH increases the hydrolysis product,  $\text{Eu}(\text{DO2A})(\text{OH})(\text{H}_2\text{O})_{q-1}$ , is formed. Lifetime measurements of each species in  $\text{H}_2\text{O}$  and  $\text{D}_2\text{O}$  allow the determination of the corresponding number of inner-sphere coordinated water molecules to be 3.0 and 2.6, consistent with the proposed structures (i.e.  $q = 3$ ). The first hydrolysis constant ( $\text{p}K_{\text{h}}$ ) is estimated to be  $8.1 \pm 0.3$ .

## Introduction

The synthesis and characterization of trivalent lanthanide ( $\text{Ln}^{3+}$ ) complexes of macrocyclic aminopolycarboxylates have been of recent interest because of many biological and medical applications, including their use as magnetic resonance imaging contrast enhancement agents,<sup>1</sup> NMR hyperfine shift reagents,<sup>2</sup> molecular luminescence probes<sup>3</sup> and DNA/RNA cleavage agents.<sup>4</sup> For example, the metal complexes of DOTA, the tetrakis(*N*-acetate) derivative of 1,4,7,10-tetraazacyclododecane (cyclen), are thermodynamically very stable and kinetically rather inert as compared to those with linear ligand structures.<sup>5</sup> The gadolinium(III) complexes of DOTA and its structural analogue, HP-DO3A [HP-DO3A is 10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetate], are well tolerated *in vivo* and have been successfully used as magnetic resonance imaging (MRI) contrast agents.<sup>1</sup> On the other hand, the thulium(III) complex of the tetraphosphonate derivative of cyclen,  $\text{Tm}(\text{DOTP})^{5-}$ , has been used as a high stability *in vivo*  ${}^{23}\text{Na}$  NMR hyperfine shift reagent.<sup>6</sup> The  $\text{Tm}(\text{DOTP})^{5-}$  complex is considered better than  $\text{Dy}(\text{TTHA})^{3-}$  (TTHA is triethylenetetraaminehexaacetate, a linear aminopolycarboxylate ligand) because of the larger NMR shift induced and less line-broadening (hence smaller quantity of shift reagent required for practical applications).<sup>7</sup>

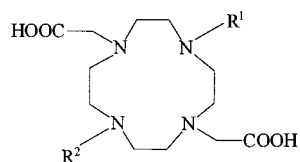
We have been interested in the study of macrocyclic lanthanide coordination chemistry and have focused on the understanding of the structural, stability and selectivity properties of such compounds. A comprehensive and systematic study

approach has been undertaken. This includes the design and synthesis of macrocyclic ligands and their lanthanide complexes, thermodynamic and kinetic studies of metal complex stability,<sup>8</sup> selectivity<sup>9</sup> and lability,<sup>10</sup> solution and solid state structural characterizations<sup>11</sup> and solvent extraction<sup>12</sup> and medical diagnostic<sup>13</sup> applications. Some interesting discoveries are (1) that many macrocyclic ligands exerted unprecedented selectivities toward lanthanide ion binding, (2) the independence upon foreign metal ions for the dissociation of macrocyclic lanthanide complexes, (3) the kinetic control of solvent extraction selectivity of lanthanides and (4) the general understanding of lanthanide solution structural properties using novel laser-excited fluorescence techniques.

Our recent research effort has been on the study of cationic, macrocyclic lanthanide complexes as potential artificial ribonucleases. To function as an artificial ribonuclease, the lanthanide complex should be cationic to favor oligonucleotide binding and have vacant inner-sphere coordination site(s) to allow transesterification of the phosphate diester linkages.<sup>14</sup> Thus, we have identified lanthanide complexes of a macrocyclic ligand, 1,7-dicarboxymethyl-1,4,7,10-tetraazacyclododecane (DO2A), among several others for this purpose. It is believed that the cationic  $\text{Ln}(\text{DO2A})^+$  complexes have enough thermodynamic stability and kinetic inertness and have several inner-sphere coordination sites to allow phosphate binding. In this paper, we report the results of the capillary electrophoresis, NMR, potentiometric and laser-excited luminescence studies of lanthanide(III) complexes of DO2A. Because the classical pH titration technique is not suitable for the determination of the kinetically inert  $\text{Ln}(\text{DO2A})^+$  complex stability constants, a newly developed capillary electrophoretic method has been employed.

It is noted that during the course of this study, the results of two other independent studies related to the ligand DO2A and some of its alkaline earth–lanthanide<sup>15</sup> and –transition metal<sup>16</sup> complexes have been reported. Although our protonation con-

<sup>†</sup> Supplementary data available: pH dependence NMR study of protonation sites and 2-D NMR assignment of C and H peaks of DO2A. For direct electronic access see <http://www.rsc.org/suppdata/dt/1998/3243/>, otherwise available from BLDSC (No. SUP 57421, 4 pp.) or the RSC Library. See Instructions for Authors, 1998, Issue 1 (<http://www.rsc.org/dalton>).



