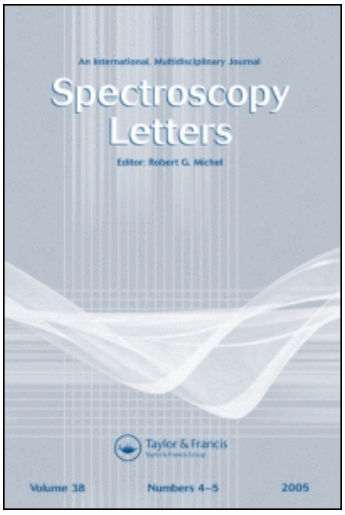

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

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Online publication date: 14 September 2004

To cite this Article Chauvin, Anne-Sophie , Gumy, Frédéric , Imbert, Daniel and Bünzli, Jean-Claude G.(2004) 'Europium and Terbium *tris*(Dipicolinates) as Secondary Standards for Quantum Yield Determination', Spectroscopy Letters, 37: 5, 517 — 532
To link to this Article: DOI: 10.1081/SL-120039700
URL: http://dx.doi.org/10.1081/SL-120039700

SPECTROSCOPY LETTERS
Vol. 37, No. 5, pp. 517–532, 2004

Europium and Terbium *tris*(Dipicolinates) as Secondary Standards for Quantum Yield Determination

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ABSTRACT

Aqueous solutions of europium(III) and terbium(III) *tris*(dipicolinates), around physiological pH are shown to be convenient secondary standards in the determination of quantum yields of lanthanide complexes containing these ions. Conditions for which a strict linearity is observed between the concentration of the solutions and the emission intensity are established. The speciation in these solutions, which contain a non-negligible amount of bis species is presented and discussed on the basis of both stability constants and lifetime determinations of the Eu($^5D_0$) level. The quantum yield Q_L^{Eu} displays a strong pH-dependence: for a solution with an absorbance of 0.30, it increases sharply from about 2% at pH

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DOI: 10.1081/SL-120039700 0038-7010 (Print); 1532-2289 (Online)

2.5 to reach 11.5–12.5% in the pH range of 6–9. The proposed standard for Eu^{III} is a solution of $Cs_3[Eu(dpa)_3]$ 7.5×10^{-5} M in tris buffer 0.1 M (absorbance = 0.20) with a quantum yield of 13.5% ± 1.5% under excitation at 279 nm. For Tb^{III}, we propose a standard solution of $Cs_3[Tb(dpa)_3]$ 6.5×10^{-5} M in tris buffer 0.1 M (absorbance = 0.18) with a quantum yield of 26.5% ± 2% under excitation at 279 nm. Despite the speciation between bis and tris complexes, these two standard buffered solutions present a constant quantum yield within a reasonably large range of concentration and they are easy to handle, which makes them adequate for laboratory use.

Key Words: Europium; Terbium; Luminescence; Quantum yield; Lifetime; Secondary standard.

INTRODUCTION

Luminescent lanthanide-containing chelates have unusual spectroscopic characteristics, such as narrow, easy-to-recognize emission bands in the visible and near infrared, as well as long-lived excited states, which make them attractive as alternative probes to organic fluorophores[1,2] in biomedical analyses.[3] As a consequence, they are being increasingly used as responsive analytical systems[4] or diagnostic tools,[2] for instance in fluoroimmunoassays,[5] for enhanced imaging of cancer[6] or in color-tailored fluorophores for simultaneous detection of multiple targets on DNA strands.[7]

These developments require the determination of quantum yield of the metal-centered luminescence upon ligand excitation. Indeed, due to the very weak oscillator strength of the Laporte forbidden f–f transitions ($\varepsilon < 1\,M^{-1}\,cm^{-1}$), the lanthanide ions have to be excited by energy transfer from chromophores attached to them, the so-called process of sensitization (or antenna effect). The overall efficiency Φ_L^{Ln} of a luminescent lanthanide-containing label is usually given as follows:

$$\Phi_L^{Ln} = \varepsilon_L(\lambda_{exc}) \times Q_L^{Ln} = \varepsilon_L(\lambda_{exc}) \times \eta_{isc} \times \eta_{et} \times Q_{Ln}^{Ln} \quad (1)$$

where $\varepsilon(\lambda_{exc})$ is the molar absorption coefficient at the excitation wavelength and Q_L^{Ln} is the quantum yield of the metal-centered luminescence upon ligand excitation. The latter quantity is the product of three terms: (i) η_{isc}, the efficiency of the intersystem crossing from the singlet to the triplet state of the ligand, which often acts as the main energy feeder for the long-lived excited states of the lanthanide ions,[8] (ii) η_{et}, the efficacy of the $^3\pi\pi^*$–Ln transfer, and (iii) Q_{Ln}^{Ln}, the intrinsic quantum yield of the lanthanide ion, upon direct excitation.

