# Luminescence Quenching of Eu(III) Carboxylates by Cu(II) in a Composite Polymer Xerogel Film<sup> $\dagger$ </sup>

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# ABSTRACT

Three Eu(III) luminescent compounds were separately entrapped in a xerogel porous silica matrix and finely ground particles of it were deposited on a glass support with polyvinylacetate (PVAc) as a binder to build a thin film sensor. These 3 devices were immersed in aqueous solutions of Cu(II) and the content of this metal was evaluated by emissionquenching experiments. The sensor containing the highly luminescent antenna chelate of diethylenetriaminepentaacetic acid (dtpa) sensitized with Coumarin120 rendered the largest Stern-Volmer constant ( $K_{SV} = 1.49 \times 10^4 M^{-1}$ ), showing no leaching of the Eu(III) complex to the aqueous solution and a reproducible value of the luminescence ratio between water and Cu(II) solution. The in situ sensor we developed can measure the concentration of Cu(II) in aqueous media down to the ppm level by emission-quenching experiments. This methodology permits a simple calibration of the sensor and an easy to use reusable device.

## INTRODUCTION

Optical sensors are attractive measuring and control devices because their designs allow *in situ* monitoring and remote sensing. Luminescence detection of fiber optics–coated sensing layers is a very adequate approach for low perturbation and fast measurement of analytes (1–5).

Lanthanide ions display a well-defined luminescence characterized by narrow and highly structured emission bands with lifetimes

- **?1** of milliseconds (6–8). The excited state long lifetime is not quenched by O<sub>2</sub>, which makes the detection possible in non-degassed media. The presence of multiple transitions enables to use
- **?2** a large difference between excitation and emission wavelengths. This possibility, together with the narrow band emission, favors correction for disperse light and broad background emission.

Lanthanide complexes are valuable alternative probes to conventional dyes because the emission is observed in the green-red region of the spectrum, in which few compounds in natural waters and biological systems emit light. Undoubtedly, lanthanide's luminescent properties make these compounds especially profitable for the design of luminescent labels (9–12) and sensors (13–14). In this later application, luminescence of Eu(III) complexes were reported as pH dependent (13) or as selective anion-binding systems (14) in aqueous solution. One of the main difficulties in the use of luminescent lanthanide complexes is their low absorption coefficient. This drawback can be overcome by including in the coordination sphere of the metal an organic chromophores with a large absorption cross-section and with suitable energy-transfer characteristics to populate the ion-centered emitting states (15–17).

Transition metal ions are known to be efficient quenchers of the emission of lanthanide complexes. Cu(II) ions act much more efficiently than any other fourth-period transition-metal cations (11,18–20). Electronic energy transfer is the quenching mechanism postulated to operate in these cases (10–12,20–22).

The build up of a sensor needs a suitable immobilization matrix for the indicator. This matrix must be reversibly permeable to the analyte while retaining the sensitive material. The sol-gel technique, based mainly on the hydrolysis and condensation of organometallic alcoxides in alcoholic solution, has been widely used to immobilize organic or inorganic molecules in a porous glass at low temperature (23). The porous and amorphous glasses thus obtained have good optical properties, are chemically inert and are hydrophilic, which makes them especially suitable for the fabrication of optical sensors. In particular, Eu(III) complexes and chelates have also been incorporated into silica matrixes to build optical sensors of pH (13,24), anions (14) and cations (25). In a recent study, Cu(II) was measured by the diffuse reflectance spectra of thin films of polyisobutylene-bound finely ground samples of a silica sol-gel matrix in which a complexing cyanine was entrapped (26).

In this article, we evaluate 3 Eu(III) complexes as potential luminescent probes to sense Cu(II) ions by emission quenching. On the basis of these results, we developed a sensor to measure the content of Cu(II) in aqueous media by using the emission quenching of a highly luminescent antenna-sensitized chelate of Eu(III). The immobilization matrix is produced by the sol-gel method to build a new *in situ* reusable Cu(II) sensor. Quenching experiments permit a simple calibration of the sensor and an easy to use reusable device. These luminescence-quenching experiments proved to be suitable to detect Cu(II) at concentration levels less

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*Abbreviations:* dtpa, diethylenetriaminepentaacetic acid; H<sub>2</sub>DPA, 2,6pyridinedicarboxylic acid; H<sub>2</sub>oda, oxydiacetic acid; PVAc: polyvinylacetate; TMOS, tetramethoxysilane.