	R <sup>1</sup>	R <sup>2</sup>
DO2A	-H	-H
DO3A	-CH <sub>2</sub> COOH	-H
DOTA	-CH <sub>2</sub> COOH	-CH <sub>2</sub> COOH

stant results (*i.e.* values and protonation sites) are similar to both reports, the calcium–lanthanide complex stability results (*i.e.* values and interpretation) as well as the number of inner-sphere coordination water molecules on the lanthanide ion are quite different from one of the two reports.

## Experimental

### Materials and standard solutions

Analytical reagent-grade chemicals and buffers, unless otherwise stated, were purchased from Sigma (St. Louis, MO, USA), Aldrich (Milwaukee, WI, USA) or Merck (Darmstadt, Germany) and were used as received without further purification. Disodium ethylenediaminetetraacetic acid (Na<sub>2</sub>H<sub>2</sub>EDTA) was purchased from Fisher. The ligand DO2A·2HCl·2H<sub>2</sub>O was prepared and purified according to a published method with minor modification<sup>17</sup> (Found: C, 38.09; H, 7.04; N, 14.88. Calc. for C<sub>12</sub>H<sub>24</sub>N<sub>4</sub>O<sub>4</sub>·2HCl·2H<sub>2</sub>O: C, 38.20; H, 6.95; N, 14.85%). Carbonate-free deionized water was used for all solution preparations.

The concentration of DO2A stock solution (*ca.* 0.01 M) was determined by acid–base titration using a standard tetramethylammonium hydroxide solution (0.1 M), and was also checked by complexometric back-titration.<sup>18</sup> The concentrations of all lanthanide nitrate stock solutions were *ca.* 0.01 M and were standardized by Na<sub>2</sub>H<sub>2</sub>EDTA titration using xylenol orange as indicator. The Na<sub>2</sub>H<sub>2</sub>EDTA solution was standardized by titrating a calcium carbonate primary standard solution (first dissolved in HCl solution) at pH 10 using calmagite as the indicator.

The 0.1 M tetramethylammonium hydroxide solution was prepared by diluting a 20% (CH<sub>3</sub>)<sub>4</sub>NOH–methanol solution obtained from Aldrich (carbonate-free). The aqueous (CH<sub>3</sub>)<sub>4</sub>NOH solution was standardized by using reagent grade potassium hydrogenphthalate. A 0.1 M HCl solution was prepared by diluting reagent grade HCl to *ca.* 1 M, then diluting the 1 M solution to 0.1 M. This solution was standardized by using the standard (CH<sub>3</sub>)<sub>4</sub>NOH solution. A 1.0 M stock solution of tetramethylammonium chloride (Aldrich) was prepared and diluted to 0.1 M for each titration to maintain a constant ionic strength (0.1 M, charge unit neglected).

### Potentiometric titrations

All titrations were carried out at a constant ionic strength of 0.10 M (CH<sub>3</sub>)<sub>4</sub>NCl. A Model 720 Metrohm Titroprocessor in conjunction with Metrohm Combination Electrode was employed to monitor the pH ( $\pm 0.001$  pH unit, however, for practical calculations, the accuracy was estimated to be  $\pm 0.01$  pH unit). Before each titration, the pH meter was standardized at pH 4.01, 7.00, and 10.00 with standard buffer solutions obtained from Fisher. A water jacketed titration vessel with 5 mL capacity (Brinkmann) along with a five-hole cover was used for the titrations. The electrode and buret were fitted into these holes. The vessel was thermostated at  $25.0 \pm 0.1$  °C using a Firstek Scientific Model B403 constant temperature circulating bath.

The sample solution was prepared by pipetting exact amounts of stock solutions into the titration vessel. For the determination of metal complex formation constants ( $K_{ML} = [ML]/[M][L]$ ), a 1:1 mole ratio of metal to ligand solution was prepared. Generally, the ligand concentration was 2.0 mM and the metal ion concentration was in slight excess ( $\approx 2\%$ ). Under this experimental condition, only 1:1 complexes were formed between each ligand and metal ion. The ionic strength of the solution was adjusted to 0.1 M using 1 M (CH<sub>3</sub>)<sub>4</sub>NCl. The (CH<sub>3</sub>)<sub>4</sub>NOH solution was delivered from a 10 mL automatic Brinkmann Metrohm Model 665 Dosimat buret with a reading accuracy of  $\pm 0.001$  mL. The titrations were performed two to four times, and the average values of reproducible results were reported.

All equilibrium calculations were performed using computer programs described elsewhere,<sup>19</sup> and double checked by published computer programs BEST and PKAS.<sup>20</sup> The pH-metric titration data were used to calculate the stepwise ligand protonation constants defined in eqn. (1), where  $n = 1-4$  for DO2A.

