Europium(III) complex formed with pyridine containing azamacrocyclic triacetate ligand: characterization by sensitized Eu(III) luminescence

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Abstract

The water soluble polyazamacrocyclic ligand, 3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene-3,6,9-triacetic acid (111Py-12N4) has been synthesized and found to be an effective antenna molecule for europium luminescence. When bound to Eu(III), excitation at the absorption band of the 111Py12N4 ligand (264 nm) leads to the well-known structured emission spectrum of the Eu(III) ion with a large Stokes shift (>250 nm). UV-light induced Eu(III) luminescence resulting from a through-space energy transfer allowed the determination of a 1:1 stoichiometry of the Eu(III) complex and the determination of the corresponding stability constant (log K_{therm} = 20.9). The luminescence lifetimes, the number of water molecules coordinated to the metal as well as the overall quantum yields were evaluated.
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1. Introduction

The design of lanthanide complexes with encapsulating ligands is an important topic in the field of supramolecular chemistry because it offers the possibility to obtain stable luminescent compounds [1]. However, lanthanide complexes suffer from the low extinction coefficient associated with Laporte-forbidden lanthanide f–f transitions so that a direct excitation of lanthanide(III) ions is only practicable with laser beams [1]. To enhance its absorption, the luminescent lanthanide ion (in the present case Eu(III)) can be chelated with ligands that have broad and intense absorption bands. In such complexes, metal-centred luminescence occurs upon light absorption by allowed ligand-centred transitions followed by ligand-to-metal intramolecular energy transfer. As a consequence, several luminescent complexes using various photosensitizers (antenna) have been developed [1–7].

Among them, tetrazamacrocyclic ligands bearing a pyridine subunit and three acidic functions offered such a chromophoric moiety as well as seven potential donor atoms of various hardness degree being able to coordinate a lanthanide ion in its first coordination sphere thus shielding it from interactions with solvent molecules. Moreover, spectroscopic investigations of pyridine containing azamacrocyclic triphosphonate or tricarboxylate ligands were reported to allow an UV-light induced Tb(III) [5] and Eu(III) [7] luminescence.

Numerous complexes of trivalent lanthanides formed with aminopolycarboxylic acid ligands [8,9] azamacrocycles [3,6] or 3,6,9,15-tetraazabicyclo[9.3.1]pentadecane-1(15), 11,13-triene-3,6,9-triacetic acid (111Py12N4) [5,7] have been characterized in terms of quantum yield and lifetime measurements. However, the determination of stability constant of the lanthanide complexes was less reported due to the slow kinetic of complexation that prevents the use of the usual experimental method (e.g. pH-potentiometric titration).

We have previously reported an original preparation of the 12-membered pyridine containing azamacrocycle triacetate ligand 111Py12N4 with 2-nitrobenzenesulfonamide as a new activating/protecting group [7]. We report upon the determination of the stoichiometry and the stability constant of the corresponding Eu(III) complex formed in aqueous medium. These evaluations were realized by UV-light induced Eu(III) luminescence measurements.

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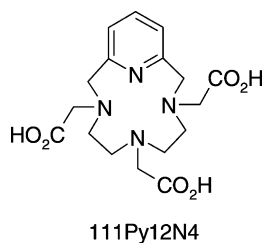
Scheme 1. Structure of ligand 111Py12N4 used as sensitizer for Eu(III) luminescence.