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than ppm in aqueous solution, a range in which this metal ion is present as pollutant in the environment (27).

## MATERIALS AND METHODS

Chemicals. Copper (II) chloride-dihydrate (compound 1) was obtained from 23 Mallinckrodt, nickel (II) nitrate hexahydrate (compound 2) was obtained from Merck and cobalt (II) chloride hexahydrate (compound 3) was obtained from Baker. All compounds were analytical grade and were used without further purification. Eu(NO<sub>3</sub>)<sub>3</sub>·6H<sub>2</sub>O (99%) was obtained from Fluka Chemie AG and used as received. Water was from a Milli-Q system. DMF was from Merck and distilled and kept in molecular sieves before use. Pyridine and ethanol were from Mallinckrodt. Dichloromethane was distilled and stored in molecular sieves.

Lanthanide compounds. Na<sub>3</sub>[Eu(oda)<sub>3</sub>] • 2NaClO<sub>4</sub> • 6H<sub>2</sub>O (1) and Na<sub>3</sub>[Eu(DPA)<sub>3</sub>] • 15H<sub>2</sub>O (2) were synthesized according to methods described elsewhere (28,29), where H2oda (HOOC-CH2-O-CH2-COOH) and H2DPA are oxydiacetic and 2,6-pyridinedicarboxylic acids, respectively (Scheme 1).

Synthesis of the Eu(dtpaCoumarin120) 3 complex. This complex is closely related to a series of antenna-sensitized compounds synthesized by Selvin et al. (15-17). A total of 0.074 mmoles of dtpa (diethylenetriaminepentaacetic acid, Aldrich) and 0.097 mmoles of Coumarin120 (7-amino-4-methylcoumarin, 99% from Aldrich) were dissolved separately in 1000 and 200  $\mu L$ , respectively, of DMF. Both solutions were mixed and 0.324 mmoles of DCC (N-N'dicyclohexylcarbodiimide, Sigma) in 0.078 g of pyridine were added. The reaction mixture was stirred for 48 h at room temperature during which time a white solid (1,3-dicyclohexylurea) separated. After discarding the solid by filtration, 27.3 mg (0.061 mmoles) of  $Eu(NO_3)_3 \cdot 6H_2O$  were added to the solution. Finally, the orange solution that was obtained was evaporated in a vacuum and the final red solid

- 24 product was washed with acetone and ethanol. The solid was further washed with a mixture of butanol/toluene (3:2) to separate free Coumarin120, followed by exposure to UV light in SiO<sub>2</sub>/TLC plates.
- The product was characterized by FTIR and emission spectroscopy, as follows: IR: 3423 cm<sup>-1</sup>, 1539 cm<sup>-1</sup> amide I stretching and deformation 25 modes, respectively. 1700 cm<sup>-1</sup> and 1614.5 cm<sup>-1</sup>, stretchings of carbonyl and carboxylate of the amide and dtpa moieties, respectively, 1384.3 cm<sup>-</sup> methyl bending of Coumarin120 moiety. Emission ( $\lambda_{exc} = 322$  nm): 577, 592, 615, 650 and 698 nm for  ${}^{5}D_{0} \rightarrow {}^{7}F_{J}$  bands for J = 0, 1, 2, 3 and 4. The emission spectrum is specified in Fig. 1.

Steady-state emission spectra and quenching measurements in aqueous solution. The steady-state emission spectra for all compounds were

?6 recorded on a PTI QuantaMaster QM-1 luminescence spectrometer. Sam-



Figure 1. Steady-state emission and excitation spectra of compound 3 in water (solid lines,  $\lambda_{exc}$ = 340 nm and  $\lambda_{em}$ = 615 nm, respectively) and of Coumarin120 in ethanol (dashed lines,  $\lambda_{exc}$ = 350 nm and  $\lambda_{em}$ = 420 nm, respectively).

ples were placed in a 1 cm square quartz fluorescence cuvette and measured in right-angle geometry. Excitation wavelengths were 394 nm, 322 and/or 532 nm. For quenching experiments, the emission wavelength was 615 nm. Excitation and emission bandwidths were set to 8 and 4 nm, respectively, for routine experiments, although in some cases emission spectra were obtained with a higher resolution (down to 0.5 nm in emission). The ?7 concentration of the lanthanide complexes in aqueous solution was ranged from 1 to 10 mM for compounds 1 and 2 and 0.025 mM for compound 3. All quenching experiments were performed in air-equilibrated solutions.

