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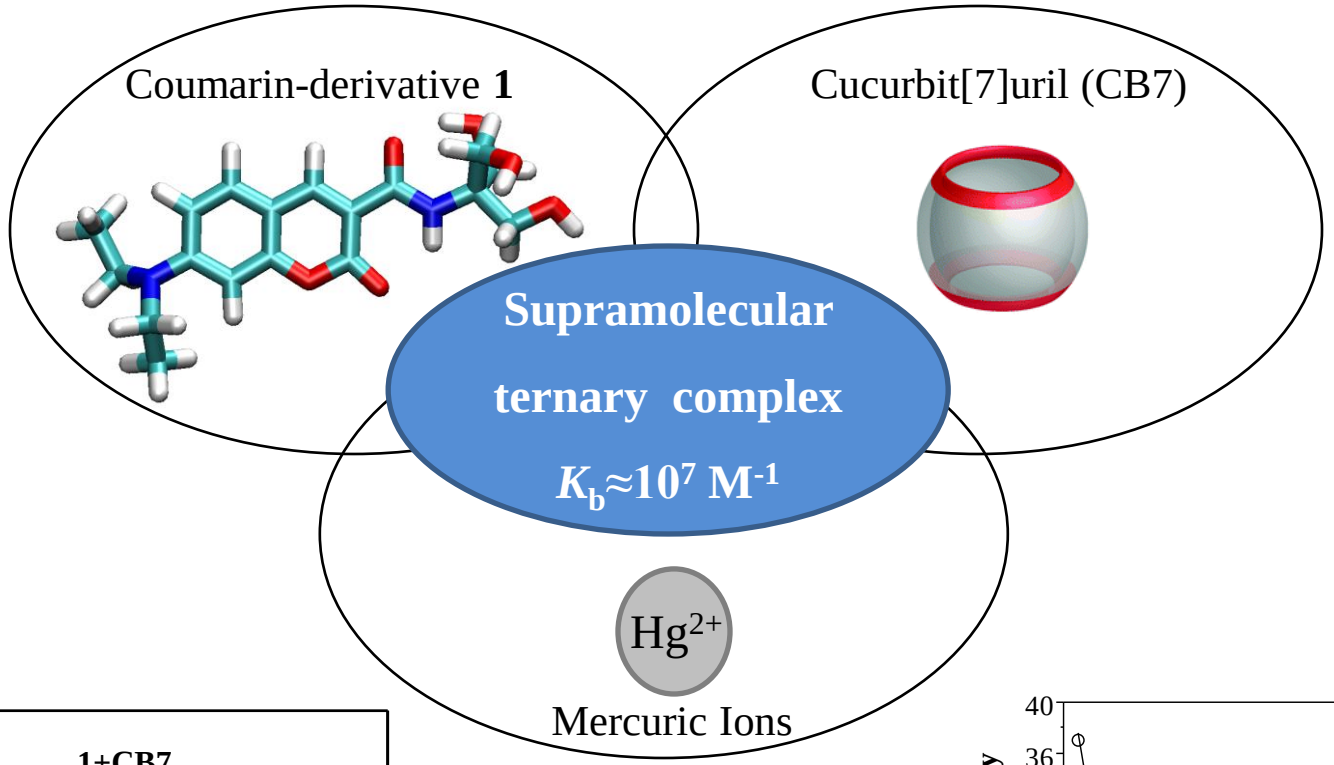
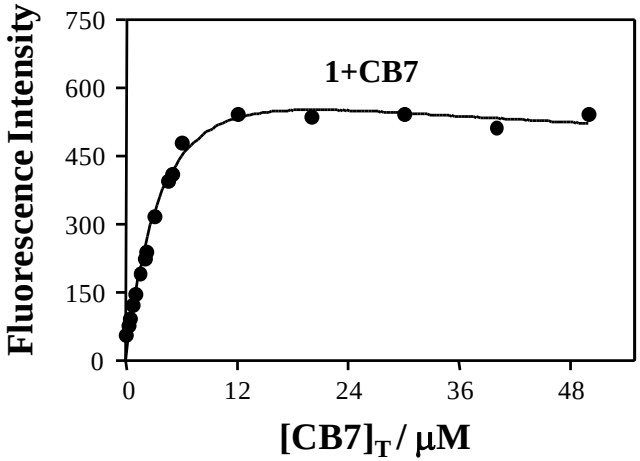
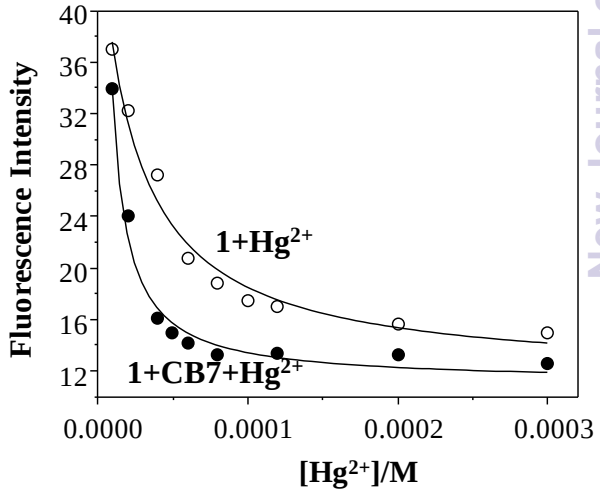
The interaction between the studied coumarin derivatives-Cucurbit[7]uril and mercuric ions displays a positive cooperative effect relative to the dyes alone.







