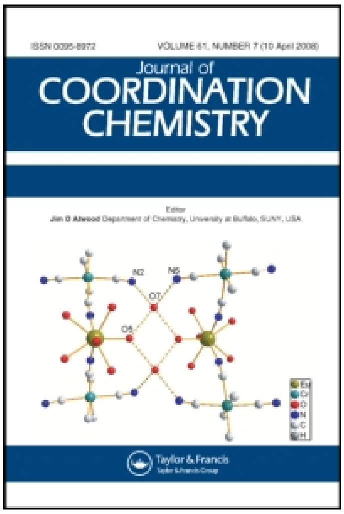

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THE $^7F_0 \rightarrow {}^5D_0$ EXCITATION SPECTRA OF EUROPIUM(III) COMPLEXES OF AMINOCARBOXYLIC ACIDS

Martti Latva[a]; Jouko Kankare[a]
[a] Department of Chemistry, University of Turku, Turku, Finland

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THE $^7F_0 \rightarrow {}^5D_0$ EXCITATION SPECTRA OF EUROPIUM(III) COMPLEXES OF AMINOCARBOXYLIC ACIDS

MARTTI LATVA* and JOUKO KANKARE

Department of Chemistry, University of Turku, FIN-20014 Turku, Finland

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The complexation of ethylenediamine, triethylenetetramine, acetic acid, glutaric acid, succinic acid, maleic acid, fumaric acid, malonic acid, iminodiacetic acid, nitrilotriacetic acid, *N*-(2-hydroxyethyl)ethylenediaminetriacetic acid, *N*^{1}-(4-isothiocyanatobenzyl)diethylene-triaminetetraacetic acid, *trans*-1,2-diaminocyclohexane-*N,N,N′,N′*-tetraacetic acid and diethylenetriaminepentaacetic acid (DTPA) with Eu^{3+} ion in aqueous solution has been studied by using the $^7F_0 \rightarrow {}^5D_0$ excitation spectroscopy of the Eu^{3+} ion. Because the energy of the $^7F_0 \rightarrow {}^5D_0$ transition of Eu^{3+} is dependent on the coordinating oxygen atoms, the "nephelauxetic" shift parameters for most typical coordinating atoms, such as in the carboxylate group, aliphatic amino nitrogen and the pyridine nitrogen atom were recalculated by multilinear regression with the present set of 22 complexes. The calculated shift parameters were used for the analysis of the excitation spectra of the complexes of diethylenetriaminepentaacetic acid, *trans*-1,2-diaminocyclo-hexane-*N,N,N′,N′*-tetraacetic acid and *N*-(2-hydroxyethyl)ethylenediaminetriacetic acid.

Keywords: lanthanides, europium, luminescence, polyaminopolycarboxylates, excitation spectra

INTRODUCTION

During the last ten years lanthanide(III) chelates have gained considerable importance because of their numerous different uses. Due to their excellent luminescence properties lanthanide(III) chelates are employed as markers in chemical and biological applications[1-3] or *in situ* fluorescence imaging[4,5]. On the other hand, they are utilized as paramagnetic shift reagents or relaxation

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*Author for correspondence.

agents in NMR spectroscopy and imaging[6,7]. Furthermore, lanthanide(III) chelates exhibit valuable catalytic activity towards the hydrolysis of internucleosidic phosphodiester bonds of RNA[8]. Considerable amounts of synthetic work have been done to design and synthesize new ligands in order to obtain chelates with better properties[9-12]. Depending on the application, different kinds of ligands have been made, *e.g.*, terdentate pyridine dicarboxylic acids[9,13], multidentate pyridine[9], bipyridine[10], terpyridine[11], and pyrazolylpyridine[12] polyaminopolycarboxylates as well as cryptands[14,15], calixarenes[16,17], cyclodextrins[18] and crown-ether derivatives[19].

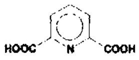
The lanthanide chelates are commonly used in aqueous solution although their precise structures and equilibria in solution are incompletely known. Luminescence excitation spectroscopy of the $^7F_0 \rightarrow {}^5D_0$ transition of Eu^{3+} using a pulsed dye laser light source has proved to be a valuable method in characterizing Eu^{3+} ion environments, particularly in solution[20-26]. The Eu^{3+} ion is especially advantageous in this regard as it has non-degenerate ground ($^7F_0$) and emitting ($^5D_0$) states, neither of which can be split by a ligand field of any symmetry. Thus the number of peaks in the excitation spectrum corresponds with the number of distinct Eu^{3+} ion environments. Although this method can be used only with europium, the chemical similarity of lanthanides allows one to draw conclusions on this basis regarding other lanthanide complexes.

The simple one-to-one correspondence between the number of excitation peaks and the number of europium(III) species present in a mixture of complexes is admittedly a considerable advantage in many analytical problems. However, at the same time, the simple nature of the excitation spectrum prevents any profound conclusions to be drawn concerning the structure of dissolved complexes. Apart from the number of peaks due to the different species, the only information is contained in the shift of the peak position. When ligands are coordinated to Eu^{3+} the atomic orbitals of europium(III) and the ligand form molecular orbitals through which the electrons can move, leading to an effective increase in the size of the orbitals (nephelauxetic effect or cloud expanding). As a consequence, electron-electron repulsion is decreased because the distance between electrons is increased. Thus the increase in the extent of covalency in the Eu^{3+}-ligand bond decreases the parameters of interelectronic repulsion and the decreased electron-electron repulsion is observed in terms of spectroscopic shifts in the $^7F_0 \rightarrow {}^5D_0$ excitation spectra of Eu^{3+}. It has been shown that the frequency of the $^7F_0 \rightarrow {}^5D_0$ transition in Eu^{3+} complexes correlates with the sum of "nephelauxetic" parameters for all of the coordinating atoms[27]. The transition frequency is dependent on the coordinating atoms according to (*1*),Equation (1) where $\tilde{\nu}_{obs}$ is the observed transition frequency, δ_i is the "nephelauxetic" shift parameter for atom of type i, n_i is the number of atoms of type i in the first coordination sphere of the Eu(III) ion and C_{CN} is the "nephelauxetic" parameter adjustment coefficient associated with the coordination number of the particular complex[27]. The energy value ($\tilde{\nu}_0$) for free-ion 17374 cm^{-1} calculated by Ofelt [28] for the gaseous Eu(III) ion was chosen to represent Eu(III) with no coordinated atoms in the first coordination sphere of Eu(III) ion, and all coordinating atoms will decrease this value.

$$\tilde{\nu}_{obs} - \tilde{\nu}_0 = C_{CN}\sum_i n_i\delta_i \tag{1}$$

Different numbers of chelating groups in a ligand produce different kinds of chelates with lanthanide(III) ions, the metal/ligand-ratio being, *e.g.*, 1:3, 1:2, 1:1 or 2:1[21,29,30]. Taking the best possible benefit from the chelates in the aforementioned applications demands exact knowledge about the conditions (*e.g.*, pH and metal/ligand ratio) under which any particular chelate species dominates as well as about the structure of the species in aqueous solutions. A study of the complex of six-dentate ethylenediaminetetraacetic acid (EDTA) with Eu^{3+} has been reported previously[31]. In the present work we have studied the complexation of ethylenediamine (En), triethylenetetramine (Trien), acetic acid (Acet), glutaric acid (Glut), succinic acid (Succ), maleic acid (Male), fumaric acid (Fuma), malonic acid (Malo), iminodiacetic acid (IMDA), nitrilotriacetic acid (NTA), *N*-(2-hydroxyethyl)ethylenediaminetriacetic acid (HEDTA), N^1-(4-isothiocyanatobenzyl)-diethylene-triaminetetraacetic acid (BDTTA), *trans*-1,2-diaminocyclohexane-*N,N,N′,N′*-tetraacetic acid (CDTA) and diethylenetriaminepentaacetic acid (DTPA) with Eu^{3+} ion.