Luminescence lifetimes. To measure the lanthanide center emission, samples were excited at 354 nm with a frequency-tripled or frequencydoubled Nd:YAG laser (Spectron), which delivered pulses of 8 ns FWHM at 10 Hz and of 30 mJ at 354 nm. Samples of compounds 1-3 in H<sub>2</sub>O were placed in a 1 cm square quartz fluorescence cuvette and measured in right angle geometry. Emitted light passed through a monochromator and was detected at 615 nm with 4 nm bandwidth. Light was measured with a R928 Hamamatsu photomultiplier and the transient signal was processed by a HP54502 digital oscilloscope and stored in a PC. The traces were fitted to a single exponential decay. The goodness of the fit was judged by a homogeneous time-distribution of residuals. The time decay of the Coumarin120-originated emission was measured in a PTI Timemaster fluorometer. The sample was excited with a 19 kHz H2-filled discharge lamp (FWHM, 1.5 ns) provided with a UG11 Schott filter (2 mm thick). Emission was detected at 390 nm with a monochromator and a strobe PMT. Decays were deconvoluted by the software provided with the equipment.

Preparation of a sensor layer. Sol-gel samples were prepared by hydrolysis of TMOS (tetramethoxysilane, Aldrich) in acid-catalyzed ethanolic medium (TMOS:EtOH:H<sub>2</sub>O = 1:4:4 in moles) at a pH of 3 (with HCl). A total of 2.5 mL of anhydrous ethanol containing 0.3 mg of the Eu(III) chelate 3 was added to 2 mL of TMOS while stirring. HCl was added and a transparent, colorless sol was obtained and left to age at room temperature in a sealed tube. After the sol gellified (5 days), it was placed in an oven at 45°C for 24 h and a transparent colorless monolith was obtained. Two similar monoliths were prepared with compounds 2 and 1, with masses of 40 mg and 35 mg, respectively.

Films were also prepared under base-catalyzed conditions by hydrolysis ?8 of TMOS in ammonia ethanolic medium at a pH of 9.0 (with a 1:4 ratio of TMOS to H<sub>2</sub>O), containing 5 g SiO<sub>2</sub>/100 mL solution (30) and 0.3 mg of compound 3.

The monoliths containing the Eu(III) complexes obtained by the sol-gel method were ground manually to a fine white powder. A total of 0.14 g of ?9 PVAc (polyvinylacetate [M = 86 000], Aldrich) was dissolved in 2.5 mL of CH<sub>2</sub>Cl<sub>2</sub>. A total of 45 mg of the ground monolith of compound 3 was suspended in 500 µL of the PVAc solution. The resulting suspension was stirred for at least 30 min and portions of 50-100 µL were placed on a microscope glass slide (surface area,  $9 \times 20$  mm) and left to dry first at

room temperature and then at 40°C for approximately 1 h. Once the sample was dry, a white spot was obtained on the glass. This spot (approximately 10 µm thick) contained the luminescent complex entrapped in the porous silica particles, which were immersed in a PVAc matrix. The spot firmly attached to the surface of the glass support was used as the sensor. Except when indicated, all layers were allowed to equilibrate with distilled water overnight before measurement.

Luminescence measurements with the sensors. The sensors were fixed firmly to a diagonal slit carved in the inside of a Teflon cap of a 1 cm square quartz fluorescence cuvette in such a way that the sensor was placed in a front-face geometry inside the cuvette containing the test Cu(II) aqueous solution. The luminescent response was measured *in situ*. The sensors were always immersed in the solutions and kept in the spectrofluorimeter in the same position. A 2 mm diameter hole in the cap enables the addition and replacement of the quencher solution while keeping the cuvette in place. The samples were stirred during the experiments.

