Effects of Silver Island Films on the Luminescent Intensity and Decay Times of Lanthanide Chelates

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Abstract

We examined the emission intensity and decay times of chelates Tb^{3+} and Eu^{3+} in micron thick samples between films of sub-wavelength size silver particles. We observed modest increases in emission intensities for the complexes between the silver particles as compared to between unsilvered quartz plates. The intensity decay times were dramatically decreased by the silver particles, which was in part mediated by diffusion toward the silver particles. These results indicated that luminescent lanthanides in close proximity to silver particles display increased rates of radiative decay. The use of luminophore-metallic surface interactions provides new opportunities for creation of luminescent probes with novel spectral properties.

Keywords

Luminescence; lanthanides; metal-enhanced fluorescence; silver islands; colloids

INTRODUCTION

Fluorescence detection and spectroscopy have become a dominant methodology in biotechnology. A vast array of fluorescence assays are available, including immunoassays, blood chemistry, intracellular physiology, and genetic analysis. In all these applications of fluorescence the fluorophores or luminophores are examined in the free-space condition. The free-space condition means that the fluorophore can be modeled as an oscillating dipole radiating energy into a transparent and mostly homogeneous medium. Although fluorescent probes can be highly sensitive to local polarity, quenching, or energy transfer, these interactions modify the non-radiative decay pathways and not the free-space spectral properties.

In several recent publications we have considered the possible advantages of using fluorophores in close proximity to conducting metallic surfaces [1-3]. Interactions of the excited fluorophore with the metallic surface (metal) alters the free-space conditions.

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Remarkably, the favorable effects can include increased quantum yields, increased photostability, and decreased lifetimes. A decreased lifetime is a unique feature of fluorophore-metal interactions, and when accompanied by an increased quantum yield, implies an increased radiative decay rate of the fluorophore. Under free-space conditions it is not possible to substantially alter the rate of radiative decay.

In the present report we extend our studies of fluorophore-metal interactions to include the luminescent lanthanides Tb^{3+} and Eu^{3+}. The lanthanides are widely used in high-sensitivity immunoassays [4-5] and in DNA hybridization assays [6-7]. The lanthanides display decay times on the ms timescale, much longer than the ns timescale of most autofluorescence. As a result, lanthanides are used with gated detection to reject sample autofluorescence. Several studies have examined the effects of silver particles on the intensities and lifetimes of lanthanides. Two reports found increases in the intensities of Eu^{3+} with no change in lifetime [8-9]. An earlier report found a 5-fold increase in intensity of a Eu^{3+} chelate near silver island films and a 1000-fold decrease in lifetime [10]. Such an effect can only be explained by an increase in the radiative decay rate. In fact, it was this report [10] that stimulated us to study the effect of metal particles on fluorescence.

In the present report we describe the intensities and intensity decays of Eu^{3+} and Tb^{3+} chelates between silver island films. Silver island films are sub-wavelength size silver particles on an inert or dielectric substrate. Given the ms decay times of the lanthanides and the approximately 1-micron-thick samples, we expected diffusion of the chelate toward the films to affect the lifetime.

MATERIALS AND METHODS

The lanthanide chelate Cs-DTPA (Scheme I) was synthesized according to published procedures [11]. Silver island films were prepared by reduction of silver ions by glucose in the presence of clean quartz slides [2-3]. The particles were 400–500 Å in diameter, covering about 25% of the surface, and displaying the expected plasmon resonance absorption. Although it is not possible to precisely control the size of the particles, the silver island films can be prepared consistently with similar optical properties. The size of the particles can be judged by the surface plasmon absorption displayed by the films (Scheme II). This absorption is due to electron oscillation in the particles as a result of the incident light, which is the same phenomenon that results in the colors of metallic colloids. The samples were placed between two silver island surfaces. The sample thickness was near 1 μ (Scheme II, bottom).

In our measurements we used 50-μM solutions of lanthanide chelate (Cs-DTPA-Tb or Cs-DTPA-Eu) in D_2O or glycerol. For preparation of the polyvinyl alcohol (PVA) films we used 50-μM solution of lanthanide chelate in 0.2% PVA (2 mg PVA (MW = 13,000–23,000, Aldrich) in 1 mL water).