IMDA
1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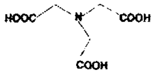
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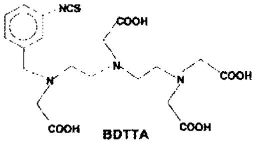
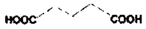

BDTTA
4


Glut
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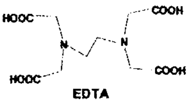
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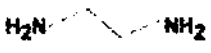
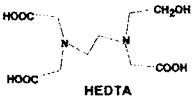
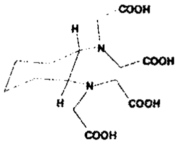

En
7


HEDTA
8


CyDTA
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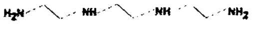
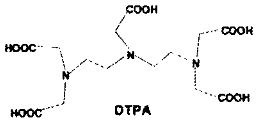
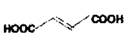
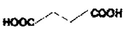

Trien
12

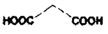
CH_3COOH
Acet
16


DTPA
10


Fuma
13


Succ
14


Male
15


Malo
17

The "nephelauxetic" shift parameters for several coordinating atoms have earlier been reported by Frey *et al.* [27] and we felt that it would be interesting to use the $^7F_0 \rightarrow {}^5D_0$ transition energies of several simple aminopolycarboxylate chelates of Eu^{3+} to obtain an independent check for the most typical coordinating atoms.

EXPERIMENTAL

Materials

IMDA (98.5 %) and malonic acid (98 %) were obtained from BDH Chemicals. NTA (99 %), $EDTAH_2Na_2$ (99 %), maleic acid (99.5 %), H_3BO_3 (99.8 %), HCl, NaOH and CH_3COOH were purchased from E. Merck. Europium(III) oxide (Eu_2O_3, 99.95 %), HEDTA (99 %), CDTA (99 %), ethylenediamine (99 %), triethylenetetramine hydrate (98 %), glutaric acid (99 %), succinic acid (99 %), fumaric acid (99 %), dipicolinic acid (DPA, 99 %), 3-cyclohexylamino-1-propanesulfonic acid (CAPS, 99 %), 4-morpholinepropanesulfonic acid (MOPS, 98 %) and 4-morpholineethanesulfonic acid (MES, 98 %) were purchased from the Aldrich Chemical Co., DTPA from Ventron GMBH and

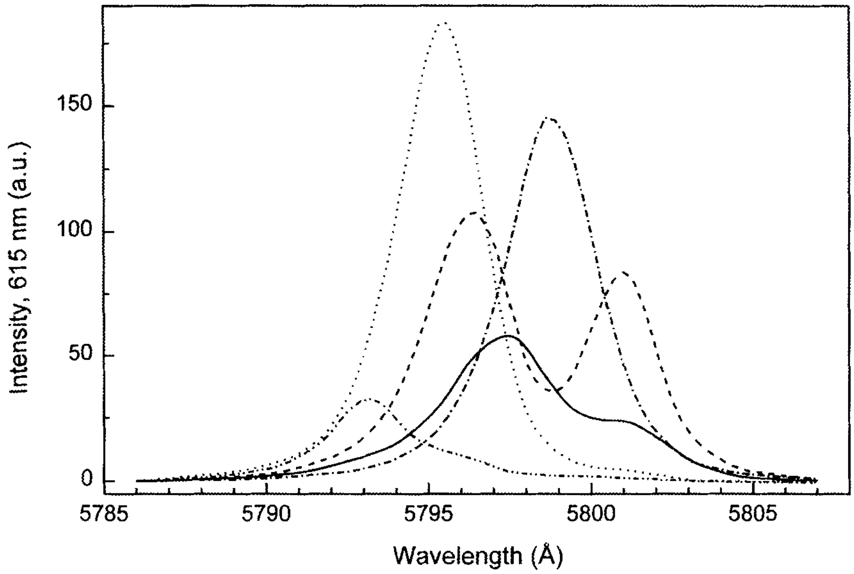


FIGURE 1 The $^7F_0 \rightarrow {}^5D_0$ excitation spectra of equimolar solutions of Eu^{3+}-NTA (-·-·-), Eu^{3+}-HEDTA (.......), Eu^{3+}-EDTA (---), Eu^{3+}-CDTA (—) and Eu^{3+}-DTPA (-·-·-·). In all solutions the concentrations of Eu^{3+} and aminopolycarboxylic acids were both 0.005 M and pH 7.2 was buffered with MOPS.

sodium perchlorate ($NaClO_4$, 99.5 %) from Fluka AG. BDTTA was a gift from Wallac Co.

Stock solutions of 0.1 M Eu^{3+} were prepared by dissolving Eu_2O_3 in perchloric acid. This solution was standardized using complexometric titrations with xylenol orange as indicator; 0.1 M solutions of complexing agents were prepared by dissolving a weighed amount of the corresponding acid in 1 M NaOH solution. Buffer solutions (0.05 M) were prepared by dissolving CAPS, H_3BO_3, MOPS, MES or CH_3COOH in triply distilled water and adjusting the pH with NaOH or HCl. Working solutions were then prepared by mixing appropriate volumes of Eu^{3+} and a suitable stock solution of the complexing agent and by diluting to the required concentration with a buffer solution of desired pH.

Methods and Apparatus

The Eu^{3+} excitation spectra and lifetime measurements were made by using a pulsed (5Hz) nitrogen-laser-pumped tunable dye laser apparatus[31]. The nitrogen laser (Model LN-1000, Photochemical Research Associates) operated at a repetition rate of 5 Hz. Rhodamine 6-G was used as a laser dye in the tunable dye laser (Model LN-107, Photochemical Research Associates), which was controlled *via* a digital drive unit (Model L-SCAN, Photochemical Research Associates). The bandwidth of the dye laser was 0.04 nm and the pulse width 500 ps. The pulsed output of the dye laser was focussed onto the sample cell and luminescence emission was collected and detected at 90° after passing through a monochromator (Model SPEX 1702, SPEX Industries, Inc.) by a photomultiplier tube (Model R928, Hamamatsu Photonics). The monochromator was controlled *via* a control unit (Model SPEX Compudrive CD2A, SPEX industries, Inc.) and 2-way RS-232 interface by a personal computer. The monochromator has a 0.01 nm resolution and focal length 0.75 m. The wavelength scale of the monochromator was calibrated by using the lines from a low-pressure Hg lamp and the dye laser in turn was calibrated by using the monochromator.

When measuring luminescence spectra, the signal from the PMT was fed into the signal input of the gated integrator and boxcar averager module (Model SR 250, Stanford Research Systems). The gate width used in the measurements was 150 μs and the delay of the gate after the trigger pulse was controlled by computer interface (Model SR 245, Stanford Research Systems). The integrated luminescence signal was fed into the computer interface. When measuring the excited-state lifetime the signal from the PMT was taken into a digitizing oscilloscope (Hewlett Packard 54503A) after amplification. The computer interface, digitizing oscilloscope and digital drive unit were connected to a personal computer *via* a GPIB bus. The measurement system was controlled by using the ASYST Language Programming package (Version 1.56, Macmillan Software Company).

The $^7F_0 \rightarrow {}^5D_0$ excitation spectra were obtained by scanning the dye laser through the $^7F_0 \rightarrow {}^5D_0$ absorption band from 5785 Å to 5815 Å whilst the luminescence intensity of the intense $^5D_0 \rightarrow {}^7F_2$ transition was recorded at 6150 Å. The excited-state lifetimes were determined by exciting the sample at a chosen wavelength and recording the emission profile on an oscilloscope at 6150 Å.