## RESULTS

### Photophysics of compound 3

The emission spectrum of compound 3 in aqueous solution shows the expected Coumarin120-centered emission, as well as the Eu(III)-centered emission when excited in the Coumarin120 absorption band (Fig. 1). The emission band of the Coumarin120 in the complex is blue shifted by approximately 12 nm, compared with the emission in ethanol. The maximum of this emission at 395 nm adequately overlaps the main absorption of Eu(III) at 394 nm for an optimum electronic energy transfer to the metal center. The Eu(III)-centered emission has a band shape that is very similar to that of closely related Eu(III) complexes (16,17). The lifetime for the emission of Coumarin120 in water is 5.1 ns, whereas it is quenched to 1.4 ns in the complex. If we assume that the difference is exclusively due to energy transfer from the sensitizer to the metal center, then the value of  $k_{ET}$  can be calculated as  $(1/1.4 - 1/5.1)^{-1}$  $ns^{-1} = 1.9 \times 10^9 s^{-1}$ . This means that 75% of the electronic excitation energy of the Coumarin120 is transferred to the Eu(III). The emission lifetime of the metal center is 0.44 ms in aqueous solution.

#### Quenching measurements in aqueous solution

Figure 2 shows the steady-state Stern-Volmer plot for the deactivation of the emission of compound 3 by Cu(II) in aqueous solution.

The dynamic rate constant for the quenching of compound 1 by Cu(II) has been reported to have a value of  $3.7 \times 10^7 M^{-1} s^{-1}$  (31). For compound 2, asimultaneous static and dynamic quenching was observed and a dynamic rate constant of  $3.7 \times 10^5 M^{-1} s^{-1}$  was estimated (31).

For compound 3, the K<sub>SV</sub> obtained from the linear Stern-Volmer plot of the quenching by Cu(II) was  $3.36 \times 10^4 M^{-1}$  and the signal was much more intense than expected for an antenna-sensitized chelate. For compound 1, the corresponding value is  $K_{\text{evi}} = k \cdot \tau$ 

**?10** chelate. For compound 1, the corresponding value is  $K_{SV} = k_q \cdot \tau_o = 3.7 \times 10^7 \ M^{-1} s^{-1} \cdot 0.865 \times 10^{-3} \ s = 3.2 \times 10^4 \ M^{-1}$  Lifetime measurements were performed in solution with UV excitation in the Coumarin120 band and alternative detection in the dye and in the metal-centered emission for the aqueous solutions of compound 3 alone ( $\tau_o$ , for an  $I_o/I$  of 1) and with Cu(II) added ( $\tau$ , for an  $I_o/I$  of 3.5). In the metal centered emission ( $\lambda_{exc.} = 354$  nm and  $\lambda_{em} = 615$  nm), the two curves were fitted by a monoexponential decay and the same lifetime was obtained as follows for both samples, within experimental uncertainty:  $\tau_o = \tau = 0.35 \pm 0.05$  ms. The lifetime for the decay of the Coumarin120 emission ( $\lambda_{exc.} = 350$  nm and



Figure 2. Steady-state Stern-Volmer plot for the deactivation of the emission of compound 3 by Cu(II) in aqueous solution ( $\lambda_{exc} = 322$  nm and  $\lambda_{em} = 615$  nm).

 $\lambda_{\rm em} = 390$  nm) were also similar with values of 1.4  $\pm$  0.1 ns. It is worth mentioning that the same lifetime measurements were performed for the Eu(dtpa) chelate without antenna and that the same results were obtained for solutions with or without added Cu(II) ion ( $\tau_o = \tau = 0.20$  ms). On the basis of these observations we confirm that that the quenching process of compound 3 by Cu(II) is almost exclusively static. This type of quenching by Cu(II) was **?11** also reported for some Ru(II) complexes 32; Mellace *et al.*, unpublished).