Emission spectra and intensity decays were recorded using a Varian Eclipse spectrofluorometer with an excitation wavelength of 328 nm for Cs-DTPA-Tb and 338 nm for Cs-DTPA-Eu. Emission spectra were collected with a 100 μs delay following the

excitation pulse. Time-resolved decays were measured using an emission wavelength of 547 nm for Cs-DTPA-Tb and 615 nm for Cs-DTPA-Eu with either no delay or a 20 µs time delay.

The intensity decays were fit to a multiexponential decay law

$$I(t) = \sum_i \alpha_i \exp\left(-t / \tau_i\right)$$

(1)

where α_i are the time-zero amplitudes of the components with decay times τ_i. The mean lifetime of the excited state is given by:

$$\bar{\tau} = \frac{\sum \alpha_i \tau_i^2}{\sum \alpha_i}$$

(2)

The amplitude weighted lifetime is given by:

$$\langle \tau \rangle = \frac{\sum \alpha_i \tau_i}{\sum \alpha_i}$$

(3)

The fractional steady state intensity of each decay time component is given by:

$$f_i = \frac{\alpha_i \tau_i}{\sum \alpha_i \tau_i}$$

(4)

RESULTS

Figure 1 shows emission spectra of the Tb^{3+} complex in deuterated water, recorded in a cuvette, between unsilvered quartz plates or between silver island films (top to bottom). The smoothly decreasing emission from 450 to 650 nm is thought to be due to the ligand. At this time we do not know why the ligand emission is higher between quartz plates and silver films than observed in a cuvette. This effect may be related to the decreased lifetime observed between the quartz plates (below). The ligand emission could be removed by off-gating the detector until 100 µs after the excitation flash (Fig. 2). However, as shown below, this gating distorts the amplitudes because of the different decay times between quartz plates and silver island films. The ungated spectra (Fig. 1) shows that Cs-DTPA displays approximated two-fold higher intensity between silver island films than between unsilvered quartz plates.

We examined the intensity decays of Cs-DTPA-Tb (Figure 3). There is a small decrease in the decay time between unsilvered quartz plates (1.5 ms) compared to the sample in a 1 cm × 1 cm cuvette (2.1 ms) (Table I). This time is adequate for the chelate to diffuse toward the quartz surface and sense the higher refractive index of quartz. Using the classic result of Strickler and Berg [12] the decay time of a 2.1-ms of the cuvette sample is expected to decrease to 1.77 ms because of the higher refractive index of quartz. The intensity decay

between the quartz plates remained moderately homogeneous in agreement with diffusive averaging of the emission during the excited state lifetime.

A dramatic increase in the decay rate was observed for Cs-DTPA-Tb between the silver island films (Fig. 3). The more rapid intensity decay is due mostly to components with decay times of 13 and 210 μs (see Table I). We believe these shorter decay times are due to complexes that are in close proximity to the silver islands or diffuse to the silver islands during the excited state lifetime. The presence of a residue 1.3-ms decay time component suggests that some of the complexes remain distant from the silver islands during their lifetimes.

To evaluate the contribution of diffusive component to the rapid decay of Cs-DTPA-Tb, we examined a glycerol solution of this complex between quartz slides and silver island films (Fig. 4). In this case the decrease in decay time was modest, confirming that diffusion toward the islands contributed to a shortening of the decay times. In an attempt to completely eliminate diffusion this complex was placed in a polyvinyl alcohol (PVA) film about 0.5 μ thick (Fig. 5). In this case we observed a 60% increase in intensity and a modest 30% decrease in lifetime. It is probable that a good fraction of the chelates are close to the metal surfaces because of the thinner of the PVA film.

To test the generality of our results, similar experiments were performed using Cs-DTPA-Eu, which in free solution between quartz slides displays a decay time of about 0.55 ms. Between silver island films we again observed increased intensity and a decreased lifetime (Fig. 6). The magnitude of the lifetime decreases was less than for Cs-DTPA-Tb, consistent with the shorter free-space lifetime of Cs-DTPA-Eu and less diffusion to the silver islands. When using glycerol as the solvent there was essentially no effect of the silver island films (Fig. 7). A decreased lifetime was observed for the Eu complex in a PVA film (Fig. 8), which may be due to the thinner sample and proximity to the islands.