2. Experimental

2.1. Chemicals and materials

3,6,9,15-Tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene-3,6,9-triacetic acid 111Py12N4 (Scheme 1) was synthesized in 78% yield following the procedure described [7,10]. The desired product was isolated as a white powder by purification by ion-exchange chromatography on Dowex® 1X8 (formate form, elution with dilute formic acid) followed by gel filtration chromatography on Sephadex® G-10 resin (elution with water). ^{1}H RMN (D$_2$O) δ: 3.0 (m, 4H), 3.51 (m, 4H), 3.59 (s, 2H), 4.05 (s, 4H), 4.82 (s, 4H), 7.48 (d, 2H, $J = 7.8$ Hz), 8.00 (dd, 1H, $J_1 = J_2 = 7.8$ Hz) ^{13}C RMN (D$_2$O) δ: 53.7, 56.5, 58.5, 60.3, 61.8, 124.8, 142.3, 152.7, 172.7, 177.7. HRMS (FAB): found m/z 381,1762 ($[M + H]^+$); calcd. for C$_{17}$H$_{25}$N$_4$O$_6$ 381,1774.

Hydrated EuCl$_3$ salt as well as HCO$_2$Na and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid sodium salt (HEPES-Na) were purchased as their puriest form from Aldrich. All glassware was thoroughly rinsed with water. The pH of the solutions was measured with a PHM 220 Radiometer Copenhagen pH-meter. EuCl$_3$ stock solutions ($\sim$10 mM) were titrated by ICP–AES. The concentrations of the ligand stock solutions ($\sim$0.1 M) were determined via titration with standardized Eu(III) solution at pH 7 on equilibrated samples using sensitized Eu(III) luminescence.

2.2. Spectroscopy

The absorption spectra were recorded on a Cary 100 Scan UV-Vis spectrophotometer operating with Cary WinUV software. UV-light induced Eu(III) luminescence measurements were recorded on a SLM Aminco 8100 spectrofluorometer. Corrected spectra were obtained taking into account the wavelength-dependent response of the instrument. Fluorescence quantum yields were determined by comparison with that of quinine sulfate in aqueous sulfuric acid solution (1N), for which a reference yield of 0.55 was taken [11]. The luminescence lifetime measurements of Eu(III) were carried out with the use of the detection system described earlier [12]. Experiments were conducted at room temperature.

2.2.1. Preparation of samples for the determination of the stoechiometry

The pH of an aqueous stock solution containing HEPES (0.015 M), KCl (0.085 M) and ligand 111Py12N4 (50 μM) was adjusted to pH 7 with an aqueous solution of HCl. A series of 11 samples was prepared in separate sealed containers with various concentration of EuCl$_3$ ranging from 0 to 200 μM. Following incubation at 60 °C overnight, the samples were allowed to cool to room temperature and were monitored by absorption spectroscopy ($\lambda = 269$ nm) until no further spectral change was detected (typically 24 h). In order to furnish a constant amount of excitation energy to the system, the set of samples were irradiated at their isosbestic point (264 nm) and the luminescence intensity of each sample was measured at 615 nm.

2.2.2. Preparation of samples for lifetime and quantum yield measurements

Samples containing HEPES (0.015 M), KCl (0.085 M), ligand 111Py12N4 (50 μM) and EuCl$_3$ (75 μM) were prepared in H$_2$O (D$_2$O). The pH (pD) was adjusted with an aqueous solution of HCl (DCl) to pH 7.0 (7.4).

2.2.3. Preparation of samples for the determination of the stability constant of the Ln(III) complex

An aqueous stock solution containing buffer (KCl or HCO$_2$Na 0.015 M), KCl (0.085 M), ligand 111Py12N4 (50 μM) and a slight excess of EuCl$_3$ (55–60 μM) was prepared. A series of 15 samples was prepared in separate sealed containers at various pH by addition of an aqueous solution of HCl leading to KCl/HCl buffer for pH 1.3 to 2.2 and HCO$_2$Na/HCO$_2$H buffer for pH 2.2–3.8. The samples were monitored by UV-light induced Eu(III) luminescence measured at 615 nm (^{5}D$_0 \rightarrow ^7$F$_2$ transition) until no further spectral change was detected. When the equilibrium was reached, the set of samples were irradiated at their isosbestic point (264 nm) and the luminescence intensity of each sample was measured. For all samples the luminescence lifetimes were determined.