#### Eu(III)-containing sensors

Attempts to sense Cu(II) ions with compound 2 entrapped in thin films of porous sol-gel silica matrix dip coated on glass slides as supports were unsuccessful. Compound 2 was incorporated into 2 types of sol-gel matrixes: an acid-catalyzed and a base-catalyzed (ammonia) porous silica sol-gel. No quenching of the luminescent signal of the entrapped compound 2 by Cu(II) was detected in the experiments when the acid-catalyzed thin films were immersed in water at neutral or acid conditions by the addition of microliters of HCl. On the contrary, when the films were immersed in aqueous solutions of ammonia (pH = 9), the deactivation of the luminescence of compound 2 was observed but the signal was very weak. For the films obtained by the base-catalyzed synthesis, the optical quality of the films was damaged, and the leaching of compound 2 to the aqueous solution was almost complete after a couple of hours. No further attempts with dip-coated films of other complexes were made.

As a new strategy, composite sensor layers containing compounds 1, 2 and 3 as the luminescent probes in grounded sol-gel monoliths dispersed in PVAc were prepared by the technique described above.

Figure 3 shows the luminescence spectra of dry sensors of compound 1, 2 and 3 measured in air. The excitation wavelengths were 394 nm, 290 nm and 322 nm, respectively. The signals were particularly intense for the sensors of compounds 2 and 3. A baseline correction was performed to the spectrum of compound 1 and all 3 spectra were normalized to their respective maximum for a better comparison. It is interesting to mention that, when sol-gel monoliths of compounds 2 and 3 were synthesized in basic



**Figure 3.** Normalized emission spectra of dry sensors of compound 1 (dotted line), compound 2 (dashed line) and compound 3 (full line) measured in air. The excitation wavelengths were 394 nm, 290 nm and 322 nm, respectively.

medium, the luminescent signals of their dry-sensor films were more intense than those of the corresponding signals in acidcatalyzed gel. However, as already mentioned for the dip-coated films, a significant leaching of these compounds to the aqueous solution was immediately evidenced during the hydration period of the sensors, reducing their luminescent response significantly. No further experiments were performed with base-catalyzed gels. All the results shown in what follows were obtained working exclusively with acid catalyzed samples.

Upon exposure to water, the sensors experienced a decrease in the emission intensity of all 3 complexes but neither the emission lifetime of the Eu(III) nor its emission spectrum changed. The decrease in the signal can be attributed to the swelling of the silica particles. The emission intensity of the sensor layer in contact with water attains a constant value after several hours.

Of all the 3 sensors, the one with compound 1 as the luminescent probe had the lowest signal and it decreases dramatically after hydration (70%). Additionally, the quenching by Cu(II) was 100 times smaller than the value obtained with the same compound in solution.

The results obtained for a sensor with compound 2 as the luminescent probe were remarkably better. After an initial period of hydration, some leaching of the complex was detected in the water by emission spectroscopy. After washing with fresh water, the sensor was rehydrated and the amount of leaching was negligible. The sensor was allowed to equilibrate with water to record the zero-quencher intensity (I<sub>o</sub>, no metal added). Afterwards, aliquots of a 5 m*M* Cu(II) solution were added to the cuvette with the sensor in place by means of a microsyringe. The time dependence of the emission intensity was recorded in the steady statefluorimeter after each addition of Cu(II) and the signals were time fitted to obtain the value of the quenched-emission intensity at long lifetimes.

Figure 4 shows the Stern-Volmer plots for the quenching of the sensor of compound 2 by aqueous solutions containing Cu(II). Contrary to the curved quenching behavior observed for this compound in solution, the plot is now linear with a slope (K<sub>SV</sub>) of  $3.86 \times 10^4 M^{-1}$ .



**Figure 4.** Stern-Volmer plot of the quenching of the sensor of compound 2 by aqueous solutions of Cu(II). The excitation wavelength was 290 nm and the emission wavelength was 615 nm. The insert shows the bands of the emission spectrum of the sensor without addition of Cu(II).

As a final test, the reversibility of the sensor was studied but no successful results were obtained: the luminescent response was not recovered even after repeated washing with water.

When a sensor containing compound 3 as the luminescent probe was evaluated, the results were even more satisfactory than those for compound 2. After a similar period of hydration, the luminescent response was still high (a decrease of approximately 20%, compared with the dry sensor) and no leaching of the Eu(III) chelate was detected by emission spectroscopy in the solution in contact with the sensor even after several hours of immersion. The lifetime of the monoexponential decay of the Eu(III) emission was 0.38 ms in the dry sensor and 0.48 ms in the water equilibrated layer.