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

The measured pH values were converted to  $p[H^+]$  values by the activity relationship:  $p[H^+] = \text{pH} + \log f$ , where  $[H^+]$  is the hydrogen ion concentration and  $f$ , 0.83, is the activity coefficient at 0.1 M ionic strength.<sup>20</sup> For the calculation of the formation constants, data points in the metal buffer region of 25–75% metal complexation were employed. The averaged values are presented together with the standard deviations calculated from those valid data points.

### Capillary electrophoresis measurements

Stock buffer solutions (100 mM) were prepared by dissolving appropriate amount of TRIS, MES (2-morpholinoethanesulfonic acid), CAPS [3-(cyclohexylamino)propanesulfonic acid], formate, borate and acetate in deionized water (at least 18 MQ cm<sup>-1</sup>). The stock electrophoretic buffer was adjusted with 0.1 M HCl or (CH<sub>3</sub>)<sub>4</sub>NOH to a desired pH value, the ionic strength was adjusted with (CH<sub>3</sub>)<sub>4</sub>NCl to 0.1 M. The stock sample solutions (100 mM) were prepared by dissolving test chemicals in the appropriate buffered solution. All electrophoretic buffer solutions were purified with 0.45  $\mu\text{m}$  syringe filters (Lida Manufacturing Corp., Kenosha, WI, USA) before use. Dilution of running buffer solutions to an appropriate concentration for the measurement was made by adding deionized water.

All measurements were performed by a BioFocus 3000 capillary electrophoresis (CE) system (Bio-Rad Labs.) with a reversible polarity power supply and an on-column multiwavelength UV/VIS photodiode absorption detector. All CE measurements were carried out in an uncoated fused-silica capillary of 25 cm in length and 50  $\mu\text{m}$  internal diameter with an effective length of 20.4 cm (obtained from Bio-Rad). The voltage applied was adjusted from 8 to 15 kV, the pressure sample injection mode was used for a uniform sample introduction for all species, at 5 psi for duration of 1 s. Electrophoretograms were recorded and processed by a locally integrated IBM PC compatible personal computer.

All samples were prepared by mixing appropriate amounts of metal ions and ligands (usually 1:1 stoichiometry) in a buffer solution and allowed to equilibrate for at least 12 h before measurements. The buffer pH values were selected at which about 50% of the metal ions were complexed and precipitation of the metal hydroxide did not occur. Calibration curves of free ligand or metal complexes were constructed using peak height data. Knowing the initial concentrations of the ligand and lanthanide ions, and with the determined equilibrium concentrations of free ligand and lanthanide complexes, one can calculate the conditional stability constant at a specific pH and then the stability constant of the complex.<sup>21</sup>

## Laser-excited Eu<sup>3+</sup> luminescence measurements

Laser-excited luminescence experiments were performed at the Regional Instrument Center of National Science Council located at the Chemistry Department, National Tsing Hua University, Hsinchu, Taiwan. A Spectra Physics PDL3 dye laser was employed using a mixture of R590 and R610 dyes. The ratio of the two dyes was adjusted to ensure that the laser power throughout the scanning region remained constant. The dye laser was pumped by a Spectra Physics DCR2A Nd: YAG laser, using its second harmonic line (532 nm). The output power of the laser was 85–90 mJ per pulse. The excitation spectra were obtained by scanning between 577 and 581 nm at a rate of 0.02 nm per step. The emission was focused into a Jobin Yvon HR320 monochromator equipped with a Hamamatsu R980 photomultiplier. The high voltage for the PMT was supplied by a Stanford Research PS325 power supply. A R610 filter was placed in front of the monochromator to eliminate laser scatter. The electronic signal was averaged by a boxcar averager constructed with a Stanford Research SR245 interface, a SR250 gate integrator and a SR280 main frame and power. The system was controlled by a program written in ASYST and run on a 486SX microcomputer. Emission decay curves were recorded on a LeCroy 9450 oscilloscope. The average of 1000 decay curves was reported.

Before new data were obtained, the laser-excited luminescence measurements were performed on Eu<sup>3+</sup>–EDTA/DTPA systems (pH 5.2). The excitation spectral maxima obtained for Eu<sup>3+</sup> (578.68 nm), Eu<sup>3+</sup>–EDTA (579.57 and 580.05 nm) and Eu<sup>3+</sup>–DTPA (579.88 nm) were consistent with literature reported findings.<sup>22</sup> For the Eu<sup>3+</sup>–DO2A system, all solutions were prepared from stock solutions of Eu(NO<sub>3</sub>)<sub>3</sub> ( $c = 9.883 \times 10^{-3}$  M) and DO2A ( $c = 1.240 \times 10^{-2}$  M). The ionic strength was adjusted to 0.1 using 1 M (CH<sub>3</sub>)<sub>4</sub>NCl solution. Control of buffer pH was achieved using buffer reagents, MES (pH 5.0–7.0), HEPES (pH 7.0–9.0) and CAPS (pH 9.0–11.0). All solutions were allowed to equilibrate for at least 4 d to ensure full reaction equilibrium prior to luminescence measurements. The sample solutions in D<sub>2</sub>O were prepared by first evaporating the aqueous solutions in appropriate volumetric flasks to dryness using a high vacuum system. To each flask D<sub>2</sub>O was added to dissolve the solids, the solution equilibrated for at least 2 h, dried, and finally dissolved in D<sub>2</sub>O to a suitable volume for luminescence measurements.