There are several known experimental procedures to determine the quantum yield of the lanthanide-containing solution. An "absolute" method is based on the dipolar scattering of monochromatic light from a series of glycogen solutions taken as standards with unit quantum yield.[9] A more commonly encountered method uses organic dyes or metal complexes[10] with known quantum yields, to which both the absorbance and the emission intensity of the sample are compared according to:

$$Q_{\text{rel}}^{\text{Eu,L}} = \frac{Q_x}{Q_r} = \frac{E_x}{E_r} \times \frac{A_r(\lambda_r)}{A_x(\lambda_x)} \times \frac{I_r(\lambda_r)}{I_x(\lambda_x)} \times \frac{n_x^2}{n_r^2} \tag{2}$$

where subscript r stands for the reference and x for the sample; E is the integrated luminescence intensity, A is the absorbance at the excitation wavelength, I is the intensity of the excitation light at the same wavelength, and n is the refractive index of the solution. A quantum yield measurement is not easy to perform for several reasons. First, when measuring in the usual right angle geometry, all the light emitted by the sample is not collected, but only a small portion of it, defined by the solid angle under which the detector sees the sample. Therefore, care has to be exercised so that the experiment geometry is exactly the same for the sample and for the reference. A better approach is to measure the entire emitted light by means of an integration sphere, but few authors perform this type of measurements. Also, when operating in photon counting mode, the spectrofluorimeter may not yield a linear response if the signal is strong, and saturation occurs as soon as the signal is larger than $5 \times 10^5 - 10^6$ counts per second (cps). More important, the linear relationship between the intensity of the emitted light and the concentration of the solution holds only if the absorbance A of the solution is smaller than 0.05.[11] Measuring solutions having a larger absorbance leads to an inner-filter effect, i.e., to re-absorbtion of the emitted light by the sample. Moreover, if $A(\lambda_x) \neq A(\lambda_r)$ the excitation light will be more absorbed by the solution having the largest value of A and less molecules will be excited. Finally, luminescence measurements are single-beam experiments; commercial instruments come with a correction function, but the latter is not always reliable at short wavelengths. To avoid most of these problems, it is strongly recommended that the same excitation wavelength be used for both the sample and the reference, as well as the sample and the reference solutions having the same absorbance. In this way, solutions with absorbance up to 0.5 can be measured without inducing errors and Eq. (2) reduces to:

$$Q_{\text{rel}}^{\text{Eu,L}} = \frac{Q_x}{Q_r} = \frac{E_x}{E_r} \times \frac{n_x^2}{n_r^2} \tag{3}$$

Or, if both the sample and the reference are diluted solutions in the same solvent, i.e., with the same refractive index:

$$Q_{\text{rel}}^{\text{Eu,L}} = \frac{Q_{\text{x}}}{Q_{\text{r}}} = \frac{E_{\text{x}}}{E_{\text{r}}} \tag{4}$$

If we focus on Q^{Ln} measurements, two other methods are available. One takes advantage of resonance energy transfer to accurately measure the quantum yield and the radiative and non-radiative decay rates. A lanthanide chelate is mixed with an acceptor of known quantum yield and the efficiency of energy transfer between them is calculated from both the lifetime and intensity measurements.[7] The last method is only valid for Eu^{III} and relies on the fact that the intensity of the purely magnetic dipole $^5D_0 \rightarrow {}^7F_1$ transition is independent of the chemical environment of the ion and that the radiative lifetime τ_{r} can be calculated from:[12]

$$k_{\text{r}} = \frac{1}{\tau_{\text{r}}} = A_{\text{MD},0} n^3 \left(\frac{I_{\text{tot}}}{I_{\text{MD}}} \right) \tag{5}$$

where $A_{\text{MD},0} = 14.65\,\text{sec}^{-1}$ is the spontaneous emission probability of the $^5D_0 \rightarrow {}^7F_1$ transition, n is the refractive index of the medium, and $I_{\text{tot}}/I_{\text{MD}}$ is the ratio of the integrated total emission from the $^5D_0 \rightarrow {}^7F_J$ transitions ($J = 0$–6) to the area of the Eu($^5D_0 \rightarrow {}^7F_1$) transition. The quantum yield is then simply equal to the ratio of the observed lifetime to the radiative lifetime:

$$Q_{\text{Eu}}^{\text{Eu}} = \frac{\tau_{\text{obs}}}{\tau_{\text{r}}} \tag{6}$$