The Cu(II) quenching of sensors of compound 3 was characterized by a downward curvature of the Stern-Volmer plots. These results can be rationalized in terms of the existence of the following 2 types of Eu(dtpaCoumarin120) populations in the sensor: a quenchable population characterized by its Stern-Volmer constant K<sub>SV</sub> and an unquenchable population whose luminescence is independent of quencher concentration (33). These 2 populations can be analyzed using a modified form of the Stern-Volmer equation. A plot of I<sub>o</sub>/ $\Delta$ I ( $\Delta$ I = I<sub>o</sub> – I) against 1/[Cu(II)] yields the fraction of quenchable sites, f<sub>a</sub>, from the intercept, f<sub>a</sub><sup>-1</sup>, and the Stern-Volmer constant from the slope, (f<sub>a</sub> • K<sub>SV</sub>)<sup>-1</sup>. The representation of this modified Stern-Volmer equation is shown in Fig. 5 for sensor S<sub>1</sub>.

The effect of aging on the gel was tested for compound 3 to determine its influence on the quenching measurements. Sensors  $S_1$  and  $S_2$  were both obtained using the same transparent silica monolith with the difference that  $S_2$  was prepared 6 months later than  $S_1$ . The sensors were hydrated for several hours before the quenching experiments were performed. Figs. 5 and 6 show the Stern-Volmer plots for the deactivation of the luminescence by Cu(II) of  $S_1$  and  $S_2$ . The values obtained for  $S_1$  and  $S_2$  were as follows:  $K_{SV} = 1.49 \times 10^4 M^{-1}$  and  $2.59 \times 10^3 M^{-1}$ , respectively, and  $f_a = 0.45$  and 0.32, respectively. Therefore, aging increases the proportion of quenchable sites diminishes.



**Figure 5.** Modified Stern-Volmer plot for the deactivation of the emission of a sensor of compound 3 (S<sub>1</sub>) by aqueous solutions of Cu(II). The lower inset shows the experimental data for the normal Stern-Volmer plot and the upper inset shows the spectra of the hydrated sensor for  $I_0/I = 1$  (solid line, no Cu(II) added) and for  $I_0/I = 3$  (dashed line).

The response of the sensor was also tested in water and the results are shown in Fig. 7. When a dry sensor of compound 3 is immersed in water (t = 0), the luminescence signal decreases as a consequence of the hydration process, as already mentioned.

**?13** When 10  $\mu$ L of an aqueous solution with a concentration of 4.6 m*M* in Cu(II) was added to the cuvette containing the sensor immersed in 2 mL of water, the emission intensity decreased by 38% (a) (I<sub>0</sub>/I = 1.6) from its initial value for a final [Cu(II)] of 2.3 × 10<sup>-5</sup> *M* in the aqueous solution. Afterwards, the Cu(II) solution was removed from the cuvette and rinsed twice with water. A volume of 2 mL of water was added to the cuvette and the luminescence signal was measured and recovered >90% of its unquenched level. Another cycle with 10  $\mu$ L (I<sub>0</sub>/I = 2) and 2 more cycles with 5  $\mu$ L of Cu(II) were performed ([Cu(II)] = 1.2.10<sup>-5</sup> *M*, I<sub>0</sub>/I = 0.7) (Fig. 7). After washing with water, the luminescence signal at *t* = 1800 s, 2400 s and 3000 s seemed to be part of a smooth decreasing response of the sensor.

## DISCUSSION

In aqueous solution, compound 1, because of its long excited state lifetime, possesses very high values of Stern-Volmer constants for the quenching by Cu(II). This enables the detection of 0.2 ppm of Cu(II) for a 10% emission deactivation. The high value of the Stern-Volmer constants and the linear plots obtained in all the experiments make compound 1 very simple to work with. A clear disadvantage of this compound is the very low emission signal observed because of the difficulty of the direct excitation of Eu(III) at 394 nm.

