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# Time-resolved Laser Fluorescence Spectroscopy of Organic Ligands by Europium : Fluorescence Quenching and Lifetime Properties

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## Abstract

Time-resolved Laser Fluorescence Spectroscopy (TRLFS) has proved its usefulness in the fields of biophysics, life science and geochemistry to characterize the fluorescence probe molecule with its chemical environment. The purpose of this study is to demonstrate the applicability of this powerful technique combined with Steady-State (S-S) measurements. A multi-mode factor analysis, in particular CP/PARAFAC, was used to analyze the interaction between Europium (Eu) and Humic substances (HSs) extracted from Saint Lawrence Estuary in Canada. The Saint Lawrence system is a semi-enclosed water stream with connections to the Atlantic Ocean and is an excellent natural laboratory. CP/PARAFAC applied to fluorescence S-S data allows introspecting ligands-metal interactions and the one-site 1:1 modeling gives information about the stability constants. From the spectral signatures and decay lifetimes data given by TRLFS, one can deduce the fluorescence quenching which modifies the fluorescence and discuss its mechanisms. Results indicated a relatively strong binding ability between europium and humic substances samples (Log K value varies from 3.38 to 5.08 at pH 7.00). Using the Stern-Volmer plot, it has been concluded that static and dynamic quenching takes places in the case of salicylic acid and europium interaction while for HSs interaction only a static quenching

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is observed.

*Keywords:* Fluorescence quenching, Europium (III), Natural Organic Matter, Time-resolved laser-induced fluorescence spectroscopy, Parallel Factor Analysis, Lifetime Analysis

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## 1. Introduction

Improved insight on the interactions between natural dissolved organic matter with lanthanides and actinides has been widely studied Kim et al. (1989); Moulin et al. (1991); Kim et al. (1993); Panak et al. (1996); Czerwinski et al. (1996); Tiseanu et al. (1998); Morgenstern et al. (2000); Monsallier et al. (2003); 5 Schmeide et al. (2006); Brevet et al. (2009); Jain et al. (2009); Lukman et al. (2012); Pacold et al. (2014). Indeed, it is of paramount importance to better understand transport and toxicity pathways of metal ions as well as creating efficient remediation techniques for contaminated environments Hiraide (1992); 10 Dube et al. (2001); Choppin (2007); Sachs and Bernhard (2011); Cobelo-García et al. (2015). Various spectroscopic techniques are used for these purposes. Fluorescence spectroscopy in S-S measurements provides quantitative and qualitative information. However, more data such as fluorescence lifetime measurements could be gathered using TRLFS. In fact, TRLFS is a convenient technique 15 when dealing with different elements in the same sample because it can offer the spectral and temporal resolution together with its high sensitivity. The discriminating capability of TRLFS relies on the fact that the different chemical species between organic matter and a fluorescent metal ion can have different decay lifetimes and spectral shapes which is adequate for the study of such a 20 complex system Collins et al. (2011). The greatest advantages of TRLFS are its relatively low detection limit and good discriminating capability for different chemical species.

An assessment of the influence of HSs on the mobility of Eu in the environment is necessary and has to include knowledge of stability constants for the formation 25 of Ln(III)-HSs complexes. In this work, complexation of  $\text{Eu}^{3+}$  by Humic Acids

(HA) and Fulvic Acids (FA) from St. Lawrence Estuary (Canada) was first studied by fluorescence quenching using Excitation Emission Matrices of fluorescence (EEMs) with a large range of europium concentration. Metal additions were performed using logarithmic increments to cover a wide range of concentration from  $2.2 \times 10^{-7}$  to  $1.5 \times 10^{-3}$  M. The obtained data was treated by CP/PARAFAC to extract the different fluorescent components using a MATLAB<sup>®</sup> home-made program Bro (1997); Luciani et al. (2009). Then a fitting based on the one-site model (1 : 1) Ryan and Weber (1982) was done on the CP/PARAFAC components to determine their stability constants. TRLFS is a complementary technique to determine the composition of the mixtures by measuring the fluorescence lifetimes and see its variation with the metal addition. Fluorescence properties of Eu(III) with Salicylic Acid (SA) and HSs complexes were studied from Eu(III) site Irving and Sinha (1970); Hasegawa et al. (1989); Tiseanu et al. (1998); Hasegawa et al. (1990); Toraiishi et al. (2005); Brevet et al. (2009); Jain et al. (2009); Kuke et al. (2010); Lukman et al. (2012); Saito et al. (2017) and also from the ligand site, but only for SA Aoyagi et al. (2004). Analysis of the literature has shown that data of humic substances complexes with Eu(III) from the ligand site is still lacking. As a matter of fact, it raises some interesting questions that have not been discussed before. Therefore, with this study, we wanted to investigate how these interactions occur focusing on the HSs fluorescence emission. Analysis of fluorescence lifetimes and Stern-Volmer plots Lakowicz (2006) will give reliable information on the quenching process that takes place. This line of study is important to settle a general relationship between organic matter properties from St Lawrence Estuary and static/dynamic quenching contributions.