3. Results and discussion

3.1. Synthesis

The synthetic procedure was already described [7]. Salt free 111Py12N4 ligand was obtained in 4 steps from diethylenetriamine with a 24% yield on a 2 g scale after purification of the resulting azamacrocycle by ion-exchange resin (Dowex® 1X8) followed by gel filtration chromatography (Sephadex® G-10).

3.2. UV-Vis spectral assays

The absorption spectra, at various pH, of equimolar aqueous solutions of EuCl$_3$ and macrocycle 111Py12N4
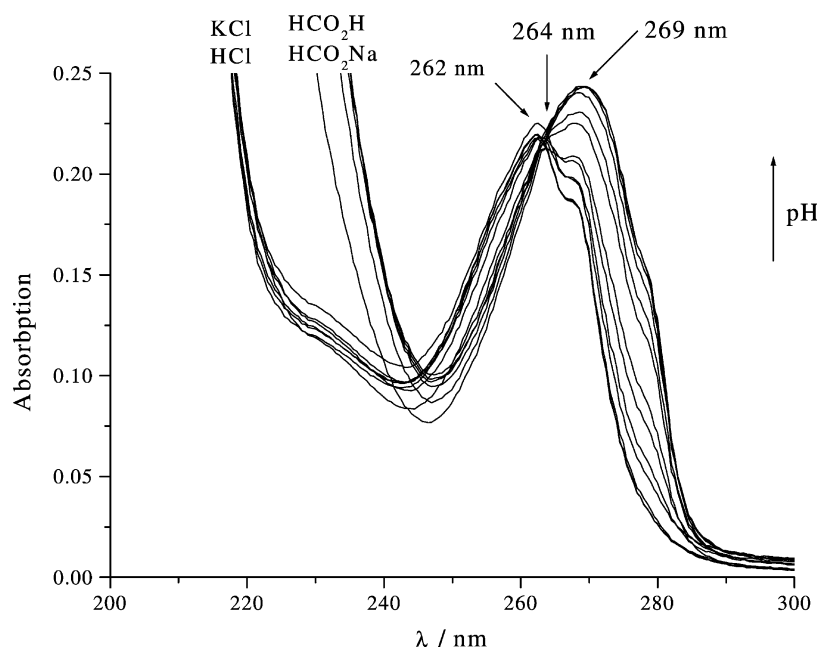
Fig. 1. UV-absorbance spectra of ligand 111Py12N4 (50 μM) in the presence of EuCl$_3$ (55–60 μM) in buffered aqueous solution and 0.085 M KCl. The pH varies from 1.3 to 2.2 (buffer: KCl/HCl 0.015 M) then from 2.2 to 3.8 (buffer: HCO$_2$Na/HCO$_2$H 0.015 M).

were monitored by UV-Vis spectroscopy (Fig. 1). Ligand 111Py12N4 is characterized by an absorption band in the UV region due to π–π^* transitions ($\lambda_{max} = 262$ nm, $\varepsilon_{max} = 4200$ dm^3 mol^{-1} cm^{-1}). Upon increasing the pH, the set of spectra exhibits a red shift of the π–π^* transitions ($\Delta\lambda = 7$ nm) which is indicative of a perturbation produced by the complexation of the coordinating metal ion ($\lambda_{max} = 269$ nm, $\varepsilon_{max} = 4600$ dm^3 mol^{-1} cm^{-1}). Moreover, the set of spectra exhibits an isosbestic point (264 nm) as expected for a two states system, the free ligand and the corresponding Eu(III) complex [13].

3.3. Luminescence assays

In the absence of ligand 111Py12N4, irradiation at 269 nm of an aqueous solution of Eu(III) did not induce Eu(III) luminescence. However, the introduction of the chromophoric ligand 111Py12N4 gave rise to the well-known structured emission spectrum of Eu(III) thus being an evidence of an efficient through-space energy transfer from the excited chromophore to the proximate lanthanide. Moreover, a large Stokes shift was observed (>250 nm) so that there is no overlap of the Eu(III) emission bands with the antenna chromophore absorption band (Fig. 2).