Host-Guest Interaction of Coumarin-Derivative Dyes and Cucurbit[7]uril: Leading to the formation of Supramolecular Ternary Complexes with Mercuric Ions.

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ABSTRACT

We investigated the photophysical behavior of the complexes formed between cucurbit[7]uril (CB7) and coumarin-derivative dyes: 7-(diethylamino)-N-(1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl)-2-oxo-2H-chromene-3-carboxamide (**1**) and N-(1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl)-11-oxo-2,3,5,6,7,11-hexahydro-1H-pyrano[2,3-f]pyrido[3,2,1-ij]quinoline-10-carboxamide (**2**), in the absence or presence of mercuric ions (Hg^{2+}). The maximum absorption of **1** shows a bathochromic shift with the addition of CB7 and the fluorescence intensity is highly increased. In contrast, addition of CB7 has no noticeable effect on the spectroscopic properties of **2**. However, a fluorescence quenching was observed in both cases after the addition of Hg^{2+}. Interestingly, in the absence of it fluorescence lifetime measurements for the **1**-CB7 complex suggest that the macrocycle is able to prevent the aggregation for **1**. The stoichiometry for these complexes, determined from the fluorescence titration measurements and mass spectrometry, indicates that 1:1 complexes are formed and the binding constants (K_b) are estimated around 10^5 M^{-1}. The NMR studies indicate that both dyes are included in the CB7 cavity but different moieties interact with it. Considering the hydrophobic effect of the cavity, and metal-ligand and ion-dipole interactions, it can be concluded that both compounds are able to form a novel supramolecular assembly that comprises CB7, **1** or **2** and Hg^{2+}. The binding observed between them displays a positive cooperative effect relative to the dyes alone, being **1**-CB7 the most efficient complex ($K_b \approx 10^7$ M^{-1}) in acidic conditions. Thus, the potential for these types of complexes to be used as multifaceted functional systems appears warranted.

Keywords: cucurbit[7]uril; coumarin-derivative dyes; host-guest interactions; supramolecular ternary complexes; mercuric ions.

1. INTRODUCTION

Host-guest supramolecular systems are ideal platforms to control photophysical and photochemical properties of organic dyes and their interactions with biomolecules.[1-3] Among the diverse kinds of supramolecular hosts studied, the cucurbit[n]urils (CB*n*) has acquired a great popularity in recent years.[4] These macrocyclic hosts molecules are composed of glycoluril units linked by a pair of methylene groups and have fairly rigid hydrophobic cavities of low polarizability.[5-7]

Cucurbit[*n*]urils occur as CB5, CB6, CB7, CB8, and CB10, and each has a different size of its inner cavity, their volumes ranging from 82 to 870 $Å^3$ for CB5-CB10, respectively.[8] CB7 displays a favorable combination of a sufficient cavity size and high water solubility (ca. 5 mM). The latter turns it into the most promising cucurbituril host for binding to fluorescent dyes.[4] In fact, CB7 can form inclusion complexes with organic guests,[9] with the strongest complexes formed with positively charged guests.[10]