## 2. Experimental setup

### 2.1. Sample preparation

The complexation of Eu(III) by SA was first studied in order to calibrate our assays and compare our results with literature Aoyagi et al. (2004). Indeed,

55 the compositions of HSs are complex and partly unknown. The HSs are composed of a complex mixture of organic and organomineral compounds and can differ depending on its type. Therefore, SA provides a carboxyl and hydroxyl group that mimic the important functionalities of HSs Stevenson (1994) and form strong complexes with Eu(III) Kuke et al. (2010).

60 Salicylic acid (NormaPur<sup>®</sup>, Prolabo) was used as received. Its aqueous solution was prepared at a concentration of  $5 \times 10^{-4}$ M with UHQ water. HA and FA were obtained from the St. Lawrence Estuary Tremblay and Gagné (2009) following the IHSS protocol Swift (1996).

Europium standard ( $1,000 \text{ mg}\cdot\text{L}^{-1}$  in 2%  $\text{HNO}_3$ , SPEX CertiPrep) was used as  
65 a quencher without further purification.

Twelve solutions with different concentrations of Eu(III) were prepared by mixing the stock solutions in a 1 cm quartz cell. The final concentrations of SA, HA and FA were kept constant at respectively  $5 \times 10^{-5}$ M, 11 and 16 mg of matter per liter. Eu(III) was added using logarithmic increments of concentrations  
70 from  $2.2 \times 10^{-7}$ M to  $1.5 \times 10^{-3}$ M. Every sample was allowed to equilibrate for at least 15 min and no precipitation was observed at higher europium concentration. The pH of the samples was adjusted to  $7.00 \pm 0.09$  using 1 mL of HEPES buffer (0.1 M). HEPES was used to maintain the pH constant and avoid pH adjustment for each sample. Preliminary measurements without metal additions  
75 were done to verify that there is no interaction between the fluorescent ligands and the HEPES buffer. The results did not show any variation of the fluorescence intensity. Thus, no interaction between the HEPES buffer and ligands was observed. A total volume of 3 mL was achieved by adding UHQ water. Necessary pH adjustments of metal solutions were done with NaOH (1M) and  
80 HCl (1 M) and the changes in the volume were taken into account.

## *2.2. Fluorescence and lifetime measurements*

Two series of experiments were done on the prepared samples. In order to perform S-S measurements, a spectrophotometer (HITACHI F4500) equipped with a Xenon lamp (450 W) and connected to a computer was used. Twelve

85 individual EEMs gathered from S-S measurements of SA, HA, and FA were collected in fluorescence quenching experiments. The excitation wavelength range was from 250 nm to 450 nm with a 10 nm slit and 5 nm excitation step. The corresponding emission wavelengths were collected at 5 nm step between 300 nm and 700 nm and 10nm slit. The scan speed was set at 2,400 nm.min<sup>-1</sup> and  
90 the photomultiplier tension was at 700 V.

TRLFS data was carried out using a pulsed Nd:YAG laser (Quanta-Ray INDI, Spectra Physics) and performed at room temperature (294 K). The Nd:YAG source provides an excitation with a pulse width of 5 ns and a repetition rate of 20 Hz. The excitation source for TRLFS was the third harmonic generation  
95 (355 nm) and the fluorescence emission was collected at 90 degrees through a bundle made of 16 optical fibers of 200  $\mu$ m into the entrance slit of a Czerny-Turner spectrograph (TRIAX 180, Horiba Jobin-Yvon). The latter has three UV-VIS gratings (300 g/mm, 900 g/mm and 2,400 g/mm). The spectral resolutions were respectively 0.27 nm, 0.09 nm and 0.034 nm. The second grating  
100 was used for these experiments.

The fluorescence signal was detected by an intensified couple charged device camera (ICCD 300V, Horiba Jobin-Yvon). For the time-resolved measurements, the initial timing of the fluorescence signal was varied by changing the delay of the trigger through a pulse generator (DG535, Stanford Research System). In  
105 order to increase the accuracy of the measurements, each laser pulse was corrected in energy fluctuation by splitting the laser beam through a pyroelectric sensor (PE10, Ophir Electronics). The power of pulse was constant within around 3-5  $\mu$ J pulse during the acquisition. Seven hundred spectra were accumulated at each time step of 0.2 ns and a time delay of 200 ns with a 35 ns gate  
110 width. These parameters were set to measure the fluorescence focusing on the ligand fluorescence emission.

Every mixture was allowed to equilibrate for at least 10 min and bubbled by a nitrogen flux to avoid photobleaching.