## Results and discussion

### Ligand protonation constants

The ligand titration curve of DO2A·2HCl (Fig. 1) shows two buffer regions: one from pH 3 to pH 5 (which corresponds to the dissociation of the two carboxymethyl acid protons) and another from pH 8 to pH 11 (which corresponds to the dissociation of two protons attached to nitrogen atoms). This assignment is consistent with that proposed for the structural analogue, DOTA.<sup>23</sup> However, unlike DOTA, two sets of nitrogen protonation sites are possible for DO2A: the secondary and tertiary amine nitrogen sites. Separate NMR measurements performed at different solution pD show that the carboxymethyl methylene proton and carbon peaks both have greater chemical shift changes in the region pD 3–5, indicating protonation at the carboxymethyl groups (the assignment of NMR peaks was achieved by 2-D experiments, see SUP 57421). Upon deprotonation, the proton peak shifts from high frequency to low frequency and the carbon peak moves in the opposite direction. The fact that both carbon and proton peaks do not show chemical shift change in the region pD 9–12 indicates that further protonation occurs preferentially at the nitrogen sites which are farther away from the carboxymethyl groups, *i.e.* the secondary amine sites. This is consistent with the general understanding that secondary amine is more basic than tertiary

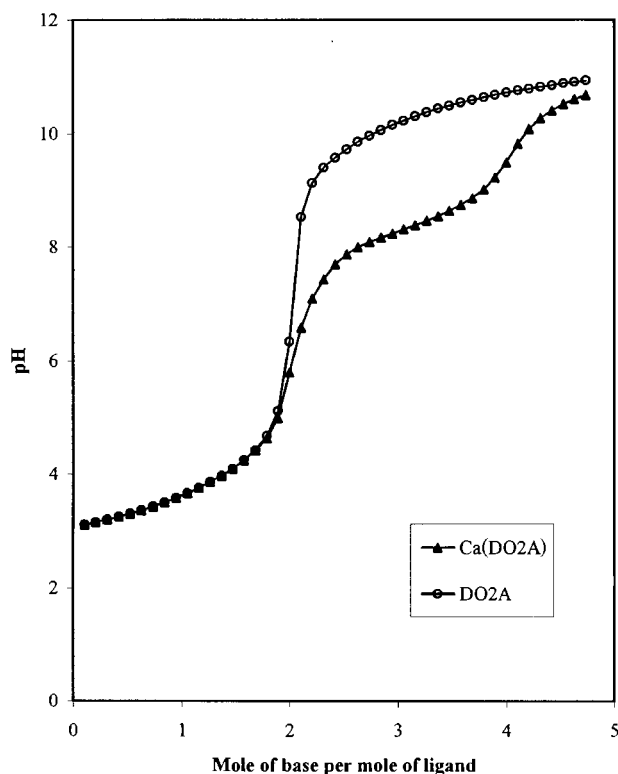


Fig. 1 Potentiometric equilibrium curves of DO2A and 1:1 ratios of Ca<sup>2+</sup>–DO2A [Ca<sup>2+</sup>] = [DO2A] = 2 × 10<sup>-3</sup> M; 25 ± 0.1 °C.

Table 1 Logarithmic protonation constants of DO2A, DO3A and DOTA ligands. 25 ± 0.1 °C,  $\mu = 0.1$

	log K <sub>1</sub>	log K <sub>2</sub>	log K <sub>3</sub>	log K <sub>4</sub>	Σlog K <sub>n</sub>
DO2A	10.94	9.55	3.85	2.55	26.89 <sup>a</sup>
	10.91	9.45	4.09	3.18	27.63 <sup>b</sup>
	11.45	9.54	4.00	2.36	27.35 <sup>c</sup>
DO3A	10.72	9.51	4.40	3.39	28.02 <sup>d</sup>
	11.59	9.24	4.43	3.48	28.74 <sup>e</sup>
DOTA	11.34	9.90	4.60	4.00	29.84 <sup>d</sup>
	11.73	9.40	4.50	4.19	29.82 <sup>e</sup>

<sup>a</sup> This work. For log K<sub>n</sub>, standard deviation ≤ 0.05 log K unit. <sup>b</sup> Refs. 15 and 24. <sup>c</sup> Ref. 16. <sup>d</sup> Ref. 11(e). <sup>e</sup> Ref. 18.