The number of water molecules in the first coordination sphere of Eu^{3+} ion was obtained by recording the luminescence decay curves in both H_2O and D_2O, from which the reciprocal excited-state lifetimes, $\tau_{H_2O}^{-1}$ and $\tau_{D_2O}^{-1}$, were determined. The number of coordinated water molecules in the case of europium can be calculated from (*2*)[32].

$$q = 1.05(\tau_{H_2O}^{-1} - \tau_{D_2O}^{-1}) \qquad (2)$$

When necessary, the luminescence excitation spectra were resolved into their components by using a curve fitting program, which was written using ASYST. In aqueous solution the peak shapes are Lorentzian-Gaussian and the product function used in this work is

$$I = I_{max} \frac{\exp\left[-0.30\left(\frac{x-a}{b}\right)^2\right]}{1 + \left(\frac{x-a}{b}\right)^2} \qquad (3)$$

where I_{max} is the maximum intensity of the peak, a is the wavelength of the peak maximum and $2b$ is the width of the peak at $I = 1/2; I_{max}\exp(-0.30) \cong 0.3704 I_{max}$[33]. Multilinear regression analysis was carried out by using the programs Origin™ (Microcal Software, Inc., MA) and Derive® (Soft Warehouse, Inc., Hawaii).

RESULTS AND DISCUSSION

The structures of the ligands treated in the present work are shown in Scheme 1. Complexes studied in this work are divided into two series. Iminodiacetic acid (IMDA) (**1**), dipicolinic acid (DPA) (**2**), nitrilotriacetic acid (NTA) (**3**), ethylenediaminetetraacetic acid (EDTA) (**6**) and N^1-(4-isothiocyanatobenzyl)diethylenetriaminetetraacetic acid (BDTTA) (**4**) form a well-defined series of complexes and their excitation spectra are easily interpreted. Although not containing either amino groups or carboxylate groups, acetic acid (Acet) (**16**), fumaric acid (Fuma) (**13**), succinic acid (Succ) (**14**), maleic acid (Male) (**15**), glutaric acid (Glut) (**5**), malonic acid (Malo) (**17**), triethylenetetramine (Trien) (**12**) and ethylenediamine (En) (**7**) were included because they provide an independent check on the shift parameter of the carboxylate group and nitrogen atom. These complexes serve as a basis which determines the values of the shift parameters. The complexes of diethylenetriaminepentaacetic acid (DTPA) (**10**), *N*-(2-hydroxyethyl)-ethylenediaminetriacetic acid (HEDTA) (**8**), and *trans*-1,2-diaminocyclohexane-*N,N,N′,N′*-tetraacetic acid (CDTA) (**9**) represent more complicated systems and their excitation spectra are analyzed by using the method presented by Frey *et al.* [27] and parameters evaluated from the complexes of the first series. The $^7F_0 \rightarrow {}^5D_0$ excitation spectra of equimolar solutions of Eu^{3+}-NTA, Eu^{3+}-HEDTA, Eu^{3+}-EDTA, Eu^{3+}-CDTA and Eu^{3+}-DTPA are shown in Figure 1.

Excitation spectra of europium complexes of DPA[30] and EDTA[31] have been previously reported and interpreted and will not be treated here in more detail. The wavelengths and the energies of the $^7F_0 \rightarrow {}^5D_0$ transitions of Eu^{3+} in aminocarboxylate complexes as well as the observed excited-state lifetimes in H_2O are listed in Table I. The excited-state lifetimes are measured also in D_2O, but they are not listed, because the excited-state lifetimes of all the complexes studied here in D_2O solutions were ~2 ms as was reported before[21,30,32,34,35]. Also, the number and type of coordinating atoms of ligands are listed in Table I.

IMDA

Iminodiacetic acid (IMDA) is a trifunctional ligand and has two carboxylate groups and one nitrogen atom through which it can form 1:1, 2:1 and 3:1 complexes with Ln(III) ions. The $^7F_0 \rightarrow {}^5D_0$ excitation spectra were measured for solutions where the concentration of Eu^{3+} ion was 0.01 M and the concentration of IMDA was 0.02 M. The effect of pH on $^7F_0 \rightarrow {}^5D_0$ spectra is shown in Figure 2. Three different peaks are seen at wavelengths 5791.5 Å (17267 cm^{-1}), 5797.0 Å (17250 cm^{-1}) and 5802.8 Å (17233 cm^{-1}). Figures 3a-3c show the intensities of these peaks as a function of the analytical concentration of IMDA at three different pH values while the concentration of Eu^{3+} is kept constant (0.01 M). The measured emission decay curves always showed single exponential behaviour and were independent of the excitation wavelength. The peak at 5791.5 Å existed at low pH values and at low IMDA concentrations. When the 5791.5 Å peak was the major component, the measured excited-state lifetime was 140 μs. This can be explained by the existence of twelve O-H vibrations of six water molecules and one N-H vibration of a coordinated IMDA in the first

TABLE I The $^7F_0 \rightarrow {}^5D_0$ transitions of Eu^{3+} in aminocarboxylate complexes.

Complex	*Coordinating atoms*†	$^7F_0 \rightarrow {}^5D_0$ *transition*	*Excited-state lifetime in* H_2O (μs)		
			cm^{-1}	Å	
Eu(IMDA)$^+$	2O$^-$, 1N, 6H$_2$O	**1a**	17266.7	5791.5	140
Eu(IMDA)$_2^{2-}$	4O$^-$, 2N, 3H$_2$O	**1b**	17250.3	5797.0	230
Eu(IMDA)$_3^{3-}$	6O$^-$, 3N	**1c**	17233.1	5802.8	375
Eu(DPA)$^+$	2O$^-$, 1N$_{Py}$, 6H$_2$O	**2a**	17264.3	5792.3	169[30]
Eu(DPA)$_2^-$	4O$^-$, 2N$_{Py}$, 3H$_2$O	**2b**	17248.8	5797.5	304[30]
Eu(DPA)$_3^{3-}$	6O$^-$, 3N$_{Py}$	**2c**	17230.9	5804.1	1640[30]
Eu(NTA)	3O$^-$, 1N, 5H$_2$O	**3a**	17261.3	5793.3	190
Eu(NTA)$_2^{3-}$	6O$^-$, 2N, 1H$_2$O	**3b**	17240.8	5800.2	725
Eu(BDTTA)$^-$	4O$^-$, 3N, 2H$_2$O	**4**	17246.4	5798.3	375
Eu(Glut)$^+$	2O$^-$, 7H$_2$O	**5**	17270.5	5790.2	135
Eu(EDTA)$^-$	4O$^-$, 2N, 3H$_2$O	**6a**	17252.3	5796.3	320[31]
	4O$^-$, 2N, 2H$_2$O	**6b**	17238.1	5801.1	320[31]
Eu(EDTA) (EDTAH)$^{4-}$	6O$^-$, 2N, 1H$_2$O	**6c**	17240.8	5800.2	1080[31]
Eu(En)$^{3+}$	1N, 8H$_2$O	**7a**	17275.6	5788.5	not possible to measure the exact lifetimes of individual species
	2N, 7H$_2$O	**7b**	17271.6	5789.8	
Eu(HEDTA)	3O$^-$, 2N, 1OH, 3H$_2$O	**8a**	17254.8	5795.5	255
	3O$^-$, 2N, 1OH, 2H$_2$O	**8b**	17238.4	5801.0	
Eu(HEDTA)$_2^{3-}$	6O$^-$, 2N, 1H$_2$O	**8c**	17241.4	5800.0	720
Eu(CDTA)$^-$	4O$^-$, 2N, 3H$_2$O	**9a**	17248.8	5797.5	340
	4O$^-$, 2N, 2H$_2$O	**9b**	17236.3	5801.7	
Eu(DTPA)$^{2-}$	4O$^-$, 3N, 1H$_2$O	**10a**	17246.1	5798.4	630
	5O$^-$, 3N, 1H$_2$O	**10b**	17241.6	5799.9	
Eu(H$_2$O)$_9^{3+}$	9H$_2$O	**11**	17280[27]	5787.0	105[27]
Eu(Trien)$^{3+}$	1N, 8H$_2$O	**12a**	17275.6	5788.5	not possible to measure the exact lifetimes of individual species
	2N, 7H$_2$O	**12b**	17271.6	5789.8	
	3N, 6H$_2$O	**12c**	17267.3	5791.2	
Eu(Fuma)$^+$	2O$^-$, 7H$_2$O	**13**	17270.5	5790.2	135
Eu(Succ)$^+$	2O$^-$, 7H$_2$O	**14**	17270.5	5790.2	135
Eu(Male)$^+$	2O$^-$, 7H$_2$O	**15**	17270.5	5790.2	135
Eu(Acet)$_x^{y+}$	1O$^-$, 8H$_2$O	**16a**	17275.2	5788.6	not possible to measure the exact lifetimes of individual species
	2O$^-$, 7H$_2$O	**16b**	17270.5	5790.2	
	3O$^-$, 6H$_2$O	**16c**	17265.8	5791.7	
	4O$^-$, 5H$_2$O	**16d**	17260.4	5793.6	
	5O$^-$, 4H$_2$O	**16e**	17255.7	5795.2	
Eu(Malo)$^+$	2O$^-$, 7H$_2$O	**17**	17274.4	5788.8	145