### 2.3. Data Treatment

115 For S-S data treatment, CP/PARAFAC algorithm was used to determine the contribution of components to the EEMs by separating independent sources after elimination of scattering phenomena Bro (1997). The first step of decomposition consists of cleaning all EEMs from the diffusion signals (Rayleigh and Raman) by cutting the Rayleigh diffusion bands (20 nm) and applying the Zepp procedure Zepp et al. (2004). No inner effect was encountered because chosen 120 concentrations were in the linear zone of fluorescence. Dilution curves were done to determine these value (data not shown). Thus, no numerical correction was applied to the EEMs. The estimation of the number of components is particularly critical because users have to test a range of model to evaluate the best number of components. In this study, it was defined by evaluating the CORCONDIA score. Decomposition investigation was done from 1 to 4 components and the number of component giving a CORCONDIA score higher than 60% was selected as optimal model. Iterations and number of runs were implemented until the algorithm converged.

130 The optimization between experimental and calculated fluorescence using one-site model with one ligand Ryan and Weber (1982) was executed by evaluating the bias errors. The bias is equal to the sum of the square of the subtraction between the experimental fluorescence intensity and the calculated fluorescence intensity.

135 Data obtained with TRLFS were analyzed using a home-made MATLAB<sup>®</sup> program. The evaluation of the best fitting of the integrated fluorescence intensity was done by time deconvolution to determine fluorescence amplitudes and fluorescence lifetimes. Supposing a system excited by an infinitely sharp pulse of light ( $\delta$ -function) and assuming the response to be linear, the measured 140 fluorescence decay is given by the convolution integral :

$$y(n) = h(n) \otimes IRF \quad (1)$$

where  $h(n)$  is the intrinsic fluorescence decay and IRF is the Instrument Response Function. Experimentally, the IRF is gathered by measuring only the

solvent diffusion.

The estimation of  $h(n)$  was achieved by time deconvolution in order to obtain  
145 the amplitudes of each fluorophore and lifetimes. Assuming a multi-exponential  
decay as follow :

$$h(n) = \sum_i \alpha_i \exp^{-t/\tau_i} \quad (2)$$

The amplitudes  $\alpha_i$  and the lifetimes  $\tau_i$  can be considered to be adjustable pa-  
rameters. They are calculated employing Simplex algorithm minimizing the  
error of the equation :

$$[y(n) - y'(n)] = \varepsilon(n) \quad (3)$$

150 It is known that quenching can occur by different mechanisms. Dynamic quench-  
ing occurs when the excited-state fluorophore is deactivated upon contact with  
other molecules in solutions due to physical collision Lakowicz (2006). In this  
case, the fluorophore returned to the ground-state during a diffusive encounter  
with the quencher. The molecules are not chemically modified in the process  
155 and can be shown by the Stern-Volmer equation 4.

$$\frac{I_0}{I_Q} = 1 + K_{SV}[Q] = 1 + k_q\tau_Q[Q] \quad (4)$$

where  $I_0$  and  $I_Q$  are the fluorescence intensities in the absence and presence of  
quencher respectively,  $\tau_Q$  is the fluorescence lifetime in the presence of metal,  
 $K_{SV}$  is the Stern-Volmer quenching constant and  $k_q$  is the bimolecular quench-  
ing constant.

160 If dynamic quenching takes place, the intensity of fluorescence and lifetime fol-  
lows the relationship:

$$\frac{I_0}{I_Q} = \frac{\tau_0}{\tau_Q} \quad (5)$$

In this expression  $\tau_0$  is the fluorescence lifetime in the absence of quencher. It  
is noteworthy that dynamic quenching is a time-dependent process.

Aside from collisional quenching, fluorescence quenching can occur due to a  
165 complex formation. Fluorophores can form non-fluorescent complexes with  
quenchers Lakowicz (2006). This process is referred to as static quenching since  
it occurs at ground-state and does not rely on diffusion or molecular collisions.

The dependence of the fluorescence intensity upon quencher concentration is given by the following expression

$$\frac{I_0}{I_Q} = 1 + K_S[Q] \quad (6)$$

170 where  $K_S$  is the association constant of the complex.

Note that the  $I_0/I_Q$  on  $[Q]$  is linear, which is identical to dynamic quenching. Hence, the measurement of fluorescence lifetimes is the most ultimate method to distinguish static and dynamic quenching.

### 3. Results & Discussions

#### 175 3.1. Steady-State and fluorescence quenching results

The CP/PARAFAC decomposition of SA-Eu<sup>3+</sup> quenching experiment shows that two components are obtained in this interaction (Fig. 1). The first component ( $\lambda_{ex}/\lambda_{em}$ )=295/410 nm shows a fluorescence intensity and is obviously the excitation/emission wavelength of SA Tauler et al. (1998) whereas, the second  
180 component does not show any fluorescence intensity variation (Fig. 2. A) nor fluorescence peak. Thus, the peak could be a noise factor and was not considered in the following discussion.