amine. Indeed, the single crystal structure of DO3A·H<sub>2</sub>SO<sub>4</sub> shows that the secondary nitrogen and its transannular tertiary nitrogen are protonated.<sup>11e</sup>

The individual logarithmic protonation constants (log K<sub>n</sub>) of DO2A and its structural analogues, DO3A and DOTA, and the overall basicities expressed by the sum of all log K<sub>n</sub> values of each ligand are listed in Table 1. (The log K<sub>n</sub> values of DO2A have also been reported previously.<sup>15,16,24</sup>) Regardless of some minor differences between our present and the previously reported log K<sub>n</sub> values, the overall basicities follow the order DOTA > DO3A > DO2A.

### Stability of metal chelates

It was found that the Ln(DO2A)<sup>+</sup> complex formation rates were too slow to permit suitable potentiometric determination of their stability constants. Instead of using a published spectrophotometric competition method,<sup>25</sup> we have developed a capillary electrophoretic method for the determination of the metal complex stability constants for complexes with slow formation rates. This method has several advantages: ample equilibration time, very small amount of sample used, direct determination of free metal ion, free ligand and complex concentrations and is less time consuming and less laborious.

**Table 2** Stability constants of lanthanide complexes of DO2A, DO3A and DOTA. 25 ± 0.1 °C, μ = 0.1 M

log $K_{ML}$	DO2A <sup>a</sup>	K21DA <sup>c</sup>	K22DA <sup>c</sup>	DO3A <sup>d</sup>	DOTA <sup>e,f</sup>	TETA <sup>g</sup>
La <sup>3+</sup>	10.94 ± 0.14	10.11	12.21	—	22.9 (22.0)	12.74
Ce <sup>3+</sup>	11.31 ± 0.03	10.89	12.23	—	23.4 (23.0)	13.12
Pr <sup>3+</sup>	12.00 ± 0.10	11.31	12.22	—	23.0 (23.5)	—
Nd <sup>3+</sup>	12.56 ± 0.10	11.60	12.21	—	23.0 (23.7)	13.76
Sm <sup>3+</sup>	12.93 ± 0.01	11.72	12.12	—	23.0 (24.1)	14.47
Eu <sup>3+</sup>	12.99 ± 0.12	11.85	12.02	—	23.5 (24.2)	14.66
Gd <sup>3+</sup>	13.06 ± 0.04 (19.42) <sup>b</sup>	11.66	11.93	21.0	24.7 (24.0)	14.73
Tb <sup>3+</sup>	12.93 ± 0.01	11.52	11.70	—	24.2 (24.2)	14.81
Dy <sup>3+</sup>	13.13 ± 0.05	11.55	11.57	—	24.8 (24.2)	—
Ho <sup>3+</sup>	13.00 ± 0.04	11.34	11.18	—	24.5 (24.1)	14.95
Er <sup>3+</sup>	13.31 ± 0.02	11.15	11.30	—	24.4 (24.1)	—
Tm <sup>3+</sup>	13.19 ± 0.11	10.79	11.10	—	24.4 (24.0)	15.15
Yb <sup>3+</sup>	13.26 ± 0.15	10.76	10.90	—	25.0 (24.0)	—
Lu <sup>3+</sup>	13.16 ± 0.18	10.33	10.84	—	25.4 (23.9)	14.77
Ca <sup>2+</sup>	7.16 ± 0.10 (15.37) <sup>b</sup>	8.74	8.29	11.26	16.37	8.53

<sup>a</sup> This work. 25 °C, ionic strength = 0.1 M; standard deviation ≤ 0.18 log  $K$  unit. <sup>b</sup> Ref. 24. <sup>c</sup> Ref. 8(b). <sup>d</sup> Refs. 11(e) and 28. <sup>e</sup> Ref. 25(a). <sup>f</sup> Values in parentheses are from ref. 25(b). <sup>g</sup> Ref. 27.

On the other hand, the Ca(DO2A) complex formation equilibrium is fast and its titration curve can be obtained by the normal pH titration method (Fig. 1). Similar to those of DO3A complexes,<sup>18</sup> the Ca(DO2A) curve is lower in pH than that of the ligand titration without calcium ions, indicating competition between calcium ions and protons for ligand binding. Noticeable calcium complexation occurs after the carboxymethyl acid protons are neutralized. This is demonstrated in Fig. 1 where the titration curves with or without calcium ions overlap with one another between 0–2 equivalents of base added. The complexation reaction after removal of carboxymethyl acid protons is best described by eqn. (2).

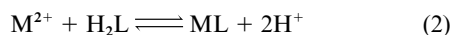


Table 2 lists the calculated stability constants (log  $K_{ML}$ ) of Ca(DO2A) and Ln(DO2A)<sup>+</sup> complexes. For comparison purposes, the stability constants of metal complexes of several other structurally similar ligands are also listed in Table 2.