Since Eqs. (5) and (6) imply several hypotheses, this procedure is not entirely reliable, although it is very convenient for solid state samples. Moreover, while relatively small, the contributions of the $^5D_0 \rightarrow {}^7F_{5,6}$ transitions must be taken into account in I_{tot}, which is not always the case in the reported literature.

Many lanthanide-containing probes make use of Eu^{III} and Tb^{III} luminescence and their quantum yield is usually determined via the "conventional" method [Eqs. (2)–(4)] by using organic dyes, such as 9,10-phenylanthracene ($Q_{\text{F}} = 93\% \pm 3\%$),[13] quinine sulfate (in 1 N H_2SO_4, $Q_{\text{F}} = 54.6\%$),[14] cresyl violet perchlorate ($Q_{\text{F}} = 54\% \pm 3\%$ in methanol),[15] rhodamine 101 ($Q_{\text{F}} = 100\% \pm 2\%$ in ethanol),[15] for instance. However, these dyes emit very broad bands, whereas Ln^{III} ion emission is quite narrow and this may create accuracy problems while comparing the two sets of band areas. Therefore, a lanthanide-containing reference sample is better suited. Some authors have been using solutions of the *tris*(terpyridine) complexes [Ln(terpy)$_3$] $(ClO_4)_3$ (Ln = Eu, Tb) 10^{-3} M in anhydrous acetonitrile, the quantum yields

of which have been determined with respect to $[Ru(bpy)_3]Cl_2$ (bpy = bipyridine, $Q_F = 2.8\%$ in air-saturated water[16]): 1.3% and 4.7%, for Eu^{III} [17] and Tb^{III},[18] respectively. However, the terpyridine complexes are very sensitive to moisture and, besides, their stability constants are not very large,[19] preventing dilutions below 1 mM: for instance, at the latter concentration, the percentage of the Eu^{III} tris complex is 90.5% and it goes down to 72.9% for a 0.1 mM concentration.

In order to avoid these problems, we have decided to investigate whether the dipicolinate complexes could replace these standards. Indeed, dipicolinate (2,6-pyridine-dicarboxylate, dpa) reacts with the lanthanide ions in water to yield nine-coordinate tris complexes (Fig. 1)[20] having appreciable stability[21] and which are quite luminescent, with reported quantum yields of 12% and 21% for Eu^{III} and Tb^{III}, respectively.[22]

EXPERIMENTAL

The complexes $Cs_3[Ln(dpa)_3]$ (Ln = Eu, Tb) were synthesized according to Brayshaw et al.[20] The tris solutions 0.1 M in bi-distilled water were

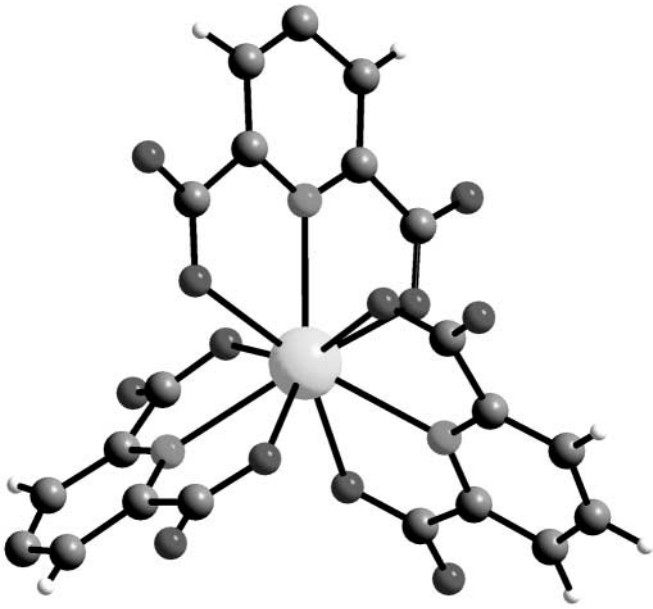

Figure 1. Structure of $Cs_3[Eu(dpa)_3]$, redrawn from Ref.[20]. (*View this art in color at www.dekker.com.*)