The relative contributions of each components of SA-Eu<sup>3+</sup> interaction are shown in Fig. 2. A. indicating the quenching of SA depending on the Eu<sup>3+</sup> concentration. The corresponding conditional stability constant (K value) for the first  
185 component is reported in Table 1 and was estimated as Log  $K_{SA} = 3.38$  based on the 1 : 1 complexation assumption between metal and ligand. This value is higher than Log  $K=2.08 \pm 0.02$  obtained by Aoyagi et al. (2004) at pH 4 from the slope of the intensity SternVolmer plot and higher than Log  $K=2.02 \pm 0.05$   
190 obtained by Hasegawa et al. (1989) at pH 6 from the glass electrode method. The stability constant of SA-Eu<sup>3+</sup> found in this study is consistent considering that the stability constant should increase when pH increases.

As for the extracted humic substances, the presence of abundant aromatic structures in organic matter provides reasonably good fluorescence characteristics. CP/PARAFAC decomposition of the EEMs obtained in the case of HA  
195

Table 1: Stability constants and average fluorescence lifetimes of SA and HSs complexed with  $\text{Eu}^{3+}$

	<b>Log <math>K_{SA}</math></b>	<b>Log <math>K_{.,C_1}</math></b>	<b>Log <math>K_{.,C_2}</math></b>	<b>Log <math>K_{.,C_3}</math></b>	<b><math>\tau_a^A</math> ns</b>	<b><math>\tau_a^B</math> ns</b>
<b>SA</b>	3.38	–	–	–	2.97	–
<b>HA</b>	–	4.43	5.08	3.63	0.61	11.33
<b>FA</b>	–	4.42	4.43	–	0.73	12.54

interaction with  $\text{Eu}^{3+}$  suggests the presence of three components (Fig. 1.B). The first component in the UV range is located at  $(\lambda_{ex}/\lambda_{em})=270/445$  nm, and has been designated as peak A. It was attributed to humic substances Coble (1996). The relative contribution of this component to the EEMs during the quenching experiment is shown in Fig. 2.B. and the corresponding stability constant is  $\text{Log } K_{HA,C_1} = 4.43$  (Table 1). The second component  $(\lambda_{ex}/\lambda_{em})=270/505$  nm shows a longer emission wavelength characteristic of a higher conjugated structure usually found in soils. This component behaves similarly to component 1 (Fig. 2.B) and has the higher stability constant ( $\text{Log } K_{HA,C_2} = 5.08$ ). The third component  $(\lambda_{ex}/\lambda_{em})=250/395$  nm is more relative to the protein-like structures and presents a weaker quenching effect. This means that the related fluorescent chemical functions are less affected by europium or less complexing. This is demonstrated by the lowest stability constant value  $\text{Log } K_{HA,C_3} = 3.63$ . In comparison, Wei et al. (2015) reported two stability constants using synchronous fluorescence spectroscopy. Their constants at pH 6 are higher than found in our study :  $\text{Log } K_1=5.51$  and  $\text{Log } K_2=5.60$ . However, the value reported by Hahn et al. (2017) using ultrafiltration and CE-ICP-MS at  $\text{pH}=5.00\pm 0.05$  seems to be also higher than ours :  $\text{Log } K=6.36\pm 0.02$  and  $\text{Log } K=6.1\pm 0.1$ . This work clearly shows that several fluorescent sites complex the europium in this particular HA from St. Lawrence estuary. More investigation should be done to compare the data, in particular for the pH conditions.

For the FA, two components were found by CP/PARAFAC showing an interesting fluorescence quenching (Fig. 2. C.) The first component (Fig. 1. C)

located at  $(\lambda_{ex}/\lambda_{em})=310/415$  nm is generally another marine humic-like component and is designated as peak M Coble (1996). The second component  
220  $(\lambda_{ex}/\lambda_{em})=265/475$  nm is larger and is designated as peak A Coble (1996) with a slight red shift in emission. The corresponding fluorescence intensities are given in Fig. 2. C. showing a homogeneous and similar behavior of both components. This is demonstrated by the values of the stability constants respectively  
225  $\text{Log } K_{FA,C_1} = 4.42$  and  $\text{Log } K_{FA,C_2} = 4.43$  of the first and second component. The study of Chung et al. (2005) reported a stability constant at pH 5 equal to  $6.4 \pm 0.2$  for FA–Eu(III) interaction using synchronous fluorescence spectroscopy. This value is higher than our stability constant. This data is in good agreement with an other published work :  $\text{Log } K=6.00 \pm 0.10$  Bidoglio et al.  
230 (1991). Furthermore, Lead et al. (1998) reported only one stability constant at a pH between 2.5 and 3.5 :  $\text{Log } K=2.36 \pm 0.13$  much like Wei et al. (2015) :  $\text{Log } K=5.84$ . The values of the stability constants found in the literature are higher due to the fact that the fulvic acids studied are mainly derived from soils or rivers and may have greater complexing capacities than the fulvic acids used  
235 in this study.