From the data in Table 2, it is seen that the Ln(DO2A)<sup>+</sup> stability increases as the atomic number increases (La<sup>3+</sup>–Sm<sup>3+</sup>) and remains roughly unchanged after Eu<sup>3+</sup>. The initial trend (La<sup>3+</sup>–Sm<sup>3+</sup>) is similar to those observed for other macrocyclic aminopolycarboxylate ligands, DOTA,<sup>22</sup> TETA, PEPA and HEHA,<sup>26</sup> presumably indicating that ionic interaction dominates the Ln<sup>3+</sup>–DO2A<sup>2-</sup> complexation (TETA = 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid, PEPA = 1,4,7,10,13-pentaazacyclopentadecane-1,4,7,10,13-pentaacetic acid, HEHA = 1,4,7,10,13,16-hexaazacyclooctadecane-1,4,7,10,13,16-hexaacetic acid). On the other hand, the later trend (Eu<sup>3+</sup>–Lu<sup>3+</sup>) seems to suggest that desolvation of the smaller lanthanide ions becomes important [similar to the case of Ln(TETA)]<sup>26</sup> in addition to other factors such as better fit between metal ion radius and ligand cavity size.<sup>8,10a</sup>

In general, if ligand structures are similar, the more basic the ligand is, as well as the more donor atoms/chelate rings the ligand contains, the more stable is the complex. Thus, for a given lanthanide ion, the order of complex stability is as observed Ln(DOTA)<sup>-</sup> > Ln(DO3A)<sup>-</sup> > Ln(DO2A)<sup>+</sup>, Table 2. On the other hand, for the dicarboxylate macrocyclic ligands which form cationic lanthanide complexes, e.g. DO2A (12-membered ring), K21DA (1,7-dicarboxymethyl-1,7-diaza-4,10,13-trioxacyclopentadecane, 15-membered ring), and K22DA (1,10-dicarboxymethyl-1,10-diaza-4,7,13,16-tetraoxacyclooctadecane, 18-membered ring), the stability trends for a given lanthanide ion are Ln(K22DA)<sup>+</sup> > Ln(DO2A)<sup>+</sup> > Ln(K21DA)<sup>+</sup> for La<sup>3+</sup>–Pr<sup>3+</sup> and Ln(DO2A)<sup>+</sup> > Ln(K22DA)<sup>+</sup> > Ln(K21DA)<sup>+</sup> for Nd<sup>3+</sup>–Lu<sup>3+</sup>. This is mainly because the larger sized macrocyclic ligands K22DA and K21DA exert more size

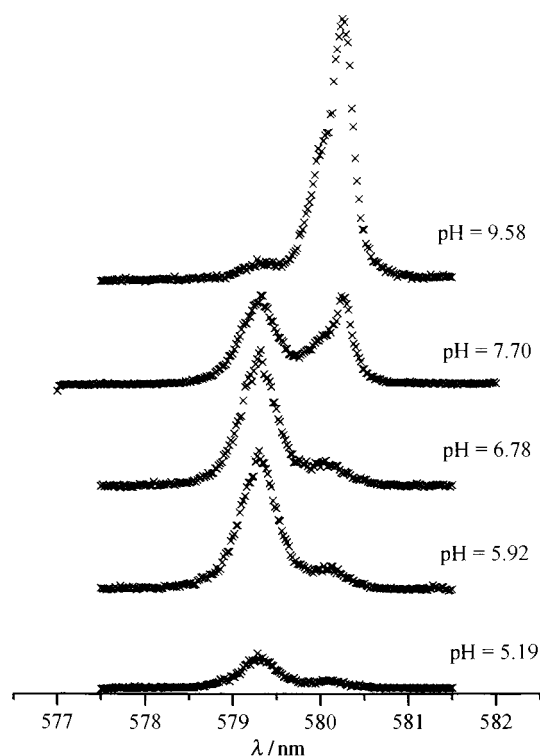
effect and because the overall basicity of DO2A is greater than that of the other two ligands (Table 2).

A previous paper also reported the logarithmic stability constants for Gd(DO2A)<sup>+</sup> and Ca(DO2A) to be 19.42 and 15.37, respectively.<sup>24</sup> These two values are quite different from those determined in the present work (*i.e.* 13.6 and 7.16, respectively). It is possible that the other reported Ca(DO2A) stability constant (log  $K_{ML}$ , 15.37) is too high. The reason is that two independently reported literature log  $K_{ML}$  values<sup>18,27</sup> for Ca(DO3A)<sup>-</sup> are 11.35 and 11.74, and Ca(DO2A) stability is unlikely to be higher than Ca(DO3A)<sup>-</sup> because DO3A has one more carboxymethyl group than DO2A. It should be noted that a new logarithmic stability constant value of 7.8 of Ca(DO2A) was later reported by the same group,<sup>15</sup> and this value is much closer to our present result.