As expected, all emissions arose from the $^5D_0$ state, and the most intense bands corresponding to the $^5D_0 \rightarrow{}^7 F_j$ ($\Delta J = 0$–4) transitions were observed. The emission spectrum is dominated by the $^5D_0 \rightarrow{}^7 F_2$ transition centred on the 615 nm peak as it represents 37% of the total emission.

3.4. Determination of the stoechiometry

The validity of the results depends both on the correctness of the assumptions made concerning the species in solution and on the attainment of equilibrium at the time of the measurement. The stoechiometry of the complex was first determined and, in all case, the solutions were allowed to equilibrate at room temperature until there was no more spectroscopic variation.

The stoechiometry of the Eu(III) complex formed with ligand 111Py12N4 was determined by a mole ratio method [13]. In order to provide a constant amount of excitation energy to the system, a set of HEPES-buffered aqueous samples containing ligand 111Py12N4 with increasing amounts of EuCl$_3$ was irradiated at the isosbestic point (264 nm, Fig. 1). By monitoring the Eu(III) luminescence at 615 nm as the function of the initial quantity of Eu(III) salt, a binding curve is obtained (Fig. 3). Since the ligand concentration is well above the dissociation constant for such lanthanide complex, the titration curve is expected to break sharply when the stoechiometry quantity of Eu(III) would be added [13]. The Eu(III) complex formed with ligand 111Py12N4 disclosed a 1:1 stoechiometry thus being consistent with the assumption of a two-state system from the absorption spectroscopic data.

3.5. Luminescence lifetime, quantum yields

The Eu(III) luminescence lifetimes as well as the overall quantum yields measured in H$_2$O and D$_2$O are given in Table 1. Such measurements allow the estimation of the
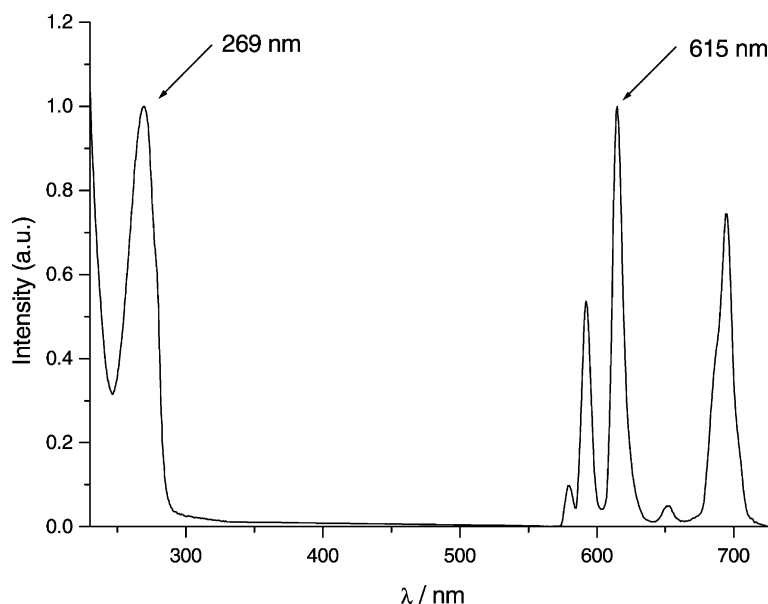
Fig. 2. Excitation and emission spectra in aqueous medium of Eu(III) complex formed with $50\,\mu M$ of 111Py12N4 at pH 7. The emission spectra resulted from a UV-light excitation at 269 nm.
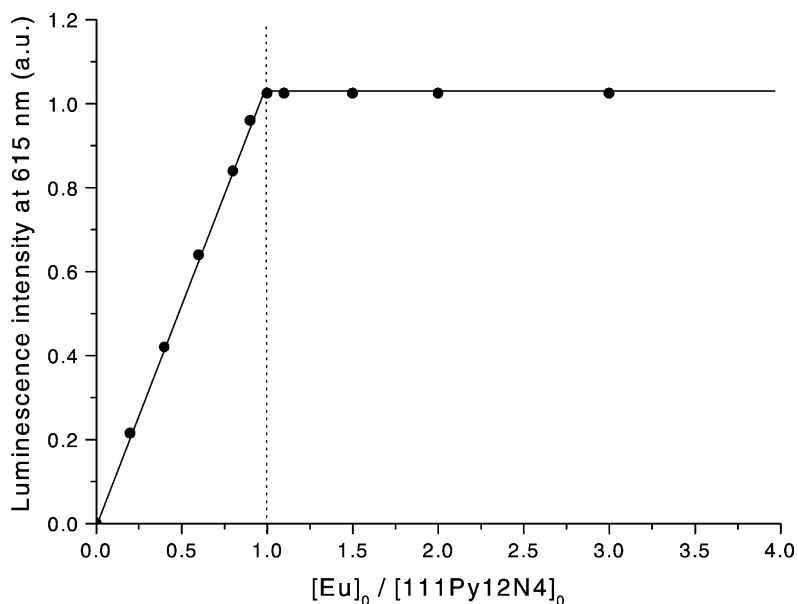