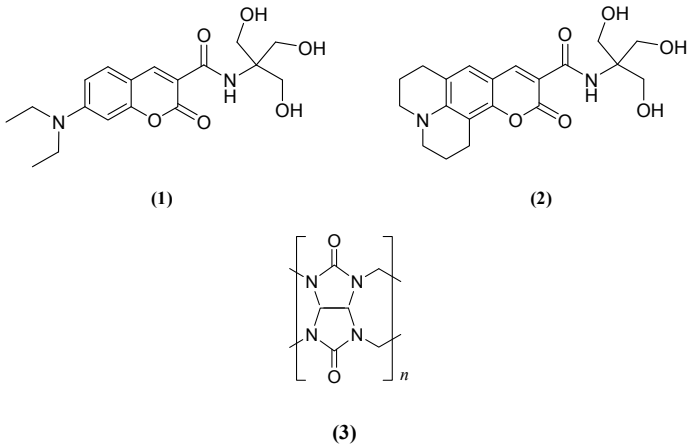
The encapsulation of cationic fluorescent dyes by CB*n* has been reported to display a whole range of advantageous effects, which include enhanced fluorescence intensity, spectral shifts, increased photostability and prevention of association and aggregation.[11-19]

Coumarin dyes constitute one of the most extensively studied systems by photophysicists[20] mainly as a consequence of their importance in biological systems and their use in various materials of commercial interest.[21-24] However, the preferred use of aqueous solutions of coumarin dyes for these applications has been limited because their low solubility, dramatic reductions in fluorescence intensity, and photochemical instability in water. These problems can be largely overcome by complex formation with the host CB7.[16,25,26] Recently there have also been reports on the interaction of a coumarin dye with a 7-alkylamino substituent, a widely used fluorescent molecule in organic solvents that is virtually nonfluorescent in water, with cucurbit[7]uril (CB7). It has been demonstrated that the macrocycle is able to increase the fluorescence intensity of aqueous solutions of 7-diethylamino-4-methylcoumarin (or coumarin 1).[27] However, no reports have been published to date describing the supramolecular interaction of the CB7 host with a bifunctional probe derived from the fluorophore 7-diethylamino-coumarin or a bridged julolidinylamino-coumarin and 2-amino-2-(hydroxymethyl) propane-1,3-diol. Thus, in the present study, we investigate the complexation behavior of cucurbit[7]uril (CB7) with these two fluorescent dyes.

On the other hand, it is known that cucurbituril forms strong complexes with different cations,[28,29] and in their presence most of the CB[n]-based systems reduce their binding affinity toward diverse guests due to competitive binding of the metal at the ureidyl C=O portal of host, resulting in dissociation of the host–guest complex.[30-33]

Interestingly, there are only a few studies where addition of metal ion to CB[n] host–guest assemblies results in a distinct three-component assembly. They include the work by Mohanty *et al*. on the system thioflavin T-CB7,[34] that of Pang *et al*.[35] about squaraine dye-CB8 systems, that of Nau *et al*.[36] on the bicyclic azoalkanes-CB7-cations and a ternary architecture driven by cooperative Hg^{2+} ion binding between CB7 and crown ether macrocyclic hosts.[37]

This cooperative effect is really attractive to us, considering our interest in the development of novel assemblies containing coumarin-derivatives as compounds capable to interact with mercuric ions.[38] Thus, in the present study we use cucurbit[7]uril (CB7) to modified the photophysical properties of novel coumarin-derivative dyes in the absence and presence of mercuric ions. Specifically, we assess the photophysical responses of the dyes 7-(diethylamino)-N-(1,3-dihydroxy-2-(hydroxymethyl) propan-2-yl)-2-oxo-2H-chromene-3-carboxamide (**1**) and N-(1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl)-11-oxo-2,3,5,6,7,11-hexahydro-1H-pyrano[2,3-f]pyrido[3,2,1-ij]quinoline-10-carboxamide (**2**) (see Scheme 1) toward mercuric ions in the presence of CB7. The aim of our work is to develop a three-component system that displays a cooperative effect and improve the interaction towards mercuric ions.



Scheme 1. Chemical structure of the studied compounds. Coumarin-derivative guests (**1** and **2**) and the cucurbit[*n*]uril (CB*n*) host, where *n*=7 for CB7 (**3**).