obtained by dilution of 1 M Sigma T 2663 solution. Solutions in D_2O (99.9%, Aldrich) were prepared under inert atmosphere in a glove-box and transferred into measuring cells by Schlenk techniques. Absorption spectra were measured on a Perkin Elmer Lambda 900 spectrometer with quartz Suprasil® cells (115F-QS) with a path-length of 0.2 cm. Luminescence spectra were measured with a Fluorolog 3 spectrometer from Jobin Yvon Horiba using the same cells, measuring the emitted light at right angle and along the long (1 cm) path length. Typical excitation and emission spectra are reported in Fig. 2. The excitation wavelength was set to 279 nm and the bandpass to 2.5 and 2 nm for Eu and Tb, respectively. The emission bandpass was set to 1 nm, the integration time to 0.1 ms, and a 370-nm filter was inserted after the sample. Quantum yields were determined with respect to two different standards. (i) Solutions of cresyl violet perchlorate (Fluka) in methanol (Spectra Pure quality, from Aldrich) in the concentration range of 4×10^{-8}–2×10^{-6} M (9 solutions, absorbance: 0.05–0.39, $Q_F = 54\% \pm 3\%$,[15] refractive index = 1.329). (ii) Rhodamine 101 (Fluka) in ethanol (Spectra Pure quality, from Fluka) in the concentration range of 3×10^{-7}–6×10^{-6} M (6 solutions, absorbance: 0.06–0.34, $Q_F = 100\% \pm 2\%$,[15] refractive index = 1.361). The refractive index of the sample solutions was 1.330. The emission intensities proved to be insensitive to degassing the solution with N_2 so that most of the measurements have been performed on non-degassed solutions. Plots of the integrated emission intensity vs. absorbance showed that linearity is achieved only in the

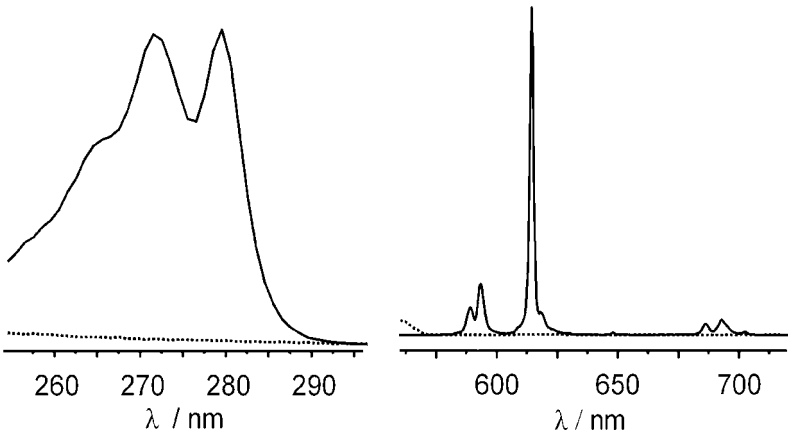

Figure 2. Excitation (left) and emission (right) spectra (—) of a solution of $[Eu(dpa)_3]^{3-}$ 3.7×10^{-5} M in water (pH 7.45, room temperature). Vertical scale: arbitrary units. The spectra of the blank are also shown (····).

absorbance range 0–0.1 for the organic dyes, while it extends at least up to 0.5 for the lanthanide complexes.

Lifetime measurements were also determined on the Fluorolog 3 spectrometer, with a 2% attenuator in order to keep the signal intensity under 5×10^5 cps, otherwise distortions occur in the calculated lifetime.

Since our luminescence measurements were recorded under experimental conditions different from those used by Grenthe,[21] we have re-determined the stability constants for Eu^{III} under our conditions, by spectrophotometric titration of dpa (Aldrich, 9.82×10^{-5} M) by $Eu(ClO_4)_3 \times 4.5\ H_2O$ 4.3×10^{-3} M in tris buffer 0.1 M (pH 7.45). Data were analyzed by Specfit[23] and factor analysis pointed to four absorbing species. Spectra could be fitted to the following stability constants (Grenthe's values are given within parentheses): $\log \beta_1 = 8.7 \pm 0.3$ (8.84 ± 0.01), $\log \beta_2 = 16.8 \pm 0.3$ (15.98 ± 0.02), and $\log \beta_3 = 22.4 \pm 0.3$ (21.49 ± 0.02). Although the calculated speciation for our solutions does not vary much between the two sets of constants, we have used our data for Eu^{III} in the tables below and Grenthe's one for Tb^{III}. The Ln content of the stock solutions was determined by complexometric titration with Titriplex III (Merck) in the presence of urotropine and xylenol orange.[24]