†Abbreviations: O = carboxylate oxygen, N = acyclic nitrogen, N$_{Py}$ = pyridine nitrogen, OH = hydroxyl oxygen and H$_2$O = water molecule.

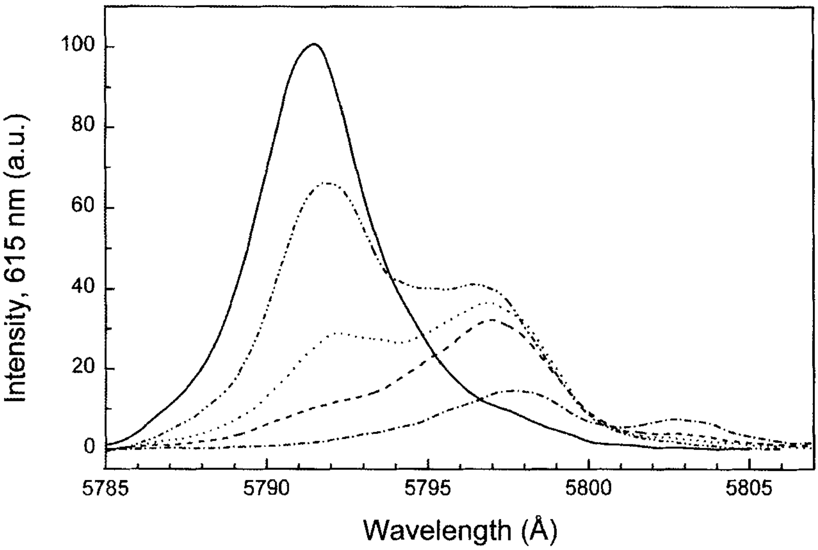


FIGURE 2 $^7F_0 \rightarrow {}^5D_0$ excitation spectra of chelates of Eu^{3+} with IMDA; the concentration of Eu^{3+} was 0.01 M and the concentration of IMDA 0.02 M; (—) pH 4.2, (-···-) pH 5.5, (.......) pH 7.0, (---) pH 7.6 and (-·-·-) pH 8.9.

coordination sphere of Eu^{3+}. The quenching effect of a bonded N-H vibrator is reported to be 1.5 times more effective for Eu^{3+} ion than that of the O-H vibrator[36]. This indicates that one IMDA is coordinated to one Eu^{3+} ion with three donor groups, supporting the structure $Eu(IMDA)^{2+}$. When only the 5797.0 Å peak existed, the excited state lifetime was about 230 μs, implying a hydration number of 4.5. This means that two IMDAs are coordinated to one Eu^{3+} ion both with three coordinating groups, the species being most likely $Eu(IMDA)_2^-$. The peak at 5802.8 Å exists at high pH values and at high IMDA concentrations. The observed excited state lifetime was 375 μs, which correlates well with the number of coordinated N-H groups being three, indicating that there are three IMDAs coordinated to one Eu^{3+} ion, the corresponding species being $Eu(IMDA)_3^{3-}$. When there were several peaks in the spectrum, it was not possible to measure accurate excited-state lifetimes of the individual components. Only the weighted average of the excited-state lifetimes of different species could be obtained. This proves that either the rate of the chemical interchange of Eu^{3+} between different species is rapid compared to the deexcitation rates in the absence of interchange, or there are energy transfer processes between different Eu^{3+} species. Both these explanations are possible because Eu^{3+} and IMDA form three different chelates, $EuIMDA^+$, $Eu(IMDA)_2^-$

and Eu(IMDA)$_3^{3-}$ with the logarithmic stability constants of 6.49, 11.65 and 15.70, respectively.[37] According to these stability constants there are, *e.g.*, in solutions of 0.01 M Eu^{3+} and 0.02 M IMDA, always species with opposite charges present at the same time, favouring the formation of ion pairs and thus enabling energy transfer between different species. Furthermore, the stability constants are low, which makes it possible that these single exponential and wavelength independent excited-state lifetimes may also be due to the fact that exchange between $Eu(H_2O)_9^{3+}$ and Eu(IMDA)$^+$, between Eu(IMDA)$^+$ and Eu(IMDA)$_2^-$ as well as between Eu(IMDA)$^-_2$ and Eu(IMDA)$_3^{3-}$, is faster than the observed excited-state lifetime. Thus, one IMDA^{2-} ligand may be coordinated and detached very rapidly as in the case of the second EDTA ligand in Eu(EDTA)(EDTAH)$^{4-}$[31]. Further support for this assumption is obtained from ^{139}La NMR studies of IMDA complexes of La(III) reported by Geraldes and Sherry, [38] which indicate rather rapid ligand exchange.

NTA

In solutions of Eu^{3+} and NTA three different peaks were seen in $^7F_0 \rightarrow {}^5D_0$ excitation spectra depending on the pH and Eu^{3+}/ligand ratio. In all cases single