2. EXPERIMENTAL

2.1 Materials and instruments. All reagents were purchased from Sigma-Aldrich and were used as received, except the host cucurbit[7]uril (CB7) was synthesized as described previously.[39] Stock solutions of CB7 were titrated with cobaltocenium by UV-vis absorption spectroscopy.[40] The solutions of mercury (II) chloride ($HgCl_2$) were prepared in Chelex-100-treated Milli-Q water. The dyes **1** and **2** also were synthesized as described in Supporting Information. Absorption and steady-state fluorescence spectra were obtained using a HP-8453 diode array spectrophotometer and spectrofluorimeter (Varian), respectively. Time-resolved fluorescence measurements for fluorescence lifetimes were carried out using a FluoTime 200 fluorometer (PicoQuant Inc.).

2.2 Electrospray ionization mass spectrometry (ESI-MS/MS). The detection of inclusion complexes was undertaken on an AB Sciex 4500 QTrap® Mass Spectrometer equipped with a Turbo Ion Spray (AB Sciex) ion source. Specific compound-dependent MS parameters for each dye were determined by direct infusion into the MS of individual standards dissolved in 10% (vol/vol) acetonitrile (concentration of 25 μM) at a flow rate of 7 μL/min. The 4500 QTRAP system was operated in positive ion mode using the multiple reaction monitoring (MRM) scan type. A declustering potential (DP) of +180, entrance potential (EP) of +10, and collision cell exit potential (CXP) of +25 were used. The ion spray voltage was set at +3500 V, source temperature was set at 300 °C, collision gas (CAD) was set to high, and source gas GS1 and GS2 were set to 10 and 20, respectively. All data were acquired using Analyst 1.6.2 (AB Sciex).

2.3 Nuclear magnetic resonance (NMR) studies. ^{1}H-NMR spectra of solutions were acquired at 25 ^{o}C on a Varian INOVA of 17.6 T (750 MHz proton frequency) spectrometer equipped with an inverse detection triple resonance probe ^{1}H/^{13}C/^{31}P and PFG gradients. The ^{1}H spectra were automatically referenced based in the deuterium lock signal. TSP was used as an internal reference for ^{1}H chemical shifts. The NMR spectra were processed with MestreNova software v9.0. All solutions were prepared by mixing appropriate volumes of stock solutions of CB7, coumarin-derivatives (**1** or **2**) and of mercury (II) chloride ($HgCl_2$).

2.4 Determining the quantum yield of emission. Fluorescence quantum yields of **1** and **2** in the absence or in the presence of CB7 and/or $HgCl_2$ were measured using a

solution of quinine sulfate in 0.5 mol/L H_2SO_4 as standard (Φ_{fl} = 0.546),[41] all values were corrected taking in account the solvent refractions index and were calculated as described in ref.[38]

3. RESULTS AND DISCUSSION

3.1 Effect of CB7 on Absorption and Emission Properties of the tested coumarin-derivative dyes. To evaluate the effect of the macrocycle (CB7) on the photophysical properties of the coumarin-derivative dyes **1-2** (Scheme 1), we first determined the absorption, excitation and emission spectra for these dyes in the absence of the CB7, as shown in Figures S1 and S2.

For the dye **1**, its absorption spectrum in aqueous solution is characterized by an intense maximum at 430 nm (Fig. S1(A)) and for the dye **2** the absorption spectra depicts a maximum at 390 nm (Fig. S1(B)). In the case of the excitation and emission spectra, the derivative **2** depicts a red shift of ≈15 nm (Fig. S2(B)) in comparison with **1** (Fig. S2(A)). This shift is in accordance with other studies,[42,43] which reported a similar shift value when the substituent of coumarin-derivative dye was modified, from a 7-dialkylamino into a bridged julolidinyl- based group binding to the coumarin scaffold.

The addition of CB7 to a solution containing either **1** or **2** gives place to a bathochromic shifts related to their absorption maximum ($\Delta\lambda_{max}$) of ≈10 nm (see Figs. S1(A) and (B)). The observation of these bathochromic shifts is in accord with other studies reported for different coumarin-CB7 complexes.[27] Gupta *et al*. (2012)[27] proposed that in spite of the low polarity cavity of CB7, there is an influence of other factors on the absorption spectrum such as rearrangement of the hydrogen bonds between the coumarin-derivative and water molecules that could justify the observed shifts.

With the objective to investigate the complexation ability of the coumarin-derivatives **1** and **2** with CB7, titration experiments were performed. As shown in Figure 1(A), the addition of increasing concentration of CB7 (0.1-12 μM) on dye (**1**) containing solutions, incremented in all cases the emission intensity. However, this intensity remained constant when the concentration of CB7 was increased beyond 6 equivalents with respect to the dye alone. In the case of dye (**2**) only a slight increase of emission intensity was observed after the addition of increasing concentration of CB7 on the dye (Fig. 2).