RESULTS AND DISCUSSION

Speciation of the Eu^{III} Solutions

We have resorted to lifetime measurements of the $Eu(^5D_0)$ level to check the speciation in our solutions. In the concentration range investigated (5.6×10^{-6}–1.9×10^{-4} M, 20 solutions), the 1 : 1 species represents always less than 0.1% of the total Eu^{III} and we have disregarded it. On the other hand, the proportion of the 1 : 2 species is in the range 11–50%, so that the luminescence decay is bi-exponential and was analyzed according to:

$$I(t) = A \cdot e^{-k_1 t} + B \cdot e^{-k_2 t} + C \tag{7}$$

Lifetimes of 1.67 ± 0.03 ($k_1 = 599\ sec^{-1}$) and 0.32–0.38 ms ($k_2 = 3125$–$2632\ sec^{-1}$) were obtained for the 1 : 3 and 1 : 2 species, respectively, which compares well with the literature data, 1.64 and 0.30–0.32 ms.[25,26] Some data are reported in Table 1 showing a good agreement between the experimentally determined speciation and the one calculated with the program HySS®[27] from our stability constants. In view of the large difference between the lifetimes of the two species and the experimental errors, data become unreliable for concentrations lower than 5×10^{-5} M. The speciation is further corroborated by the average number q of water molecules

Table 1. Speciation of some $[Eu(dpa)_3]^{3-}$ solutions as determined from lifetime measurements and as calculated from the stability constants.

			$[Eu(dpa)_2]^-$ (%)		$[Eu(dpa)_3]^{3-}$ (%)	
$[Eu^{3+}]_t$ (M)	A[a]	B[a]	b	c	b	c
1.90×10^{-4}	29,137	2,410	10.2	11.1	89.8	88.9
1.32×10^{-4}	21,585	1,887	10.7	13.1	89.3	86.9
8.28×10^{-5}	14,371	1,664	13.7	16.3	86.3	83.7

Notes: [a]Coefficients determined from Eq. (7), with $k_1 = 599\,\text{sec}^{-1}$ and $k_2 = 3{,}125\,\text{sec}^{-1}$. b, from the lifetime measurements, using a corrective factor of 0.73 for A $[=\varepsilon([Eu(dpa)_2]^-)/\varepsilon([Eu(dpa)_3]^{3-}) = 9{,}800/13{,}400$ at 279 nm]; c, from

bound to Eu^{III}, as calculated from Eq. (8) and from mean lifetimes (single exponential analysis) determined both in water and deuterated water:[28]

$$q = 1.05 \cdot \Delta k_{obs} \quad \text{with } \Delta k_{obs} = k_{obs}(H_2O) - k_{obs}(D_2O) \tag{8}$$

In the concentration range $1\text{–}1.7 \times 10^{-4}$ M, $\tau_{obs}(H_2O) = 1.56 \pm 0.02$ ms and $\tau_{obs}(D_2O) = 3.0 \pm 0.1$ ms, leading to $q = 0.32 \pm 0.02$. Since one dpa^{2-} ligand replaces three water molecules in the inner coordination sphere of Eu^{III}, this corresponds to $11\% \pm 1\%$ of $[Eu(dpa)_2]^-$, which compares reasonably well with the percentages calculated from the stability constants (14.6–11.1%).

Quantum Yield of the $[Eu(dpa)_3]^{3-}$ Solutions

First, the quantum yield Q_{dpa}^{Eu} of a solution 3.06×10^{-5} M (Eu1) has been determined from Eq. (2) with respect to two organic standards, cresyl violet perchlorate (CV) and rhodamine 101 (RH). Care has been exercised that both the sample and the reference solutions had almost the same absorbance. The excitation wavelength was set to 279 nm and the refractive index correction (n_x^2/n_r^2) amounted to 1.001 for CV and to 0.955 for RH. The calculated values of the Eu1 quantum yield were $Q_{dpa}^{Eu} = 12.4\% \pm 2\%$ and $12.5\% \pm 2\%$, with respect to CV and RH. Then, 19 solutions have been investigated in the concentration range of $5.6 \times 10^{-6}\text{–}1.9 \times 10^{-4}$ M and their quantum yield were determined with respect to Eu1. Results, along with the speciation of the solutions, are reported in Table 2. As expected, the quantum yield increases with increasing concentration of the solutions

Table 2. Quantum yields of $[Eu(dpa)_3]^{3-}$ solutions in water at pH 7.45 (Tris buffer 0.1 M) and 295 K, vs. the concentration of the solutions.