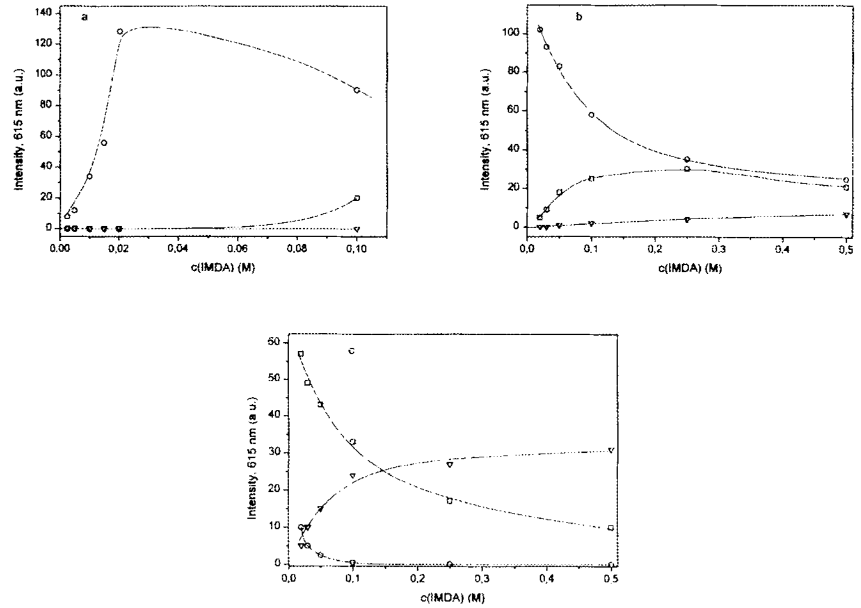


FIGURE 3 The intensities of the peaks formed in the solutions of Eu^{3+} and IMDA as a function of c(IMDA) at three different pH values while the concentration of Eu^{3+} is 0.01 M. In fig 3a pH is 4.7, in fig 3b pH is 5.7, and in fig 3c pH is 7.5; the peak at 5791.5 Å ((), the peak at 5797.0 Å ({) and the peak at 5802.8 Å (~) is marked as shown.

exponential luminescence decay curves and wavelength-independent excited-state lifetimes were observed. Two peaks were observed at 5793.2 Å (17262 cm^{-1}) and 5796.5 Å (17252 cm^{-1}) in equimolar solutions at pH values lower than 3, and at higher pH values a peak at 5800.0 Å (17241 cm^{-1}) was observed. The intensity of the latter increased when the concentration of NTA was increased relative to Eu^{3+}. This also caused the excited-state lifetimes to increase and when this 5800.0 Å peak was the only component in the spectrum, the excited-state lifetime was 725 μs, indicating its origin to be a chelate where one europium is coordinated to two NTA ligands. The peak at 5793.2 Å must be due to a 1:1-complex of Eu^{3+} and NTA because this peak was the major one in 1:1 solutions over the pH range 2 to 8, and the observed excited-state lifetime was 190 μs when this peak was the only peak seen in the spectrum. At pH values higher than 8, the peak at 5800.0 Å (1:2-complex) was the major component. The origin of the peak at 5796.5 Å was difficult to study because this small shoulder did not exist alone in the spectrum of any solution. It is possible it is due to a species where two Eu^{3+} ions are chelated by three NTA-ligands. The existence of a 2:3 complex is also supported by the logarithmic stability constant of 36.84 for $Eu_2(NTA)_3^{3-}$ as reported by Noddak and Oertel [39].

BDTTA

BDTTA is a seven-dentate ligand and its europium complex is extensively used as a labelling agent in immunoassay and other binding assays[40]. The excitation spectrum of this complex consists only of a single peak at 5798.3 Å (17246.4 cm^{-1}) while the observed excited-state lifetime was 375 μs. Hence, there are four carboxylate oxygens, three nitrogen atoms and two water molecules in the first coordination sphere of the Eu^{3+} ion.

Carboxylic Acids

Acetic acid can coordinate to Eu^{3+} only through its carboxylate group. When altering the acetic acid concentration at constant pH (4.5) and at constant Eu^{3+} concentration (0.005 M) different peaks were found. Usually several peaks were observed at the same time and after resolving the spectra into their components, in all, five different peaks were found. The transitions were at the wavelengths 5788.6 (17275.2 cm^{-1}), 5790.2 (17270.6 cm^{-1}), 5791.8 (17265.8 cm^{-1}), 5793.6 (17260.4 cm^{-1}) and 5795.3 Å (17255.4 cm^{-1}). The position of the peak dominating the spectra changed from lower to higher wavelengths as the concentration of acetic acid increased. Simultaneously, the observed excited-state lifetime, which was always wavelength independent and an average of the excited-state lifetimes of individual complexes present at the same time in the solution, increased. Depending on the ligand concentration, each peak in turn except the peak at 5788.6 Å was clearly distinguished as a major component in the spectra. Hence, their wavelengths could be obtained without resolving the spectra into components. The extremely small peak at 5788.6 Å existed only at low ligand concentrations. Although the intensity of the transition at 5788.6 Å is low because of both the high symmetry around the Eu^{3+} ion and the low luminescence quantum yield, the concentration of the corresponding complex must be very low. In equimolar solutions of acetic acid and Eu^{3+} only the 5790.2 Å peak was clearly seen without resolving the spectra into its components. As we take into account the reported wavelength 5787.0 Å for $Eu(H_2O)_9{}^{3+}$[27], we note that one acetic acid shifts the $^7F_0 \rightarrow {}^5D_0$ transition 3.2 Å to lower wavelengths. It has been reported that an isolated carboxylate coordinates in a bidentate fashion[41], and most likely the 5790.2 Å transition is due to a bidentate complex while the 5788.6 Å transition is due to a monodentate complex. When more than one acetic acid was coordinated to Eu^{3+}, the wavelength difference between the peaks was ~1.7 Å, indicating that the extra acetic acids coordinate to Eu^{3+} also in a monodentate fashion.

In equimolar (0.005 M) solutions of Eu^{3+} and succinic acid (or fumaric acid, glutaric acid or maleic acid) at pH 4.5, one major peak at 5790.2 Å was observed while the excited-state lifetime was ~135 μs. When the concentrations of ligands were increased, more peaks at higher wavelengths were formed, and after resolving the spectra into their components, peaks at wavelengths 5791.8 (17265.8 cm^{-1}), 5793.6 (17260.4 cm^{-1}) and 5795.3 Å (17255.4 cm^{-1}) were found. Simultaneously the observed excited-state lifetime increased. The Eu^{3+} complex of malonic acid was exceptional because in equimolar (0.005 M) solution of malonic acid and Eu^{3+} at pH 4.5 the $^7F_0 \rightarrow {}^5D_0$ transition was observed at 5788.8 Å. The observed excited-state lifetime was 145 μs. Usually, Eu^{3+} complexes of dicarboxylic acids have their $^7F_0 \rightarrow {}^5D_0$ transitions at the same wavelengths as Eu^{3+} complexes of acetic acid because of similar coordinating groups. However, it is not likely that only one carboxylate group is coordinated to Eu^{3+} in the malonic acid complex as a monodentate in spite of the fact that the wavelength 5788.8 Å is close to the one observed when the carboxylate group of acetic acid is coordinated in such a way. This is because the Eu^{3+}-malonic acid complex has a longer excited-state lifetime[35,42] and a higher stability constant[37] than other dicarboxylate complexes of Eu^{3+}. Probably the complex formed by Eu^{3+} and malonic acid has an extraordinary coordination geometry around the Eu^{3+} ion, where Eu^{3+} and malonic acid form a six-membered ring so that both carboxylate groups are coordinated to Eu^{3+} via monodentate fashion. Consequently there is less space in the first coordination sphere of Eu^{3+}, and water molecules are forced further away. Due to the increased distance between water molecules and Eu^{3+}, the higher energy of the $^7F_0 \rightarrow {}^5D_0$ transition and smaller quenching effect of water molecules based on O-H vibrations was observed. The excitation spectra of equimolar (0.005 M) solution of Eu^{3+} and malonic acid at pH 4.5, equimolar (0.005 M) solution of Eu^{3+} and succinic acid at pH 4.5 and solution of 0.001 M Eu^{3+} and 1.3 M acetic acid at pH 4.5 are shown in Figure 4.