Among the compounds we studied, compound 3 seems to be the most suitable ligand to detect Cu(II) by means of quenching

**?14** experiments. In this antenna-sensitized chelate, which has already been studied by Selvin and coworkers (15–17,34), the incoming energy is transferred from the chromophore Coumarin120 to the emissive lanthanide ion very efficiently (while the dtpa ligand minimizes the nonradiative mechanisms) by excluding water in the



**Figure 6.** Modified Stern-Volmer plot for the deactivation of the emission of a sensor of compound 3 ( $S_2$ , see text) by aqueous solutions of Cu(II). The inset shows the plot for the normal Stern-Volmer relation.

coordination sphere, thus yielding a remarkably high intensity of luminescence (35). The formation of a 1:1 association complex between Cu(II) and compound 3 should be the main factor responsible for the static quenching of the luminescence of this compound. Although no value for the equilibrium constant associated with the formation of this association complex is available, it is known that the dtpa ligand forms very stable complexes with Eu(III) (36) and Cu(II) (37) ions in aqueous solution ( $K_{Eu(dtpa)2^-}$  =  $2.45 \times 10^{22}$  and  $K_{Cu(dtpa)3} = 1.2 \times 10^{21}$ ). It seems reasonable to think that the association of the negatively charged Eu(dtpa)<sup>2-</sup> ions with the positively charged Cu(II) ions should be favored. On the basis of the inset in Fig. 5, it is evident that the emission of the Coumarin120 is quenched by Cu(II). Given that, in solution, the quenching is static with a high efficiency, we can postulate that the deactivation is also static in the sensor, taking into account the more restricted mobility of the quencher in the gel. There is no further deactivation of the Eu(III)-centered emission by Cu(II), as evidenced by the linear modified Stern-Volmer plot (Fig. 5).



**Figure 7.** Reversibility of a sensor of compound 3 to Cu(II) in aqueous solutions. At points a and b, the final Cu(II) concentrations were  $2.3 \times 10^{-5} M$  and  $1.2 \times 10^{-5} M$ , respectively.



Figure 8. Emission spectra of dry sensors  $S_1$  (dashed line) and  $S_2$  (solid line) of compound 3. The excitation wavelength was 322 nm.

Furthermore, the advantages of compound 3 over compound 2 include the very low mass of Eu(III) chelate (in the order of sub-mg) used in the synthesis of the silica monolith, the absence of leaching of the chelate from the matrix of the sensor to the aqueous solution and the high percentage of reversibility of the luminescent signal.

The linearization of the Stern-Volmer plots obtained for the quenching experiments of the 2 sensors  $S_1$  and  $S_2$  of compound 3 by Cu(II) indicates that, as expected, not all the Eu(dtpaCoumarin120) sites of the sensor matrix are equally exposed to the metal ions. Only the fraction (approximately one-third) of accessible complexes is the responsible for the static quenching of the Eu(dtpaCoumarin120) centers of the sensors. The remaining active centers are sufficiently buried in the matrix of the sensors and do not undergo any luminescence deactivation even after long periods of hydration. This gives in turn a narrow quenchable range of  $I_0/I$  in the Stern-Volmer plots shown in Figs. 5 and 6.

?15

The simplest heterogeneous model for quenching involves only 2 types of sensing sites: those that can and those that cannot be quenched. In the context of a more general microheterogeneous model with a distribution of quenching sites that is physically meaningful to describe the system (38,39), one can interpret that this distribution is well represented by a bimodal distribution of quenchable and nonquenchable sites.

The difference of an order of magnitude between the values of the Stern-Volmer constants K<sub>SV</sub> for the sensors S<sub>1</sub> and S<sub>2</sub> is certainly surprising. A much higher sensibility for sensor  $S_1$  (K<sub>SV</sub> =  $1.49 \times 10^4 M^{-1}$ ) prepared with the monolith recently obtained was observed, compared with that for sensor S<sub>2</sub> (K<sub>SV</sub> =  $2.59 \cdot 10^3 M^{-1}$ ), which was obtained from the same monolith 6 months later. This is in keeping with the fact that the ratio of the luminescent intensity at 615 nm of Eu(III) to the luminescent intensity at 395 nm (bound Coumarin120) is higher for sensor  $S_1$  than for  $S_2$  when the sensors are dry (Fig. 8). Considering that the only difference in the preparation of these 2 sensors is the duration of aging for the monolith itself, it is reasonable to consider that the silica matrices of these 2 sensors are somehow different as a result of structural changes. Studies by <sup>29</sup>Si FTMAS (Fourier Transform Magic Angle Spinning) NMR spectra of silica gels prepared under several conditions with different gelled and dried temperatures and times