Nr.	A	$[Eu]_t$ (M)	$[Eu(dpa)_2]^-$ (%)	$[Eu(dpa)_3]^{3-}$ (%)	Q^{Eu}_{dpa} (%)
1	0.510	1.90×10^{-4}	11.1	88.9	9.5
2	0.448	1.67×10^{-4}	12.1	88.0	10.0
3	0.390	1.46×10^{-4}	13.2	86.9	10.7
4	0.355	1.32×10^{-4}	13.1	86.9	11.0
5	0.325	1.21×10^{-4}	13.6	86.4	11.1
6	0.295	1.10×10^{-4}	14.6	85.4	11.5
7	0.243	9.07×10^{-5}	16.0	84.0	13.0
8	0.222	8.28×10^{-5}	16.3	83.7	13.6
9	0.198	7.39×10^{-5}	17.4	82.7	13.5
10	0.182	6.79×10^{-5}	18.0	82.0	13.8
11	0.155	5.78×10^{-5}	19.1	80.9	14.5
12	0.143	5.34×10^{-5}	20.4	79.6	14.7
13	0.133	4.96×10^{-5}	20.8	79.2	13.3
14	0.115	4.29×10^{-5}	22.0	78.0	13.4
15	0.105	3.92×10^{-5}	22.8	77.2	14.0
16	0.099	3.69×10^{-5}	23.5	76.5	12.9
17	0.090	3.36×10^{-5}	24.6	75.4	12.7
18 (Eu1)	0.082	3.06×10^{-5}	25.8	74.2	12.5
19	0.026	9.70×10^{-6}	40.9	59.1	11.8
20	0.015	5.60×10^{-6}	49.7	50.3	10.3

(Fig. 3), since the proportion of the tris species becomes larger. Indeed, three water molecules are bound in the inner coordination sphere of the metal ion in the bis complex, which necessarily leads to a smaller quantum yield. The maximum is reached around $[Eu]_t = 5–6 \times 10^{-5}$ M, but the quantum yield is fairly constant in the range $4–8 \times 10^{-5}$ M. For concentrations larger than 8×10^{-5} M, Q^{Eu}_{dpa}(obs) decreases regularly down to 9.5%, probably because of inner-filter effects.

Finally, Fig. 4 displays the quantum yield dependence vs. pH for a solution 1.32×10^{-4} M. The observed variation is quite important, since at pH 2.5, the quantum yield is as low as 2.3%, a consequence of the competition between Eu^{3+} and H_3O^+ for the carboxylate di-anions. The pK_as of the dipicolinic acid are 2.16 and 4.76[29] or 2.03 and 4.49,[30] which means that it is totally dissociated at pH larger than 6.5. As a result, the speciation is constant in the pH range 6.5–9.5 and so is the measured quantum yield. Therefore, the choice of the Tris buffer yielding a pH of 7.45 close to the physiological pH for the solutions reported in Table 2 is quite adequate.