Amines

Ethylenediamine and triethylenetetramine can both be coordinated to Eu^{3+} ion only through amine nitrogens. The intensities of the $^7F_0 \rightarrow {}^5D_0$ excitation transitions of Eu^{3+}-En and Eu^{3+}-Trien were clearly lower than transitions of Eu^{3+} complexes of carboxylic acids because of the lower stabilities of amine complexes. In solutions of Eu^{3+} and ethylenediamine only one peak at 5789.8 Å (17271.8 cm^{-1}) was clearly observed although the concentration of Eu^{3+} ion was 0.01 M and the concentration of En 0.2 M. After resolving the spectra into their components a second peak at a lower wavelength was noticed, in spite of the fact that due to the low luminescence intensity the signal/noise-ratio was low. The wavelength 5788.5 Å (17275.6 cm^{-1}) was obtained with some uncertainty. The observed excited-state lifetime 120 μs is most likely a weighted average of the excited-state lifetimes of $Eu(H_2O)_9{}^{3+}$, $Eu(En)(H_2O)_8{}^{3+}$ and $Eu(En)(H_2O)_7{}^{3+}$. The intensities of the $^7F_0 \rightarrow {}^5D_0$ transitions of Eu^{3+}-Trien complexes were higher than the intensities of Eu^{3+}-En complexes and three different peaks were found. In equimolar (0.01 M) solution of Eu^{3+} and Trien at pH 5.5 the main peak was found at 5789.8 Å while the minor peak at 5788.5 Å was found only after resolving the spectra into their components. At higher Trien concentrations a third peak at 5791.3 Å (17267.3 cm^{-1}) was found but no other complexes were observed despite the high concentration of Trien (0.18 M) and Eu^{3+} ion (0.01 M). We are able to interpret these transitions at 5788.5 Å, 5789.8 and 5791.3 Å as being due to $Eu(Trien)(H_2O)_8{}^{3+}$, $Eu(Trien)(H_2O)_7{}^{3+}$ and $Eu(Trien)(H_2O)_6{}^{3+}$, respectively.

DTPA

In equimolar solutions of Eu^{3+} and DTPA there was one peak 5799.4 Å (17243.1 cm^{-1}) and the measured excited-state lifetime was 630 μs, conforming with 1.25 water molecules in the first coordination sphere of Eu^{3+}. The position of this peak shifted to slightly lower wavelengths as the europium(III)/ligand ratio was increased and simultaneously the excited-state lifetime decreased (240 μs in 2:1-solutions). Using a curve fitting program it was possible to deconvolute the peak found in equimolar solutions into two components centred at 5798.4 Å (17246.1 cm^{-1}) and 5799.9 Å (17241.6 cm^{-1}), referring to two different chelates. The intensity of the lower wavelength component was increased at low pH and with increasing the Eu/DTPA ratio.

HEDTA

HEDTA forms rather stable complexes with Eu^{3+}, having three carboxylic acid groups, two nitrogen atoms and one CH_2OH group. In order to study the complexes of Eu^{3+} with HEDTA, the $^7F_0 \rightarrow {}^5D_0$ excitation spectra and excited-state lifetimes were measured as a function of pH at a concentration of 0.001 M for both Eu^{3+} and HEDTA. In addition, a series of measurements at constant pH of 7.7 was performed, where the concentration of Eu^{3+} was 0.001 M and the concentration of HEDTA was varied. In equimolar solutions there is one major peak at 5795.5 Å (17254.8 cm^{-1}) and a small peak at 5801.0 Å (17238.4 cm^{-1}). The observed excited-state lifetime was 255 μs, which is in agreement with a hydration number of 3.5. This hydration number probably originates from the six O-H vibrations of three water molecules and the O-H vibration of the CH_2OH group of HEDTA. When the wavelengths of these peaks are compared with the peaks obtained in EuEDTA-solutions, it is observed that the major peak is 2.4 cm^{-1} higher than in the EuEDTA-chelate[31]. This shift is caused by the replacement of the coordinating group COO^- of EDTA with CH_2OH of HEDTA. The minor peak at 5801.0 Å is almost at the same wavelength as the second major peak in EuEDTA chelates. This is attributed to a species, where the total coordination number of Eu^{3+} is eight and there are six donor atoms of EDTA and two water molecules in the first coordination sphere of Eu^{3+}[31]. The relative intensity of this 5801.0 Å peak is much smaller than in the case of $Eu(EDTA)^-$ (see Figure 1). It is reported that in the case of LaEDTAH the loss of H^+ from the ten-coordinate molecule is accompanied by tightening of all chelate linkages and the rejection of one water molecule[43]. In a similar way the chelates of Eu^{3+} and Gd(III) are transitional between nine-coordinated $Sm(EDTA)(H_2O)_3^-$ and eight-coordinated $Tb(EDTA)(H_2O)_2^-$. According to this the proton of the CH_2OH-group of HEDTA prevents the tightening of chelate linkages and the structure in EuHEDTA solutions is mainly nine-coordinate causing the relative intensity of the peak at 5795.5 Å to be much higher than the peak at 5801.0 Å. When the concentration of HEDTA is higher than the concentration of Eu^{3+} the formation of a new peak at 5800.0 Å is observed. As in the case of EuEDTA, the excited-state lifetime becomes simultaneously longer. This new peak can be assigned to a complex where two HEDTA ligands are coordinated to Eu^{3+}, $Eu(HEDTA)_2^{3-}$.

CDTA

As in the case of EDTA, in equimolar solutions of Eu^{3+} and CDTA complexes give rise to two peaks in the $^7F_0 \rightarrow {}^5D_0$ excitation spectra. The positions of the peaks are 5797.5 Å (17248.8 cm^{-1}) and 5801.7 Å (17236.3 cm^{-1}) (in the case of EDTA 5796.3 and 5801.1 Å). Besides this 1 Å difference between the wavelengths of the higher energy peaks of EuEDTA and EuCDTA complexes, these ligands differ from each other in that CDTA, unlike EDTA, is not able to form chelates where two CDTA ligands are coordinated to one Eu^{3+}, because both the $^7F_0 \rightarrow {}^5D_0$ excitation spectra and the observed excited-state lifetimes remained unchanged when the concentration of CDTA was increased while the concentration of Eu^{3+} was constant. Both ligands have the same six chelating groups but the structural difference between EDTA and CDTA is the cyclohexane ring, which makes CDTA less flexible than EDTA. In equimolar solutions of CDTA and Eu^{3+} at pH 7.5, the observed excited-state lifetime was 340 μs, which is almost the same as in equimolar EuEDTA solutions[31]. The 1 Å difference between the peaks in $^7F_0 \rightarrow {}^5D_0$ excitation spectra is most likely due to the cyclohexane ring which changes the coordination geometry so that the effect of ligating atoms is changed. Most probably this change occurs in the "nephelauxetic" shift parameters of two coordinated nitrogen atoms, because they are bonded directly to the cyclohexane ring; due to the rigidity the distance between these atoms and Eu^{3+} is shorter than in EuEDTA chelates, causing the peak at $^7F_0 \rightarrow {}^5D_0$ excitation to be at a higher wavelength.

Spectral Shift Parameters of the $^7F_0 \rightarrow {}^5D_0$ Transition

On the basis of the correlation between the energy of the $^7F_0 \rightarrow {}^5D_0$ transition and the sum of "nephelauxetic" parameters (shift parameters) for all of the coordinating atoms suggested by Frey *et al.*, the shift parameters are scalar, mutually independent quantities, and can be estimated for entire ligands and for different donor groups[27]. The energy of the $^7F_0 \rightarrow {}^5D_0$ transition is decreased from the energy calculated for free Eu^{3+} ion linearly when the number of equal coordinating groups is increased[27] assuming that the total coordination number of Eu^{3+} is not changed.