Fig. 3. Binding curve obtained by monitoring the luminescence intensity (615 nm) of samples containing $50\,\mu M$ of ligand 111Py12N4 and different amounts of Eu(III) in 0.085 M KCl and 0.015 M HEPES-buffered medium (pH 7.0). The luminescence resulted from an UV-light excitation at 264 nm. The filled circles represent actual data points.

Table 1
Luminescence lifetimes of Eu^{3+} complex formed with 111Py12N4, average number of water molecules coordinated to the complex and luminescence quantum yield

	Lifetime τ (ms)[a]		$^q H_2 O$[b]	$^q H_2 O$[c]	Luminescence quantum yield[d]	
	H_2O	D_2O			H_2O (%)	D_2O (%)
[Eu $\subset$ 111Py12N4]	0.37	2.12	2.3	2.38	2.9	19.1

[a] Measured at 293 K; excitation into the lowest energy ligand-centred absorption band (269 nm) in correspondence with the hypersensitive $^5 D_0 \rightarrow ^7 F_2$ transition (615 nm); experimental error 5%.

[b] Estimated using the Horrock's equation [14]: $q = 1.05(\tau^{-1}H_2O - \tau^{-1}D_2O)$; uncertainty ± 0.5.

[c] Estimated using the Parker's equation [15]: $q = 1.2[(\tau^{-1}H_2O - \tau^{-1}D_2O) - 0.25]$.

[d] Measured at 293 K with reference to quinine sulfate [11]; excitation into the lowest energy ligand-centred absorption band (269 nm); experimental error 10%.

number of coordinated water molecules by the use of the empirical relation proposed by Horrocks and Sudnick [14] or Parker and coworkers [15]. The presence of 2.3 coordinated water molecules for [Eu $\subset$ 111Py12N4] is in agreement with the expected seven-coordinating nature of ligand 111Py12N4 since Eu(III) prefers a coordination number of 8–9 [1].

3.6. Determination of the stability constant

The determination of stability constants of lanthanide ion chelates is fundamental to understand their coordination chemistry. Knowledge of these constants has practical importance given that such chelates found applications in medicine both as diagnostic [16] and assignation of the properties and functions of biochemical systems [17,18] and therapeutic agents [19]. The stability constants of complexes formed with 111Py12N4 and alkaline-earth as well as first-row transition-metal ions have been determined by potentiometric method [20] but this could not be applied to lanthanide ions since the kinetics of complexation are slow. Stability constants could be determined by spectrophotometry using a competition reaction between ligand and Arsenaso(III) [21] but limitations of this technique have been already discussed [22]. Proton relaxation rates [10,23] and laser-excited Eu(III) luminescence spectroscopy [22] have been used for the determination of stability constant of Gd(III) and Eu(III) chelates, respectively, but the drawback of these methods is that they require the use of sophisticated analytical apparatus.