(ranging from hours to months) show that more-extensive drying increases the extent of condensation of the gel (30). This reorganization of the matrix (*i.e.* aging) may cause the network to stiffen via the formation of additional cross-links, which could interfere in the energy transfer process from the Coumarin120 to the Eu(III) by a nonconvenient relative orientation of the donoracceptor system. These results show that the aging mechanisms of the silica matrix are crucial because they are capable of interfering with the accessibility of the quencher due to the pore collapse of the material. This tendency is already evident in the decrease of the ratio of the Eu(III)-centered emission vs the Coumarin120 emission between the complex in water (Fig. 1) and for sensor S<sub>1</sub> (Fig. 8).

The reproducibility of the Stern Volmer constant for 5 sensors with similar preparation times was in the order of  $\pm$  20%. The sensor of compound 2 gives the lowest detection limit for Cu(II) (0.2 ppm of Cu(II) for 10% deactivation;  $K_{SV} = 3.84 \cdot 10^4 M^{-1}$ ), but in contrast, it shows the nonreversibility of the luminescent signal after washing. We do not discard compound 2 for the fabrication of a nonreusable Cu(II) sensor, in view of the low cost of the DPA ligand and the easy preparation of compound 2.

Compound 1 is not a suitable luminescent probe for sensing Cu(II), because of the poor performance obtained in the quenching measurements and the small absorption cross-section of the complex even in the UV.

The improvement of the performance of the sensors can be rationalized in terms of the larger area of the ground sol-gel silica particles, which greatly enhances (by at least 1 order of magnitude) the luminescent response of the entrapped Eu(III) complexes, compared with experiments in thin films. The PVAc polymer **?17** shows the suitable features of an immobilization matrix for retaining the silica particles while permitting the entrance and exit of ions in aqueous solution and, as a binder, conferring high mechanical stability to the system.

The better and faster response of base-catalyzed matrices from **?18** silica sol-gels has already been reported and was compared with the response for matrices obtained from acid-catalyzed synthesis for an entrapped Eriochrome Cyanine R (26). In this study, the sensors were able to detect Cu(II) in the 1–10 ppm range by means of diffuse reflectance techniques. In the study by Sommerdijk *et al.* (25), detection limits of 0.1 ppm were achieved for Cu(II), but the measurements were not *in situ* and not reversible. In our case, the control of the leaching of the Eu(III) complex is essential because the sensor tests the luminescent signal *in situ* with the sensor continuously immersed in the Cu(II) aqueous solution. We observed **?19** significant signal differences when the sensor was removed from the solution due to the dehydration process.

With respect to our results in the experiments with dip-coated thin films, a possible explanation can be given in terms of simple electrostatic considerations and pore sizes. The entrance of Cu(II) ions from the solution to the thin film of acid-catalyzed gel, immersed in acid solution (at pH < pH<sub>iep</sub>), is not facilitated because of the positive net surface charge of the silanol groups (pH<sub>iep</sub> = 2.2) (30). Only after the pH of the aqueous solution is increased to much higher values with ammonium hydroxide is the activity of the quencher Cu(II) facilitated, because of the favorable electrostatic interactions. In films prepared from base-catalyzed media, the mean larger size of the pores, compared with the pore size in acid catalyzed gels, cannot prevent the leaching of the entrapped probe when it is continuously immersed in aqueous solution, and the optical quality of the film is critically damaged. It is known that the size of the pores in acid-catalyzed xerogels is much smaller than

that in base-catalyzed xerogels (30). This prevents the leaching of compound 2 from the acid-catalyzed film to the aqueous solution.

In conclusion, composite sensor layers containing Eu(III) complexes as luminescent probes in grounded acid-catalyzed solgel monoliths dispersed in PVAc proved to be suitable devices to detect Cu(II) ions by means of quenching experiments in aqueous solution down to the ppm range. The small pore sizes of the acid-catalyzed matrix prevent the leaching of the Eu(III) complex to the aqueous solution, while still permitting the entrance and exit of the Cu(II) ions. These results open the possibility for *in situ* Cu(II) measurements.

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