In order to recalculate δ_i values for the different kinds of coordinating atoms, we have included in this study the $Eu(H_2O)_9^{3+}$ ion, the complex of EDTA, two complexes of NTA, three complexes of IMDA, three complexes of DPA, the complex of BDTTA, four complexes of acetic acid, one complexes of glutaric acid, fumaric acid, maleic acid and succinic acid, two complexes of triethylenediamine and one complex of ethylenediamine. We tried to keep the number of different coordinating atoms as low as possible to ensure the calculation of the correct shift parameters for the coordinating atoms mostly used in ligands complexed with lanthanides. Thus we have concentrated only on the simplest aminopolycarboxylates and, aza macrocycles were not included because of the possible difference in their shift parameter for the nitrogen atom comparing to acyclic amines. DTPA and CDTA are not included because the excitation peaks are not well resolved. Hence we have 22 components and their experimental values of transition energies having as donor groups water molecules, carboxylate, secondary amine, tertiary amine, and pyridine nitrogen atoms.

The fact that the effect of donor groups on the shift is additive in a scalar sense can be seen by comparing the effects of 1:1, 1:2 and 1:3 complexes of Eu^{3+} with IMDA and DPA. In these complexes at least the 1:3 geometry is symmetric, and if instead of scalar, the vectorial additivity, *e.g.*, the position of two identical donors at opposite locations of europium ion cancels the effect, is valid, the transition energy should be unchanged from the aquo complex $Eu(H_2O)_9^{3+}$. The vectorial additivity can be expected if the effect of ligating atoms is purely electrostatic. However, by plotting the transition energy *versus* the ligand-to-Eu ratio for these complexes (Figure 5) we obtain two straight lines which have nearly the same intercept at *ca* 17282 cm^{-1}. These lines show that each ligand in these two series of complexes gives the same contribution to the shift, not depending on the presence of other ligands in the complex. According to these plots one DPA shifts the spectra ~17.2 cm^{-1} and one IMDA ~16.8 cm^{-1}. Further support to this rationale is obtained by adding the 1:1 and 1:2 complexes of Eu^{3+} and NTA to the same plot. The line drawn through these two points intercepts the axis at about the same point, 17282 cm^{-1}. This intercept corresponds to the transition energy of the aquo complex $Eu(H_2O)_9^{3+}$. The exact value 17280 cm^{-1} of the transition energy of this ion has been reported in the literature[27].

The statistically "best" shift parameters can be estimated by using standard methods of multivariate analysis. In order to obtain the exact shift parameters we have exploited matrix algebra to solve equations where the number of equations is greater than the number of parameters. Twenty two equations were generated, *(4)*

$$\nu_{obs} - \nu_0 = n_{1i}\delta_{COO-} + n_{2i}\delta_{N_{alif}} + n_{3i}\delta_{N_{Py}} + n_{4i}\delta_{H2}O \quad \mathrm{i} = 1, 2, \ldots, 22 \qquad (4)$$

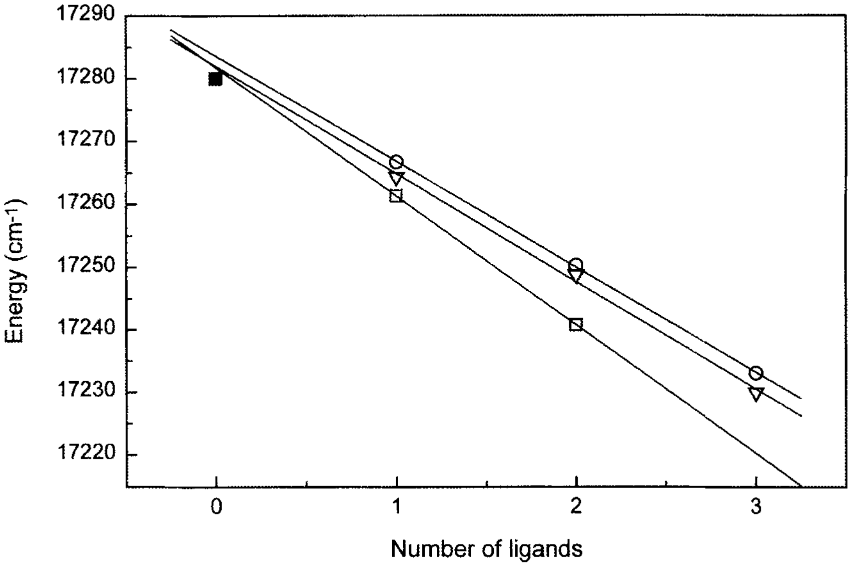


FIGURE 5 The $^7F_0 \rightarrow {}^5D_0$ transition energies of the peaks of Eu(IMDA)$^+$, Eu(IMDA)$_2{}^-$, Eu(IMDA)$_3{}^{3-}$, Eu(DPA)$^+$, Eu(DPA)$_2{}^-$ and Eu(DPA)$_3{}^{3-}$ plotted as a function of ligand/lanthanide(III) ratio. Transition energies of Eu(H$_2$O)$_9{}^{3+}$, Eu(NTA) and Eu(NTA)$_2{}^{3-}$ are inserted in the same Figure, suggesting the existence of a similar linear dependence. Symbols: IMDA((), DPA(~), NTA({) and Eu(H$_2$O)$_9{}^{3+}$(}).

The values of the shifts of the $^7F_0 \rightarrow {}^5D_0$ transition for different ligating atoms was then obtained by multilinear regression. Whether further improvement in correlation is obtained by a more detailed parametrization can be studied by using the analysis of variance (ANOVA). A further separation of the amino groups into secondary and tertiary amines did not prove to be statistically significant in spite of the fact that their shift parameters can differ from each other slightly because the surroundings of the nitrogen atoms are different. Hence we can write (*5*)

$$\begin{aligned} \nu_{obs} &= \nu_0 + n_{COO-}\delta_{COO-} + n_{N_{alif}}\delta_{N_{alif}} + n_{N_{Py}}\delta_{N_{Py}} + n_{H_2O}\delta_{H_2O} \\ &= 17374 - 15.53n_{COO-} - 15.08n_{N_{alif}} - 16.67n_{N_{Py}} - 10.34_{n_{H2O}} \end{aligned} \quad (5)$$

which allows estimation of excitation maxima at a standard deviation of 1 cm^{-1}. The values of shifts of the $^7F_0 \rightarrow {}^5D_0$ transition for different ligating atoms are listed in Table II along with standard deviations. The range of the shifts and the fit obtained by using the parameters from Table II are demonstrated in Figure 6, which shows the observed excitation energies *vs* the calculated energies with a correlation coefficient of 0.9982. In Figure 7 the "nephelauxetic" shift parameters

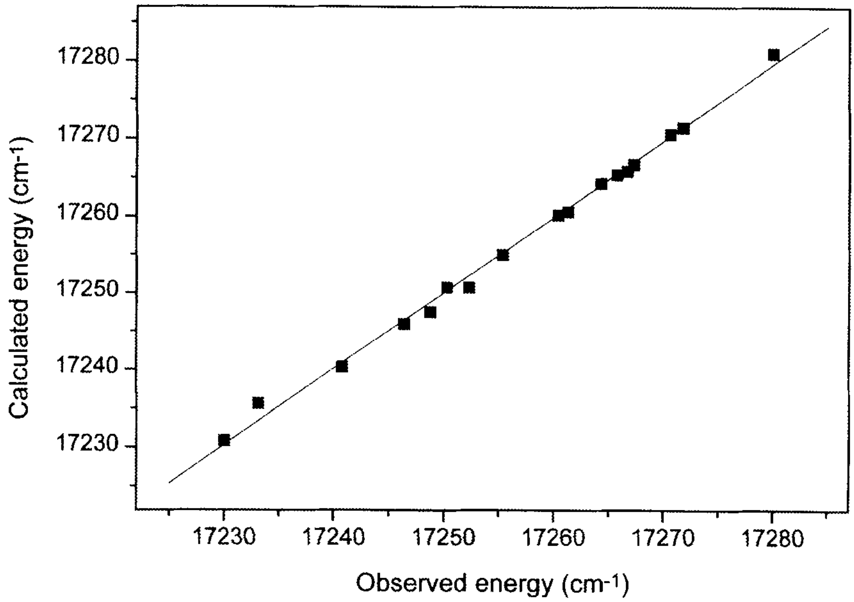


FIGURE 6 Plot of the observed excitation energies *vs* energies calculated according to equation (1) by using parameters from Table II. The obtained linear fit with correlation coefficient 0.9982 is shown.

from Table II are compared with Jørgensen's h-values[44] derived from studies of transition metal complexes. As seen in Figure 7, the parameters obtained here agree clearly better with h-values than the parameters calculated earlier[27]. Hence, the parameters calculated for amine nitrogen and carboxylate oxygen are approximately of equal size.