In this work, we set out to simplify the latter technique by using UV-light induced Eu(III) luminescence resulting from a through-space energy transfer. The complexation of the Eu(III) salt with ligand 111Py12N4, represented as L^{3-}, leads to an ion-exchange process with the protons of the ligand (Eq. (1)).

$$[Eu^{3+}] + [L^{3-}] \rightleftarrows [EuL] \tag{1}$$

The equilibrium constant for Eq. (1) can be expressed as follows:

$$K_{\text{therm}}(Eu^{3+}) = \frac{[EuL]}{[Eu^{3+}][L^{3-}]} \tag{2}$$

Depending on its intrinsic basicity, each ligand has a different response to the proton competition that limits the metal ion binding leading to the conditional stability constants (Eq. (3)):

$$K_{\text{cond}}(Eu^{3+})$$
$$= \frac{[EuL]}{[Eu^{3+}]\{[L^{3-}][HL^{2-}][H_2L^-][H_3L][H_4L^+]\}} \tag{3}$$

The thermodynamics and conditional stability constants are related by Eq. (4).

$$K_{\text{cond}}(Eu^{3+}) = K_{\text{therm}}(Eu^{3+})\alpha_H \tag{4}$$

with

$$\alpha_H^{-1} = 1 + K_{a1}[H^+] + K_{a1}K[H^+]^2 + K_{a1}K_{a2}K_{a3}[H^+]^3$$
$$+ K_{a1}K_{a2}K_{a3}K_{a4}[H^+]^4 \tag{5}$$

where K_{ai} are the successive protonation constants from the fully deprotonated ligand which were obtained from potentiometric titration [20].

In order to provide constant excitation energy to the system, the set of samples previously studied by UV-spectroscopy was irradiated at their isosbestic point (264 nm, Fig. 1). Consequently, the luminescence intensity (I) is proportional to the concentration of the Eu(III) complex [EuL] and may be expressed as a function of the pH and of the stability constant of the complex K_{therm} by Eq. (6)

$$I = \frac{I_{\max}}{2L_0} \left\{ [Eu]_0 + [L]_0 + \frac{\alpha_H^{-1}}{K_{\text{therm}}} \right.$$
$$\left. - \left[\left([Eu]_0 + [L]_0 + \frac{\alpha_H^{-1}}{K_{\text{therm}}} \right)^2 - 4[Eu]_0[L]_0 \right]^{1/2} \right\} \tag{6}$$

with $[Eu]_0$ and $[L]_0$ stand for the initial concentrations of Eu(III) and ligand, respectively, and $I_{\max}$ is the luminescence intensity for a total complexation of the ligand.

For all the samples, the emission spectrums are homothetic with the ration I_{593}/I_{615} constant. The Eu(III) luminescence lifetimes was measured for all solution. The luminescence intensity decreases according to mono exponential kinetics (Fig. 4). Thus, under the conditions of our experiments, only 1:1 complexes will be present and we were able to measure the conditional formation constant [24].

By plotting the luminescence intensity I, measured at 615 nm, as a function of the pH, a binding curve is obtained (Fig. 5).
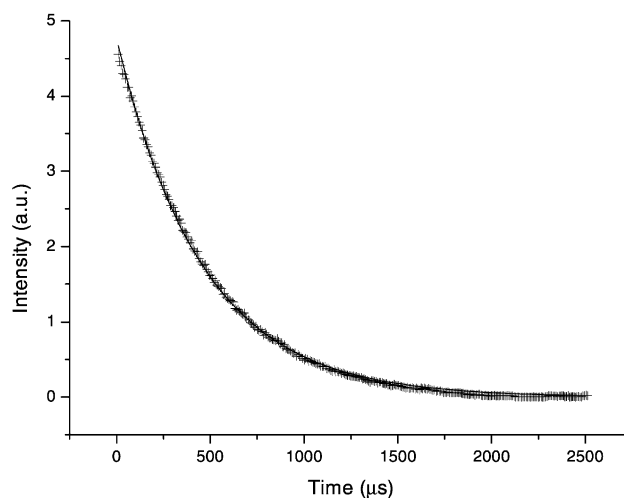