DISCUSSION

Several reports have examined lanthanide decays near silver particles. For Eu^{3+} in a silica sol gel containing silver particles, the intensities increased but the lifetimes were unchanged [8-9]. In the case of an Eu^{3+} complex dip coated onto silver island films, Weitz *et al.* [10] observed a 5-fold increase in intensity and a 1000-fold decrease in lifetime. These authors suggest these effects were due to an increased rate of radiative decay near the silver particles. Our results are in quantitative agreement with these results from Weitz et al. [10].

It is of interest to calculate the increase in the radiative decay rate that is needed to explain our results. The free-space quantum yield of a fluorophore is given by:

$$Q = \frac{\Gamma}{\Gamma + k_{nr}}$$

(5)

where Γ is the radiative decay rate and k_{nr} is the non-radiative decay rate. The free-space lifetime is given by:

$$\tau = \frac{1}{\Gamma + k_{nr}} \tag{6}$$

Suppose the silver particles change Γ by a factor n and do not affect k_{nr}. The quantum yield and lifetime are then given by:

$$Q = \frac{n\Gamma}{n\Gamma + k_{nr}} \tag{7}$$

$$\tau = \frac{1}{n\Gamma + k_{nr}} \tag{8}$$

Using decay times of 1.5 ms and 0.56 m for Cs-DTPA-Tb in free space and between silver island films, and assuming a quantum yield of unity, one can readily calculate that Γ is increased by about 3-fold ($n = 2.7$) by the silver particles. However, if we assume that the short lifetime component $\tau_1 = 0.013$ ms is due to complexes near the silver particles, then the radiative rate of this population is increased by over 100-fold. Such large effects appear to be consistent with theoretical predictions for fluorophores near small silver particles [13]. The effect on the quantum yield of the lanthanide is modest because the free-space quantum yield is already high.

Although we also observed a modest increase in fluorescence intensity, the effects of silver particles can be explained only by an effect on the radiative rate. The observed effects cannot be due only to a field enhancement effect because this would not change the lifetimes. The absence of a large enhanced excitation field is consistent with the nature of our samples, in which complexes are excited throughout the sample volume and then diffused to the silver particles where the free-space conditions are altered. It is not clear why a field enhancement was not observed by Weitz *et al.* [10] because their sample was dry thus restricting diffusion. In any event an effect on the radiative rate, without an increase in the excitation rate or the quenching rate, is a favorable result. This implies that it should be possible to dramatically modify the quantum yield and lifetimes of fluorophores using metallic particles.

It is interesting to speculate on the potential application of lanthanide-metallic surface interactions. Lanthanide-based immunoassays usually contain washing steps to remove the probes that are not bound to the surface. If the surface were coated with silver particles the surface-bound fraction could be resolved from its shorter lifetime. Another possibility is to use the increased rate of emission to improve detectablity. Because of their long lifetimes the lanthandie can emit about 1000 photons per second at saturating light levels. A 100-fold decrease in lifetime would allow the lanthanides to undergo 10^5 excitation-emission cycles per second. The use of these effects may allow design of a new class of metal particle-fluorophore components with exceptional spectral properties.