### 3.2. TRLFS results

As mentioned above in section 2.3, the quenching process cannot be assessed using steady-state measurements. Therefore, fluorescence lifetime measurements using TRLFS are the most ultimate method to distinguish whether static,  
240 dynamic or both quenching processes occur based on Stern-Volmer plots Lakowicz (2006).

After S-S quenching experiments, eight samples out of twelve from the quenching curves were selected for lifetime measurement, because of the time required for each measure. The first sample does not contain any concentration of  $\text{Eu}^{3+}$   
245 while the seven other samples contain an increasing volume of it at a concentration varying from  $2.2 \times 10^{-5}\text{M}$  to  $2.2 \times 10^{-3}\text{M}$ .

For the interaction between SA- $\text{Eu}^{3+}$ , the decay was found to be mono-exponential. The average lifetime  $\tau_a$  recorded was  $2.97 \pm 0.2$  ns. In Fig. 3.A

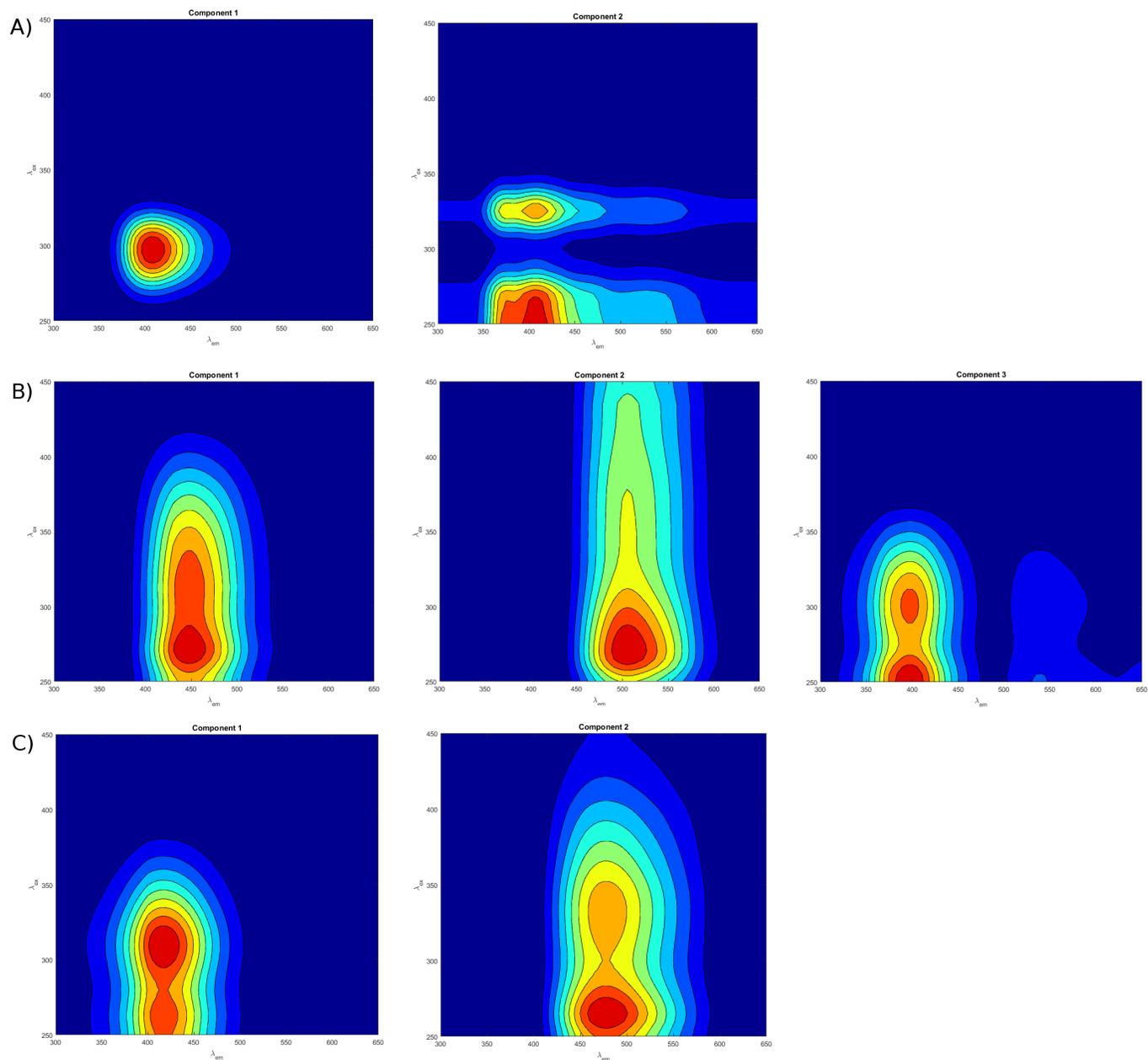


Figure 1: Components obtained for A) Salicylic acid-Eu<sup>3+</sup> B) Humic acid-Eu<sup>3+</sup> and C) Fulvic acid-Eu<sup>3+</sup> interaction after CP/PARAFAC decomposition

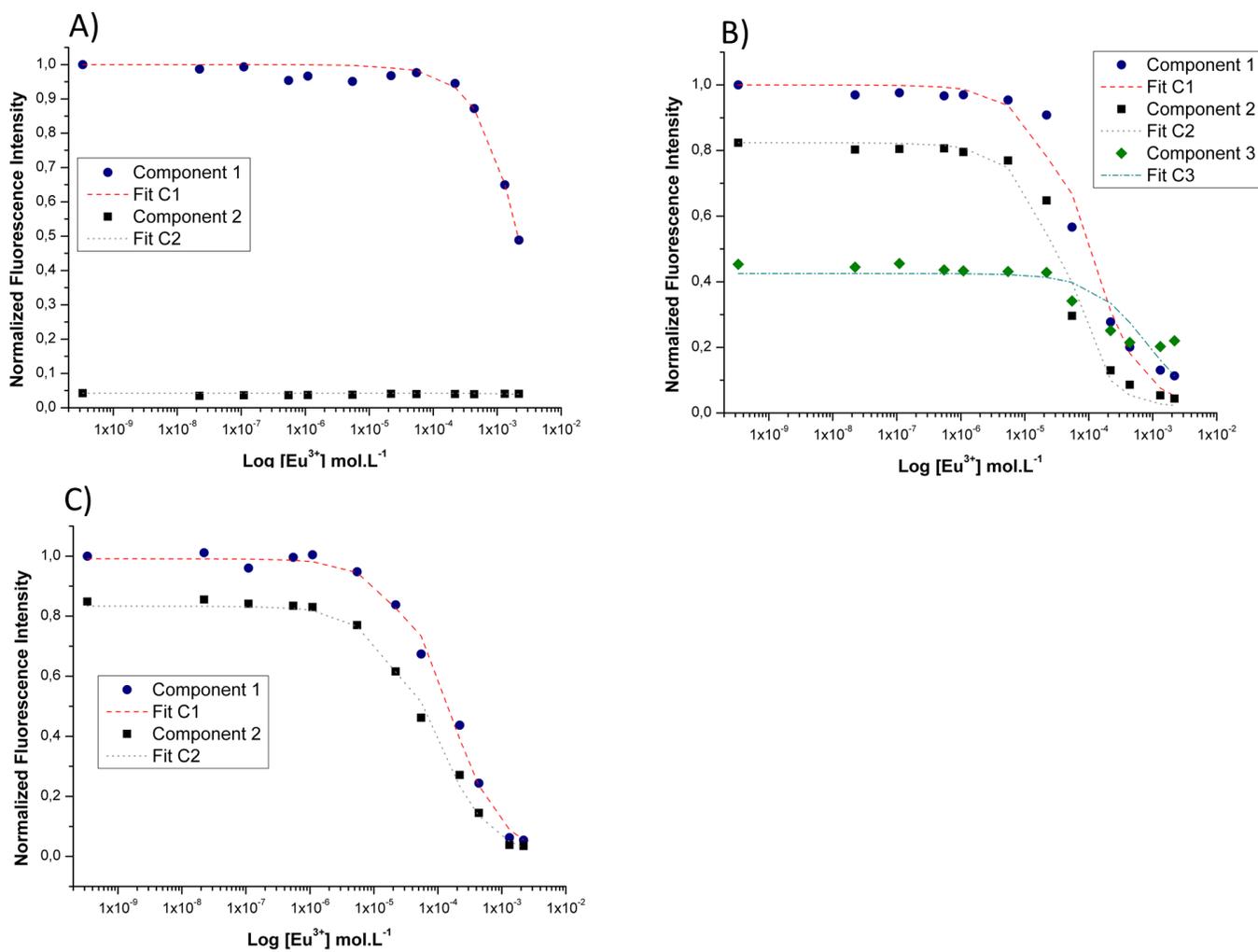


Figure 2: Variation of the CP/PARAFAC components contributions to the EEMs of A) Salicylic acid B) Humic acid and C) Fulvic acid as a function of  $\text{Eu}^{3+}$  concentration