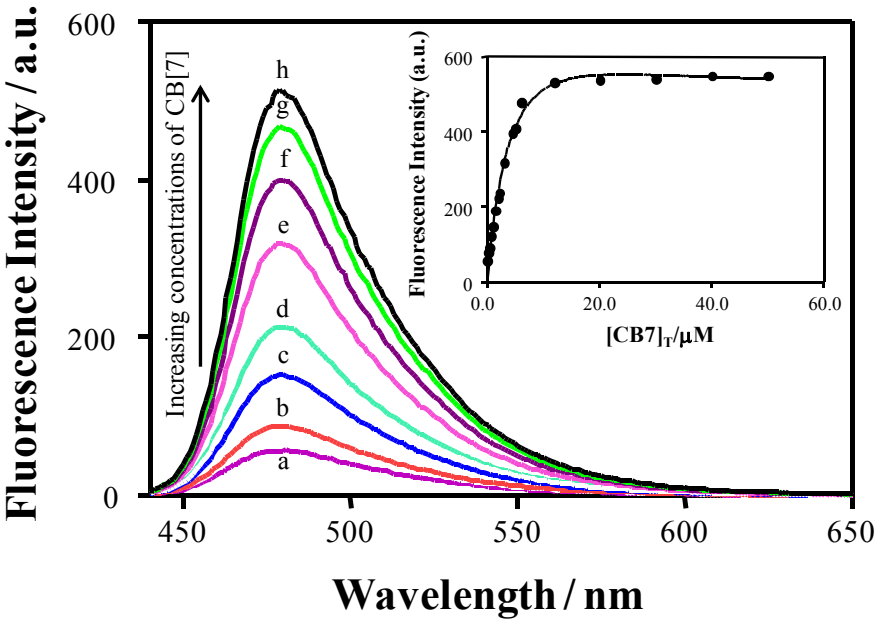


Figure 1. Fluorescence spectra of the coumarin-derivative **1** with CB7. (A) **1** dye (2.0 μM) with [CB7]/μM: (a) 0, (b) 0.4, (c) 1.0, (d) 1.5, (e) 3.0, (f) 4.5, (g) 6.0, and (h) 12. Inset show the fluorescence titration curves for the respective dye with CB7 (in aqueous solution at pH = 5). Excitation and emission slits of 5 (nm) were used.

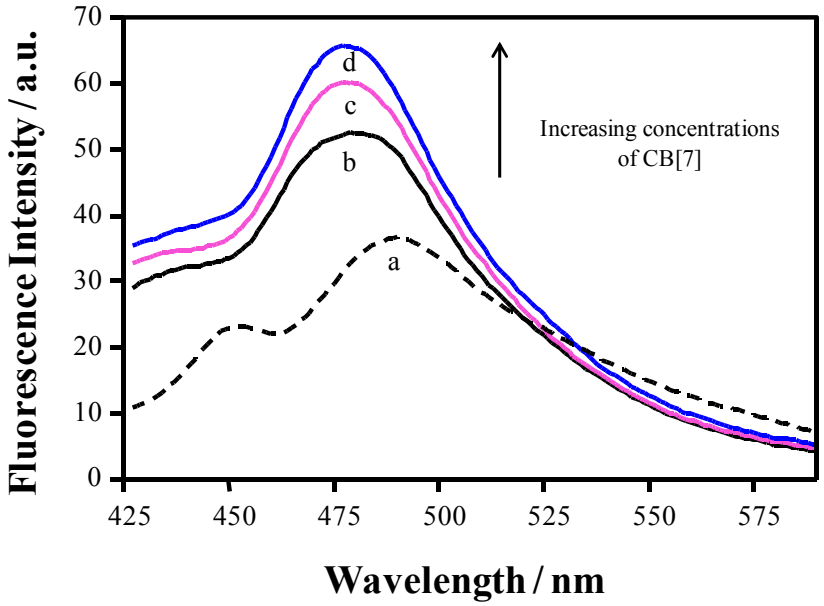


Figure 2. Fluorescence spectra of the coumarin-derivative **2** with CB7. (A) **2** dye (4.0 μM) with [CB7]/μM: (a) 0, (b) 50, (c) 100 and (d) 150; at pH = 5. Excitation and emission slits of 5 (nm) were used.