When the total coodination number of the Eu^{3+} ion decreases from nine to eight, the simultaneous tightening of all chelate linkages decreases the distances between coordinating atoms and Eu^{3+}[43]. Hence the covalent natures of the bonds are increased and the change in the "nephelauxetic" shift parameters is observed[27]. It has been reported that the values of the shift parameters are increased by a factor of 1.06 as the total coordination number is changed from nine to eight[27]. When taking into account the C_{CN} of 1.06 for eight-coordination, the reported "nephelauxetic" parameters[27] and (1) give the transition energy 17253.4 cm^{-1} for the complex existing at 17238.1 cm^{-1} in equimolar solutions of Eu^{3+} and EDTA, interpreted as $EuEDTA(H_2O)_2^-$[31]. The observed energy difference between the peaks at 17252.3 and 17238.1 cm^{-1} is -14.2 cm^{-1} for the removal of one water molecule from the coordination

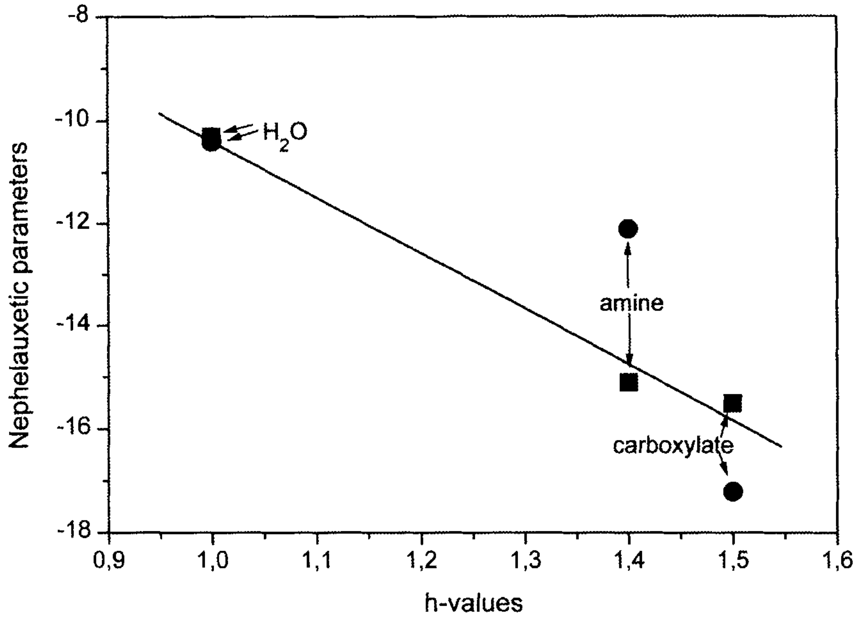


FIGURE 7 Plot of nephelauxetic parameters developed in this work (}) and earlier by Frey *et al.*[27] (&cirf;) *vs* Jørgensen's nephelauxetic h-values[44] derived from studies on transition metal complexes for coordinating atoms that are common to these studies.

sphere and shortening of all chelate linkages. The separation of the two peaks in the excitation spectrum of Eu(HEDTA) is 16.4 cm^{-1} and 12.5 cm^{-1} in the case of Eu(CDTA)$^{-}$. The most probable explanation is the same as with EDTA, *i.e.*, the lack of one water molecule from the species giving rise to the peaks at lower energies. By using these energy separations the calculated C_{CN} is 1.21 for total coordination number eight.

The higher energy peak of Eu(HEDTA) at 17254.8 cm^{-1} allows us to compute an estimate for the shift parameter of the CH_2OH group using the parameters in Table II. Although the value -11.8 cm^{-1} (-11.6[27]) thus obtained is based on a single compound and consequently rather doubtful, it is probably of the right magnitude because of the similarity of oxygen in water and the alcoholic hydroxyl group.

Application of the shift parameters of Table II to DTPA gives a value of 17240.8 cm^{-1} for the octadentate species. This would correspond to the experimental value of the main peak at 17241.6 cm^{-1}. It is tempting to assume that the weaker peak at 17246.1 cm^{-1} is due to the species in which one carboxylate group is detached and replaced with a water molecule, because the calculated value for this species would be 17246.0 cm^{-1}. However, the influence of Eu/ligand ratio on this peak and the decrease in the decay time with this ratio indicate that this peak may be due to the 2:1 complex of Eu^{3+} with DTPA.

The $^7F_0 \rightarrow {}^5D_0$ transition is dependent on the coordinated atoms is the first coordination sphere of Eu^{3+}. The shift values for several ligating atoms were calculated statistically on the basis of 22 complexes. It should be noted that the shift parameters derived in this work originate from simple open-chain compounds. It can be expected that ligands having steric constraints such as cyclic structures may force donor atoms closer or further from europium and consequently the shift parameters for these atoms may not fit within this short list of parameters. On the other hand, the apparent incompatibility may serve as a diagnostic structural tool for deducing the conformation of the complex. One simple example in this work concerns CDTA which requires use of a slightly different value for its nitrogen atoms in order to give an acceptable fit with the experimental shift. Furthermore, excitation spectra of Eu^{3+} complexes are composed very often of several peaks, against expectations. This is due to the presence of different species with originate from dynamic equilibria with different numbers of ligand donors and water molecules in the coordination sphere. $^7F_0 \rightarrow {}^5D_0$ excitation spectroscopy and the use of shift parameters may be the only method for elucidating the structure of these species.

Simple polyaminopolycarboxylic acids like IMDA, NTA, EDTA, and DPA are suitable ligands to be used to create a model to evaluate more difficult structures. They have unambiguous peaks with excellent luminescence intensities. The number of compounds in the present work is high compared to the number of different parameters, giving excellent statistical reliability for the shift parameters. Although the number of different ligating groups is presently rather small it is likely that the most important groups have been covered.

Lanthanide(III) complexes of polyaminopolycarboxylic acids remain an important area of research because of their numerous applications. Most of the properties of these complexes depend on the number of coordinated atoms and therefore knowledge about the structure around lanthanide(III) ion is important. The $^7F_0 \rightarrow {}^5D_0$ excitation spectroscopy of Eu^{3+} ion is probably the best way to solve these questions in spite of the few disadvantages such as the luminescence intensity being dependent on the symmetry around the Eu^{3+} ion.

Presently we are extending this work to hydroxycarboxylic acids, their ternary Eu^{3+} complexes with aminocarboxylic acids and more complicated structures such as highly luminescent Eu^{3+} complexes of pyridine, bipyridine and terpyridine tetracarboxylates having potential practical applications in the field of labelling agents for binding assays.

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