On the other hand, a good linear correlation exists between the log  $K_{CaL}$  values and log  $K_{GdL}$  values ( $r^2 = 0.95$ ) for a number of selected macrocyclic and linear aminopolycarboxylate ligands.<sup>27</sup> Assuming our Ca(DO2A) stability constant is correct, by using the published linear plot (Fig. 2 of ref. 27), we estimate the log  $K_{GdL}$  value should be in the range 12–13, consistent with what we obtained.

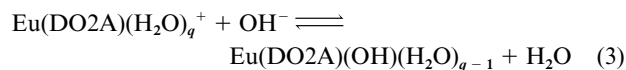
#### Laser-excited luminescence studies

Laser-excited luminescence spectroscopic studies of the solution structural features and properties of Eu<sup>3+</sup> complexes of macrocyclic ligands are well documented.<sup>28</sup> For example, the number of inner-sphere coordinated water molecules, the number of equilibrium isomeric species, the metal–ligand complexation stoichiometries, the values of formation constants, the ternary complex formation and the metal–metal distances (for binuclear complexes) can all be determined. In general, the transition between the ground (<sup>7</sup>F<sub>0</sub>) and excited (<sup>5</sup>D<sub>0</sub>) states of Eu<sup>3+</sup> is accessed by a tunable dye laser in the range 577–581 nm, while the “hypersensitive” <sup>5</sup>D<sub>0</sub> → <sup>7</sup>F<sub>2</sub> emission band is monitored at 614 nm. Since both ground and excited states are nondegenerate, a single excitation band is observed for each unique Eu<sup>3+</sup> environment with an intensity proportional to the complex concentration. Fig. 2 shows the laser-excited fluorescence spectra of the Eu(DO2A)<sup>+</sup> complex solutions at various pH (pH 5.19–9.58). The Eu(DO2A)<sup>+</sup> complex has one major characteristic excitation spectral maximum at 579.33 nm and a minor one at 580.06 nm. As the solution pH increases, the 579.33 nm peak intensity decreases and a new peak at 580.25 nm arises. As it is possible that Eu(DO2A)<sup>+</sup> has several inner-sphere coordinated water molecules ( $q$ ), this observation is attributed to the following reaction in which the peak at 579.33 nm corresponds to Eu(DO2A)(H<sub>2</sub>O) <sub>$q$</sub> <sup>+</sup> and the



**Fig. 2** Laser-excited luminescence spectra of  $\text{Eu}^{3+}$ -DO2A system at various pH.  $[\text{Eu}^{3+}] = [\text{DO2A}] = 2 \times 10^{-5} \text{ M}$ ;  $25 \pm 0.1 \text{ }^\circ\text{C}$ .

peak at 580.25 nm corresponds to  $\text{Eu}(\text{DO2A})(\text{OH})(\text{H}_2\text{O})_{q-1}$ , eqn. (3). This assignment is consistent with the observation that



upon coordination by additional negatively charged donor groups, the luminescence peak maximum shifts to higher wavelength.<sup>22,28c</sup>

The excited-state lifetimes, measured in both  $\text{H}_2\text{O}$  and  $\text{D}_2\text{O}$ , are diagnostic of the number of coordinated water molecules, owing to an isotope effect.<sup>29a</sup> These properties have been determined and reported for a number of  $\text{Eu}^{3+}$  complexes with K21DA, K22DA<sup>11a</sup> and several amide-based macrocycles.<sup>11b</sup> For example, for the  $\text{Eu}(\text{III})$  complex of K22DA (an eight-coordinate ligand), it has been found that one inner-sphere coordinated water molecule is present, making the overall  $\text{Eu}(\text{III})$  complex nine-coordinated. On the other hand, for the seven-coordination ligand, K21DA, there are two inner-sphere coordinated water molecules on the resulting  $\text{Eu}(\text{III})$  complexes. In almost all cases, the results are consistent with those found by other means whether solid state structural determination or thermodynamic studies.

The present determination of the  $\text{Eu}(\text{DO2A})^+$  complex lifetimes in  $\text{H}_2\text{O}$  and  $\text{D}_2\text{O}$  ( $\tau_{\text{H}_2\text{O}}$  and  $\tau_{\text{D}_2\text{O}}$ , respectively) were found to be:  $\text{Eu}(\text{DO2A})(\text{H}_2\text{O})_q^+$ ,  $\tau_{\text{H}_2\text{O}} = 0.280 \text{ ms}$  and  $\tau_{\text{D}_2\text{O}} = 1.34 \text{ ms}$ ;  $\text{Eu}(\text{DO2A})(\text{OH})(\text{H}_2\text{O})_{q-1}$ ,  $\tau_{\text{H}_2\text{O}} = 0.313 \text{ ms}$  and  $\tau_{\text{D}_2\text{O}} = 1.46 \text{ ms}$ . The inner-sphere water coordination numbers can therefore be calculated using eqn. (4). The results show that  $\text{Eu}(\text{DO2A})$ -

$$q = 1.05 (\tau_{\text{H}_2\text{O}}^{-1} - \tau_{\text{D}_2\text{O}}^{-1}) \quad (4)$$