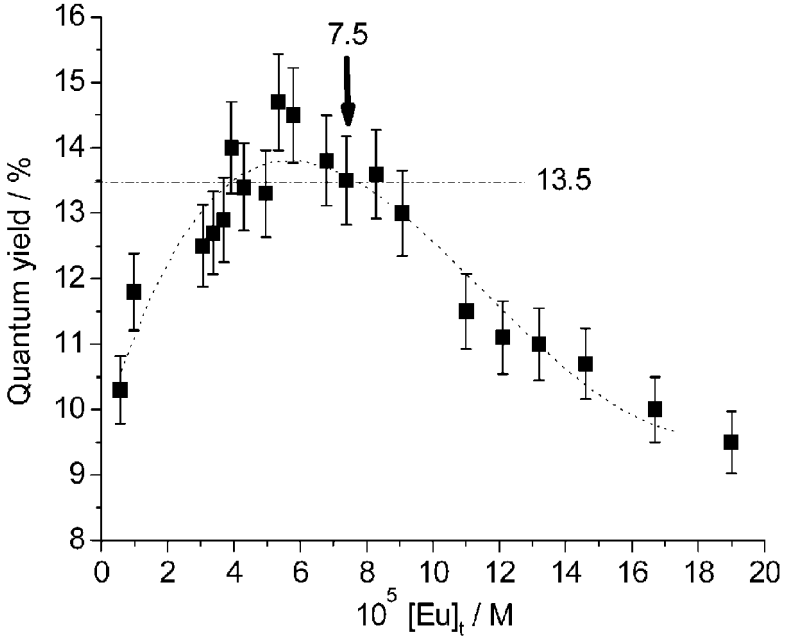

Figure 3. Quantum yield of $[Eu(dpa)_3]^{3-}$ solutions in water at pH 7.45 (tris buffer) vs. the total concentration in europium. The arrow points to the proposed standard solution.

Quantum Yield of the $[Tb(dpa)_3]^{3-}$ Solutions

Similarly to the study of the europium complex, the quantum yield Q_{dpa}^{Tb} of a solution 2.96×10^{-5} M (Tb1) has been determined first from Eq. (2) with respect to the two organic standards. The calculated values of the Tb1 quantum yield were $Q_{dpa}^{Tb} = 24.3\% \pm 2\%$ and $24.4\% \pm 2\%$, with respect to CV and RH. Then, 10 more solutions of the Tb^{III} complex in the concentration range 1.6×10^{-4}–3×10^{-5} M, both in water and in deuterated water, have been investigated and their quantum yield determined with respect to Tb1; data are reported in Table 3 and Fig. 5. As for Eu^{III}, the quantum yield first increases with concentration, when more of the better luminescent tris species is formed; it becomes relatively constant in the range of 4–8×10^{-5} M and finally decreases because of inner-filter effect. These variations are, however, less pronounced than for Eu^{III}, although the proportion of the 1 : 2 species is approximately the same. The quantum yield is approximately twice as large as Eu^{III}, a consequence of the larger energy gap $Tb(^5D_4 \rightarrow {}^7F_0)$ compared with $Eu(^5D_0 \rightarrow {}^7F_6)$. One also notes that the

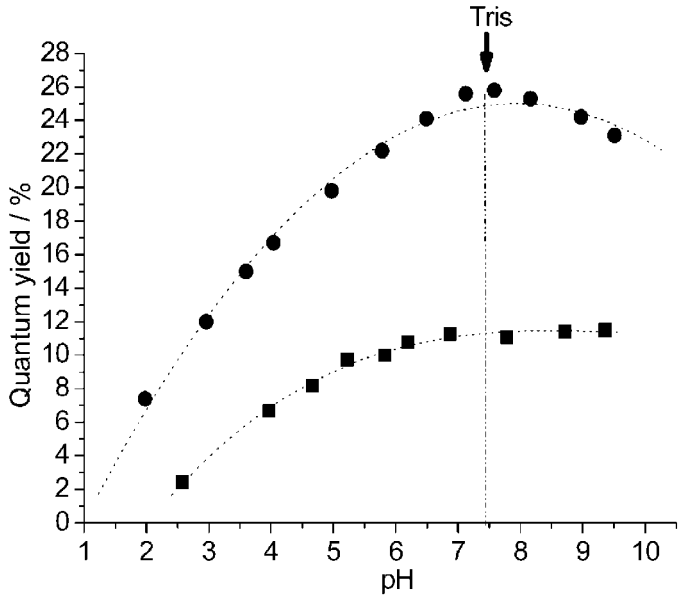

Figure 4. pH dependence of the quantum yield of a solution of $[Eu(dpa)_3]^{3-}$ 1.32×10^{-4} M (■) and $[Tb(dpa)_3]^{3-}$ 6.65×10^{-5} M (●) in water, at room temperature.

quantum yield determined in deuterated water is the same as in water, within experimental errors, pointing, on average, to almost no water molecule coordinated in the inner coordination sphere of the metal ion. This is in line with a small proportion of the 1 : 2 species (9%–12%) in the concentration range investigated (8×10^{-5}–1.5×10^{-4} M). The pH dependence of Q^{Tb}_{dpa}(obs) is also similar to the one shown for Eu^{III}, with stable values in the pH range 6.5–11 (Fig. 4).