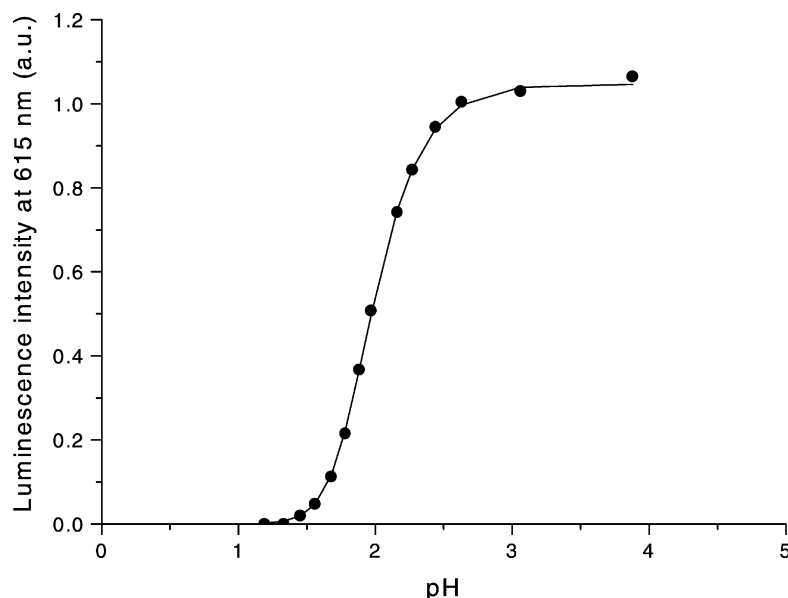Fig. 4. Excited-state luminescence decay for the system containing 111Py12N4 (50 mM) and EuCl$_3$ (55 µM) at pH 3.18.

Fig. 5. Binding curve obtained by monitoring the luminescence intensity I at 615 nm of samples containing ligand 111Py12N4 (50 mM) and EuCl$_3$ (55–60 µM) as the function of pH. The luminescence resulted from a UV-light excitation at 264 nm. For experimental conditions see Fig. 1. The filled circles represent actual data points and the solid line represents the theoretical fit of data with log K_{therm} = 20.9.

The fitting (least square method) of the theoretical curve obtained from Eq. (6) to the experimental data (Fig. 4) leads to a Log K_{therm} value of 20.9 for Eu(III) complex formed with ligand 111Py12N4.

4. Conclusions

In conclusion, we report here the characterization of the unique mononuclear Eu(III) complex formed with the antenna ligand 111Py12N4 in aqueous medium by sensitized Eu(III) luminescence methodology. Indeed, by mixing Eu(III) salt and 111Py12N4 ligand in aqueous medium, a complex is formed in situ and displays the europium characteristic sharply-spiked luminescent emission spectrum with a excited-state lifetime of 0.37 ms. The 1:1 stoichiometry of the complex was determined by a mole ratio method using UV-light induced Eu(III) luminescence and the stability constant of the [Eu ⊂ 111Py12N4] chelate (log K_{therm} = 20.9) was evaluated by plotting the luminescence intensity as a function of the pH. The successful outcome of this preliminary work encouraged us to embark on a program aimed at characterizing other Eu(III) complexes by sensitized Eu(III) luminescence. Indeed, such a study could be of interest to determine the structural requirements of ligands to produce stable and/or lanthanide selective complexes.

Acknowledgements

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