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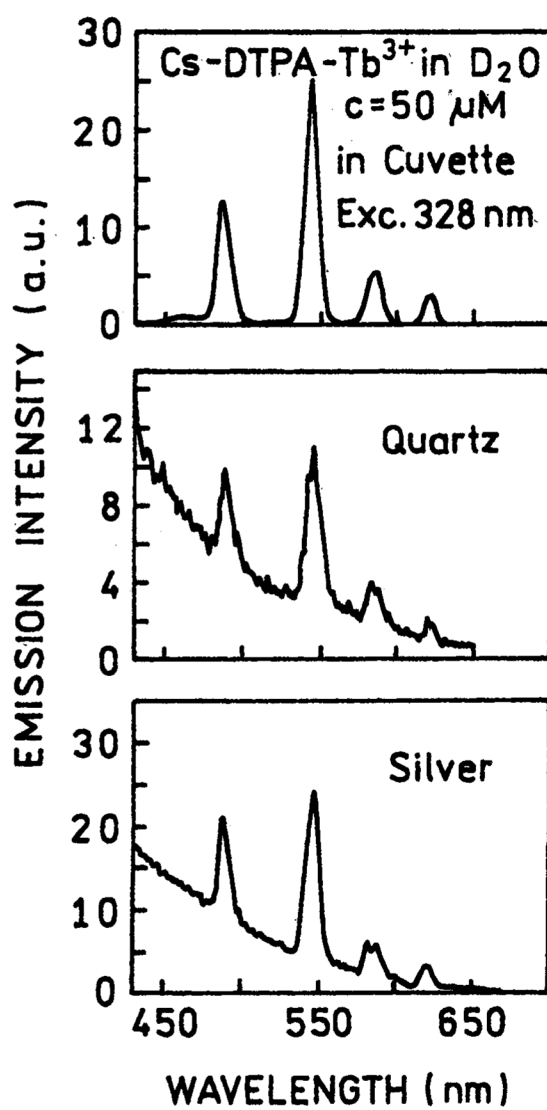
Fig. 1.
Emission spectra of Cs-DTPA-Tb^{3+}, in a cuvette (top), between quartz plates without silver (middle) and between quartz plates with silver (bottom).
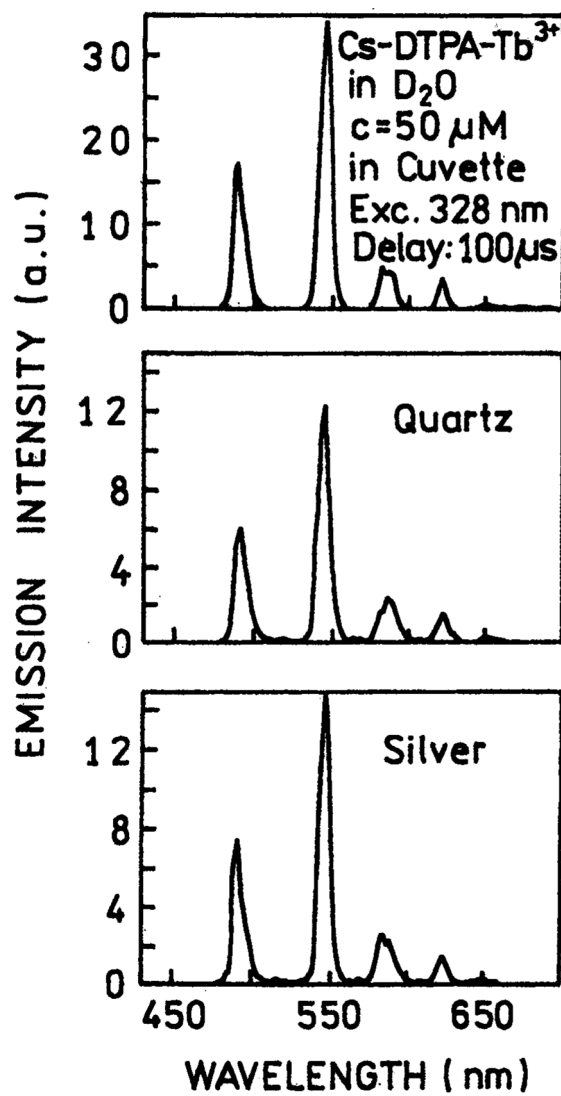