$(\text{H}_2\text{O})_q^+$  has 3.0 inner-sphere water molecules and  $\text{Eu}(\text{DO2A})(\text{OH})(\text{H}_2\text{O})_{q-1}$  has 2.6 inner-sphere water molecules. This is consistent with a  $q$  number equal to 3 and the overall coordination number for  $\text{Eu}^{3+}$  is nine (assuming DO2A is six-coordinated).

It should be noted that a number of studies have recently been reported concerning the importance of the fluorescence

quenching effect of other XH (*e.g.* NH and CH) oscillators in the  $\text{H}_2\text{O}/\text{D}_2\text{O}$  lifetime measurement.<sup>29</sup> For example, the respective reduction of the luminescence decay constant per inner-sphere O-H, amide N-H and acetate C-H oscillator for a few  $\text{Eu}(\text{DOTA})^-$  structurally related complexes<sup>29</sup> have been estimated to be 450, 75 and  $25 \text{ s}^{-1}$ . Unfortunately, these limited estimated data are not always easily applicable to other systems. However, it is possible that some of the  $q$  values estimated using eqn. (4) could be somewhat higher than the actual values. Given the fact that the equation is empirical and certainly carries with it some uncertainty and because of the successful applications of it in estimating the  $q$  values for many  $\text{Eu}(\text{III})$  complexes, we feel our current results are quite reasonable. A separate laser excited luminescence quenching study on  $\text{Tb}(\text{III})$ -DO3A and -DOTA systems reveals that the  $\text{Tb}(\text{DO3A})$  complex has two inner-sphere coordinated water molecules and  $\text{Tb}(\text{DOTA})^-$  has one.<sup>30</sup> [ $\text{Eu}(\text{DOTA})^-$  has also only one inner-sphere coordinated water molecule.] This is consistent with our present finding because DO2A has one less donating group than DO3A and three inner-sphere coordinated water molecules should be present for the  $\text{Eu}(\text{DO2A})^+$  complex, making the total coordination number around  $\text{Eu}(\text{III})$  ion to be nine.

A reviewer of this paper also suggested that a newly published correlation<sup>31</sup> [eqn. (5)] between  $q$  and  $k_{\text{obs}}(\text{Eu})$  is better

$$q = 1.1 k_{\text{obs}}(\text{Eu}) - 0.71 \quad (5)$$

than eqn. (4). In our view, this correlation is even more empirical because it assumes that the quenching effect in  $\text{D}_2\text{O}$  is the same for all  $\text{Eu}(\text{III})$  complexes. This is certainly not always true. For example, the  $\tau_{\text{D}_2\text{O}}^{-1}$  values for the respective  $\text{Eu}(\text{DO2A})^+$ ,  $\text{Eu}(\text{K21DA})^+$ ,  $\text{Eu}(\text{K22DA})^+$  and  $\text{Eu}(\text{EGTA})^-$  [EGTA = ethylenebis(oxyethylenetriamino)tetraacetic acid] complexes<sup>11a</sup> are 0.75, 0.67, 0.84, and  $0.49 \text{ ms}^{-1}$ . Indeed, the use of this equation predicts even higher numbers of inner-sphere coordinated water molecules, *i.e.* 3.2 and 2.8.

Finally, by using the relative peak intensities of the peaks at 579.33 nm and 580.25 nm at various pH, we have estimated the first hydrolysis constant ( $\text{p}K_{\text{h}}$ ) for  $\text{Eu}(\text{DO2A})(\text{H}_2\text{O})_3^+$  to be  $8.1 \pm 0.3$ . The same technique has been applied to the  $\text{Eu}(\text{DTPA-DIEN})$  system and a value  $8.3 \pm 0.2$  is obtained,<sup>22</sup> consistent with our reported value [DTPA-DIEN = 1,4,7-tris(carboxymethyl)-9,17-dioxo-1,4,7,10,13,16-hexaazacyclo-octadecane]. The value is also similar to that of  $\text{Eu}(\text{EDDA})(\text{H}_2\text{O})_4^+$ ,  $\text{p}K_{\text{h}} = 8.06 \pm 0.05$ , determined by a potentiometric pH titration method (EDDA = ethylenediamine diacetic acid).<sup>32</sup> Note that due to the kinetic inertness, the potentiometric determination of the hydrolysis constant of the  $\text{Eu}(\text{DO2A})(\text{H}_2\text{O})_3^+$  complex was not successful.

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