$I_0/I_Q$  and  $\tau_0/\tau_Q$  are shown as a function of the concentration of  $\text{Eu}^{3+}$ . The  
250 solid red line shows the linear regression of the Stern-Volmer plot intensity for  
 $I_0/I_Q$  and  $\tau_0/\tau_Q$ . The value of the lifetime ratio seems to be dependent on  
metal addition. This indicates that the observed quenching involves a dynamic  
quenching process or a mix of static and dynamic quenching, as the  $I_0/I_Q$  and  
 $\tau_0/\tau_Q$  should have the same slope in case of pure dynamic process Lakowicz  
255 (2006). On the other hand, Aoyagi et al. (2004) found a fluorescence lifetime  
equal to  $2.02 \pm 0.01$  ns and a static quenching occurring for SA- $\text{Eu}^{3+}$  interaction.  
This discrepancy could be due to the pH value as they worked at pH 4.

For HA- $\text{Eu}^{3+}$  interaction, CP/PARAFAC decomposition gives three com-  
ponents while the time deconvolution suggests a bi-exponential decay and two  
260 lifetimes denoted by exponent indexes  $\tau^A$  and  $\tau^B$ . One very short, indicated by  
 $\tau_0^A/\tau_Q^A$  in Fig. 3. B and the second one, specified by  $\tau_0^B/\tau_Q^B$ , longer than the first  
one (11.33 ns). The two fluorescence lifetimes turned out to be independent on  
total concentration of  $\text{Eu}^{3+}$ . This suggests a static quenching. The dependence  
of  $I_0/I_Q$  of component 1 and component 2 on  $[\text{Eu}^{3+}]$  is therefore non-linear.  
265 In the case of static and dynamic quenching, the characteristic feature of the  
Stern-Volmer plot is an upward curvature, concave towards the y-axis. The  
concave pattern of the Stern-Volmer plot is interpreted as the manifestation of  
fluorophores that are not accessible for quenching Lakowicz (2006). Thus, the  
data cannot be accurately fitted using the linear Stern-Volmer equation 4 in this  
270 situation. However, Reiller and Brevet (2010) observed a bi-exponential decay  
for the luminescence of  $\text{Eu(III)}$ -HSs complexes and reported a deactivation of  
at least two different excited-states from  $\text{Eu(III)}$  site. The reported values of  
fluorescence lifetime cannot be compared to our study since they measured the  
fluorescence lifetime values from  $\text{Eu(III)}$  site.

275

The intensity ratios of the fulvic acid with  $\text{Eu}^{3+}$  in Fig. 3. C. seem to be  
linear except the penultimate point which could be attributed to an error in the  
measurement. According to Tiseanu et al. (1998), the existence of a tri-modal  
decay time distribution with time values centered around 0.7, 3 and 11 ns was