CONCLUSION

We have demonstrated that buffered solutions of europium and terbium *tris*(dipicolinate) in water are stable, easy to handle, and can be used as secondary standards for quantum yield determination of the metal-centered luminescence of Eu^{III} and Tb^{III}. Since the pH provided by the tris buffer (7.45), which interacts weakly with the Ln^{III} ions (cf. $\log K = 2.44 \pm 0.07$ for Eu^{III})[31] is close to the physiological pH, the proposed secondary stan-

Table 3. Quantum yields of $[Tb(dpa)_3]^{3-}$ solutions in water and deuterated water at pH 7.45 (tris buffer 0.1 M) and 295 K, vs. the concentration of the solutions.

Nr	A	$[Tb]_t$ (M)	$[Tb(dpa)_2]^-$ (%)	$[Tb(dpa)_3]^{3-}$ (%)	Q^{Tb}_{dpa} (H_2O) (%)	Q^{Tb}_{dpa} (D_2O) (%)
1	0.448	1.62×10^{-4}	9	91	19.4	18.6
2	0.390	1.41×10^{-4}	9	91	20.7	20.1
3	0.355	1.29×10^{-4}	9	91	21.3	19.6
4	0.325	1.18×10^{-4}	9	91	21.5	20.0
5	0.295	1.07×10^{-4}	10	90	22.3	20.1
6	0.220	8.03×10^{-5}	12	88	26.3	26.0
7	0.182	6.58×10^{-5}	12	88	26.7	24.9
8	0.155	5.60×10^{-5}	13	87	28.1	25.6
9	0.105	3.79×10^{-5}	14	86	27.0	24.8
10	0.099	3.60×10^{-5}	17	83	25.0	23.4
11 (Tb1)	0.082	2.96×10^{-5}	18	82	24.3	18.5

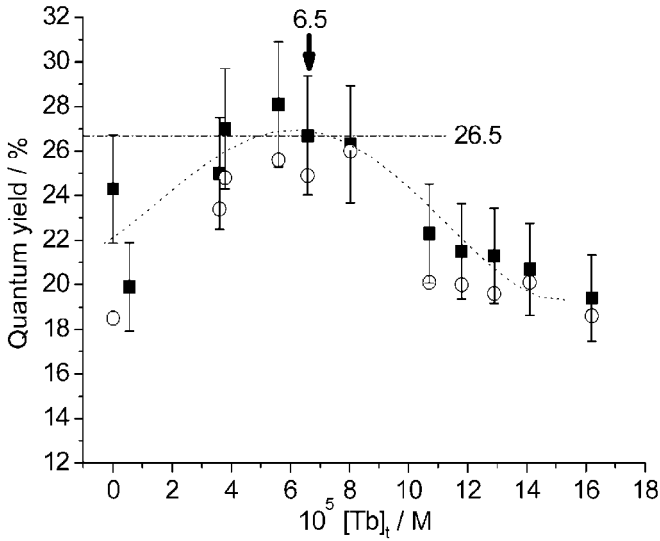

Figure 5. Quantum yield of $[Tb(dpa)_3]^{3-}$ solutions in water at pH 7.45 (■) and deuterated water (○) vs. the total concentration in terbium. The arrow points to the proposed standard solution.

dards are therefore quite adequate for testing Eu^{III} and Tb^{III} chelates developed for biomedical applications.

The proposed standards are solutions of (i) $Cs_3[Eu(dpa)_3]$ 7.5×10^{-5} M in tris buffer 0.1 M (absorbance = 0.20) with the quantum yield of 13.5% ± 1.5% under excitation at 279 nm, and (ii) a solution of $Cs_3[Tb(dpa)_3]$ 6.5×10^{-5} M in Tris buffer 0.1 M (absorbance 0.18) with the quantum yield of 26.5% ± 2% under excitation at 279 nm. The given uncertainties are rather conservative but we think they reflect the difficulty of this type of measurements. For solution Eu1 (see above), we have taken into account the integrated intensity of all the $^5D_0 \rightarrow {}^7F_J$ ($J = 0-6$) transitions. However, the contribution of the $^5D_0 \rightarrow {}^7F_{5,6}$ transitions to the total emission is less than 0.5% so that in practice it can be indeed neglected, as is often the case in the reported literature. It is noteworthy that the use of buffered solutions eliminate any variation related to pH and that the solutions being easy to prepare and to handle thus they represent valuable standards, despite the quantum yield dependence upon concentration due to the speciation between bis and tris complexes.

The present work is now being extended to the other luminescent lanthanide ions, including those emitting in the near infrared.

ACKNOWLEDGMENT

This work is supported by grants from the Swiss National Science Foundation.

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Received February 21, 2004
Accepted April 19, 2004