Fig. 2.
Phosphorescence spectra of Cs-DTPA-Tb^{3+} in a cuvette (top), without silver (middle) and with silver (bottom). The delay time was 100 μs.
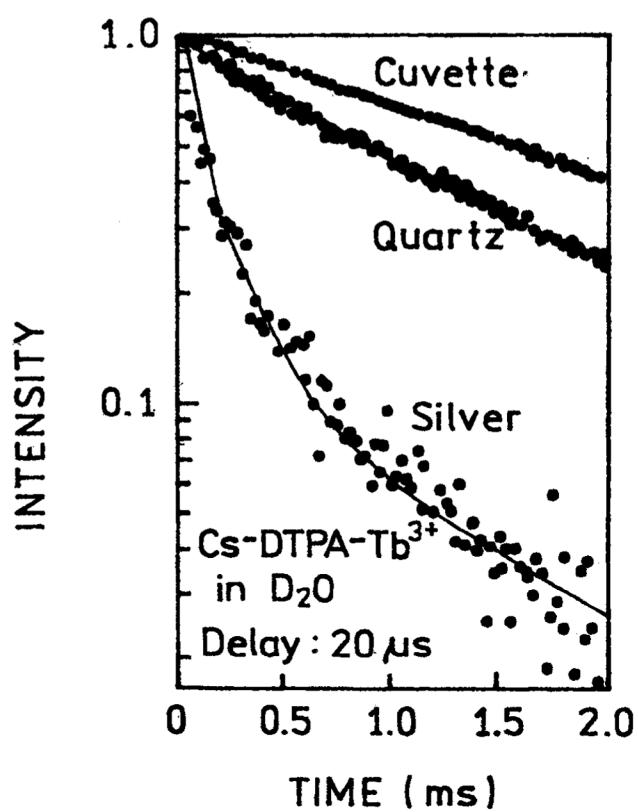
Fig. 3.
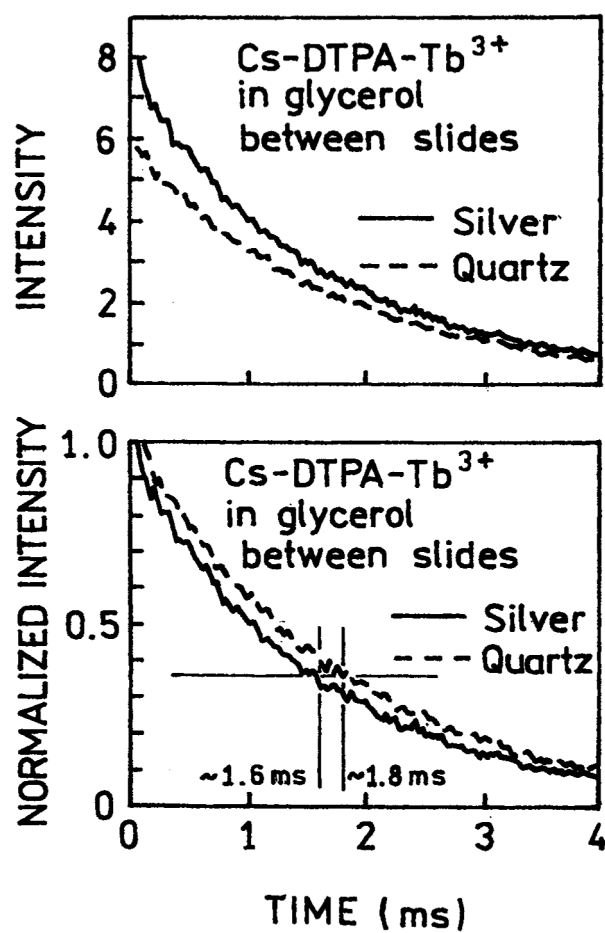Cs-DTPA-Tb^{3+} emission intensity decays.