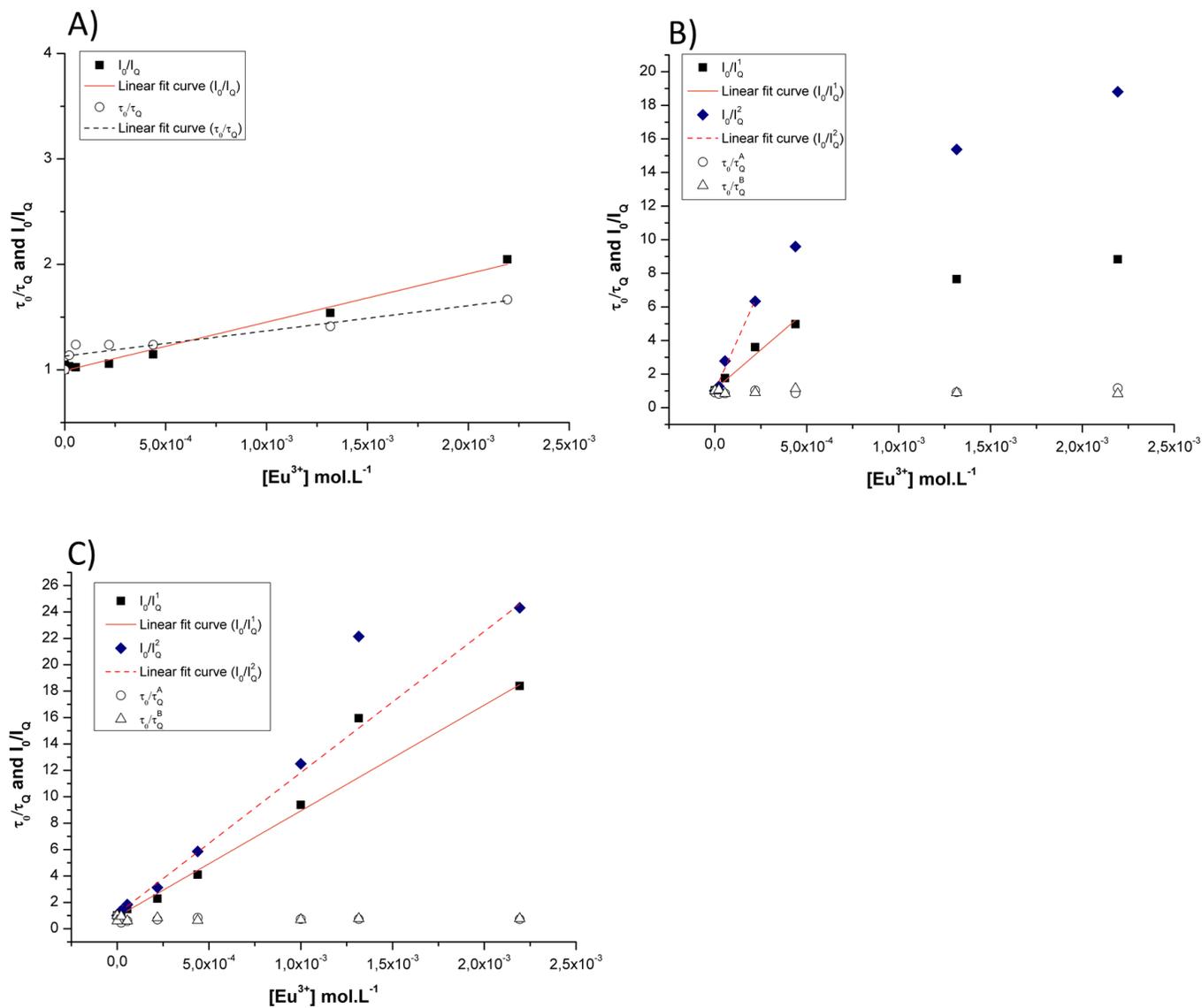


Figure 3: Stern–Volmer plot showing the intensities ratio and the fluorescence lifetimes ratio of A) Salicylic acid–Eu<sup>3+</sup>, B) Humic acid–Eu<sup>3+</sup> and C) Fulvic acid–Eu<sup>3+</sup> interactions

280 reported. In this study, we found a bi-exponential time decay. The first average  
decay time component (0.7ns) referred to as  $\tau_0^A/\tau_Q^A$  may be assumed as typical  
for FA of different origins Kumke et al. (1998) and similar to Tiseanu et al.  
(1998). The second lifetime indicated by  $\tau_0^B/\tau_Q^B$  in Fig. 3. C. is around 12 ns  
and is similar too to the literature. In the presence of  $\text{Eu}^{3+}$ , the dependency of  
285 the relative amplitudes of the fluorescence decay of FA was almost unchanged  
showing a limited decrease for the shorter lifetime and a great increase for the  
longer lifetime. The resulting plot is linear and can be fitted using the linear  
Stern-Volmer equation. The invariance of the decay time values of the FA upon  
metal addition was not related to energy transfer between FA singlet states and  
290 the  $\text{Eu}^{3+}$ . Quenching of FA fluorescence induced by  $\text{Eu}^{3+}$  thus indicates a static  
quenching. The previous study ( Tiseanu et al. (1998)) also reported a static  
quenching in the case of FA-Eu(III) interaction.

#### 4. Conclusion

In this work, the interactions of salicylic acid, humic acid and fulvic acid with  
295  $\text{Eu}^{3+}$  were studied by steady-state and time-resolved laser-induced fluorescence  
spectroscopy focusing on the ligand fluorescence emission using a nanosecond  
pulsed laser. Lifetime analysis using Stern-Volmer plot showed that part of dy-  
namic quenching process or both static and dynamic quenching contributed to  
the global quenching of fluorescence in the case of salicylic acid- $\text{Eu}^{3+}$  interaction.  
300 Data obtained is not exactly in agreement with literature Aoyagi et al. (2004)  
since they found a static quenching in the case of SA-Eu(III) interaction. This  
discrepancy with SA should be investigated at different pH to define precisely  
the quenching process dependence on pH. Static quenching process took place  
for humic acid and fulvic acid extracted from St. Lawrence Estuary. Tiseanu  
305 et al. (1998) also found a static quenching in the case of FA-Eu(III) interaction,  
which is in a good agreement with our study. The stability constants of all the  
interactions studied were calculated using the one-site 1 : 1 model and are in  
concordance with literature for SA and HA. For FA, we found constants slightly

lower than those in the literature which may be due to the origin of FA. It is  
310 noteworthy that TRLFS successfully discriminates the fluorescence quenching  
process that takes place and gives original lifetimes of HSs and constant of com-  
plexation with Eu(III). Further application can be extended to quantitatively  
analyze other lanthanides and actinides.

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