Fig. 4.
Intensity decays of Cs-DTPA-Tb^{3+} in glycerol between quartz plates with (——) and without silver (- - - -). There is only a modest change in intensity decay time.

Fig. 5.
Emission spectra (top) and intensity decays (bottom) of Cs-DTPA-Tb^{3+} in PVA spin-coated on quartz plate with (——) without silver (- - - -).
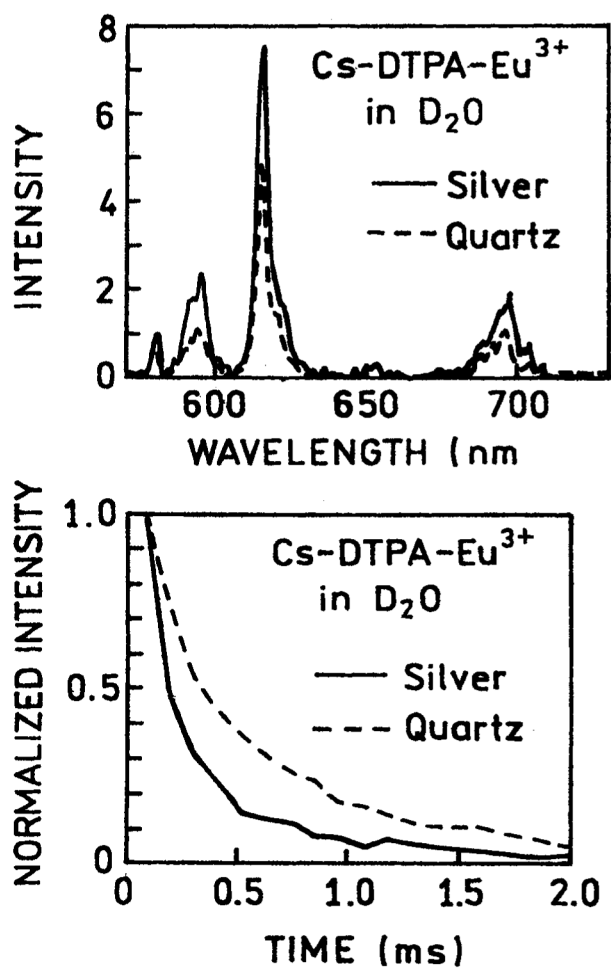
Fig. 6.
Emission spectra (top) and intensity decays (bottom) of Cs-DTPA-Eu^{3+} in D$_2$O between slides without (- - - -) and with (——) silver island film.
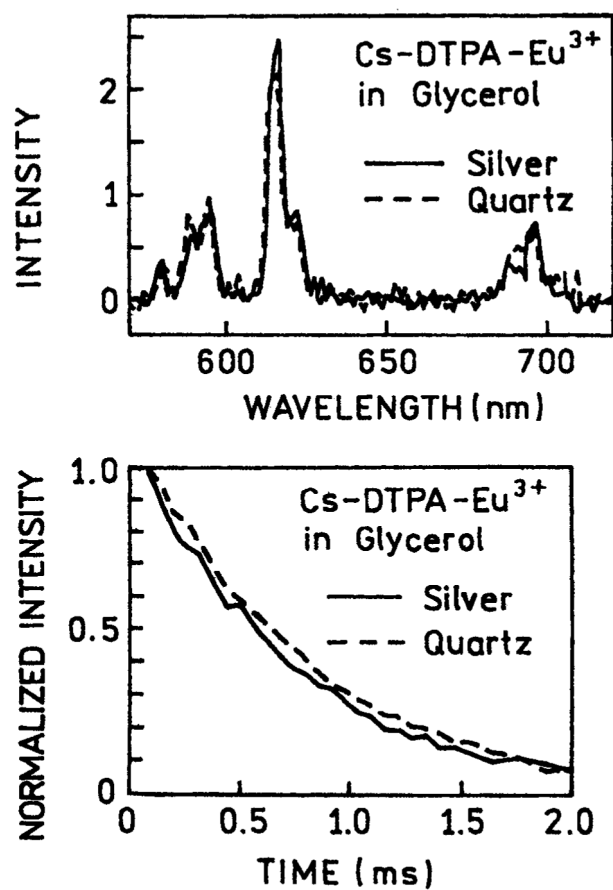
Fig. 7.
Emission spectra (top) and intensity decay (bottom) of Cs-DTPA-Eu^{3+} in glycerol between slides without (- - - -) and with (——) silver island film.
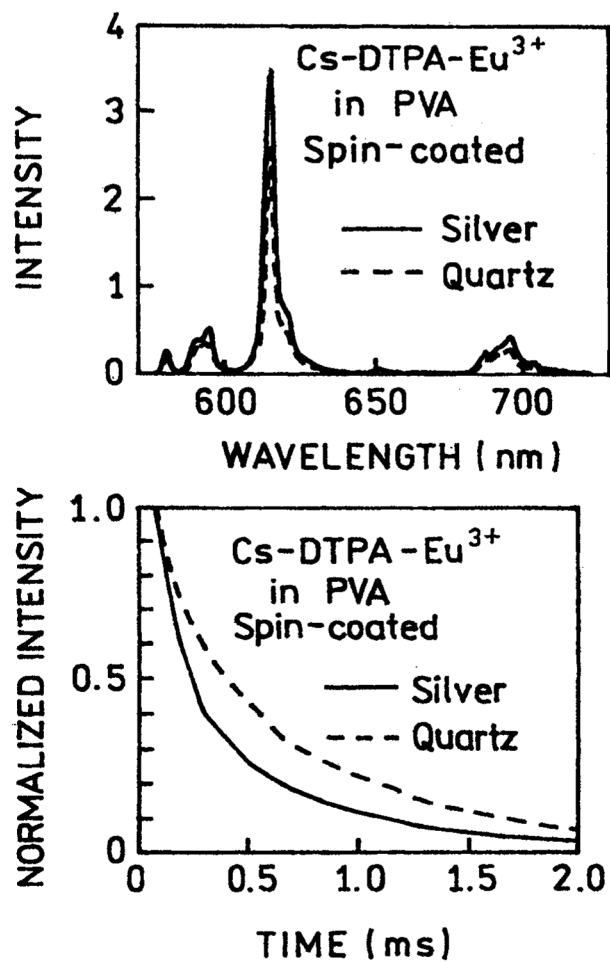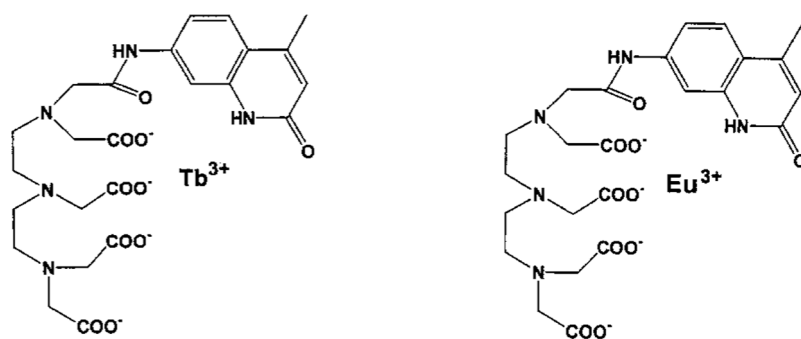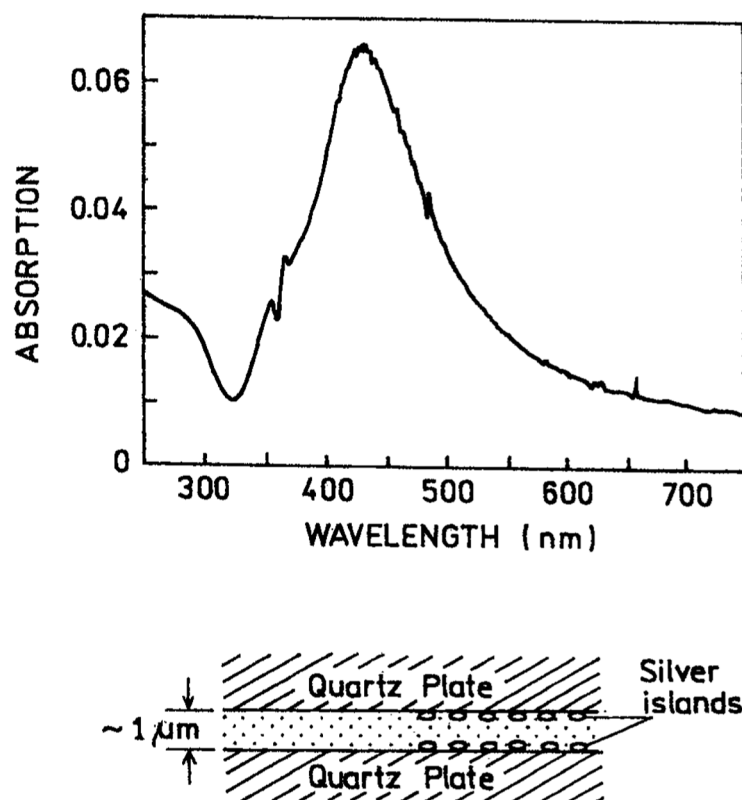
Fig. 8.
Emission spectra (top) and intensity decays (bottom) of Cs-DTPA-Eu^{3+} in PVA spin-coated on slide without (- - - -) and with (——) silver island film.

Scheme I.
Structures of the Cs-DTPA chelates of Tb^{3+} and Eu^{3+}.

Scheme II.
Adsorption of a single quartz slide coated with silver island film (top) experimental arrangement of two slides half coated with silver island films.

Table I.

Multiexponential Analysis of Emission Intensity Decays of Cs-DTPA-Tb^{3+} in D$_2$O

Conditions	$\bar{\tau}$ (ms)	$\langle \tau \rangle$ (ms)	α_1	τ_1 (ms)	α_2	τ_2 (ms)	α_3	τ_3 (ms)
Cuvette	2.155	2.155	1.0	2.15	-	-	-	-
Quartz	1.504	1.335	0.135	0.134	0.865	1.522	-	-
Silver	0.562	0.037	0.938	0.013	0.051	0.210	0.011	1.312