It is known that the encapsulation of organic dyes by hosts is often accompanied by the modification of physical properties of the encapsulated guests.[44] Indeed, previous reports showed enhancements or modifications in the fluorescence yield and lifetime of coumarin-derivative as consequences associated with the complex formation with the CB7 host.[43,45] Thus, from data shown in Table 1 we observe that the essentially non-fluorescent coumarin-derivative **1** ($\Phi_f \approx 0.013$) was converted into a highly fluorescent ($\Phi_f \approx 0.24$) entity in aqueous solutions and a similar effect was observed in acidic media. Probably, this implies that inclusion of **1** within CB7 may take place in our experimental conditions. In fact, other authors have reported that as a consequence of the trapping of the diethylamino group inside the hydrophobic cavity of CB7, the formation of a twisted intramolecular charge transfer (TICT) state is restricted.[44] The last mention would explain the increase of fluorescence emission quantum yield and negligible shift in the emission peak observed in this study for dye **1** in the presence of CB7.

Table 1. Photophysical parameters of the tested fluorescent coumarin-derivative dyes in the absence and presence of 200 μM CB7 in aqueous solution.

pH	[CB7] (μM)	λ_{abs}^{max}/nm	λ_{em}^{max}/nm	ε/(10^4 $M^{-1}cm^{-1}$)	Φ_{fl}	τ_1 /ns a_1(%)	τ_2 /ns a_2(%)
			Coumarin-derivative 1				
2	**0**	430	480	3.48	0.015	0.50(79.0)	3.20 (21)
2	**200**	445	480	6.46	0.299	0.58(99.4)	3.13(0.6)
5	**0**	430			0.013	0.50(64.0)	3.24(36)
5	**200**	445			0.241	0.59(99.3)	3.15(0.7)
			Coumarin-derivative 2				
2	**0**	390	490	4.29	0.009	0.89(99.8)	3.95(0.2)
2	**200**	455	485	3.43	0.014	1.05(97.1)	4.63(2.9)
5	**0**				0.005	0.89(97.0)	4.00(3.0)
5	**200**				0.012	1.16(96.2)	4.88(3.8)

On the other hand, Jones *et al*.[42] have reported that the fluorescence quantum yield of the coumarin-153, which structure displays a restricted amine similar to dye **2**, is

moderated (Φ_f < 0.4) in protic solvents such as water or ethanol but reaches higher values in nonhydroxylic organic solvents, like acetonitrile (Φ_f = 0.56), and is almost unity in the nonpolar cyclohexane (Φ_f = 0.9).[42] Therefore, considering the low polarity of the cavity of CB7, we propose that if the compound **2** were interacting with this cavity, a considerate increase in its quantum yield could be expected, which do not occur in our experimental conditions (see Table 1). The latter suggest that the dye **2** is not able to form an inclusion complex with CB7, or at least if so it would involve another group that is not the coumarin chromophore present in its structure.

To gain further understanding on the photophysical properties of coumarin-derivative **1-2** with or without CB7, experiments using time-resolved fluorescence at room temperature were carried out both, in the absence and presence of the host and in an aqueous and acidic media.

Fluorescence lifetime measurements were performed by a time correlated single photon counting (TCSPC) system at two different pH values. All the fluorescence decay profiles obey to a biexponential function, deducing the presence of two species in solution as shown in Figures S3-S4 (Supporting Information). The lifetime values obtained by the fitted curves are shown in Table 1. For the dye **1**, the shorter lifetime component of ~0.5 ns (τ_1) in aqueous solution was associated with the monomer of the dye as previously described by other authors for a compound containing a coumarin scaffold.[46] Regarding the longer lifetime component of ~3.2 ns (τ_2), it would be attributed to a dimeric form of the dye.[46] It is important to note, that when the pH decreases no variations in the lifetime components were observed, however, the free monomer contribution increases from 64% to 79%. We believed that this is likely a consequence of an electrostatic repulsion between the protonated moiety of 7-diethylamino binding to the coumarin at low pH which favors its monomeric form. In the presence of CB7, the dye **1** at pH=2.0 and 5.0 shows little increase in the lifetime values (τ_1) associated with the monomer compared with those obtained in the absence of CB7. Interestingly, the percentage of monomer associated with τ_1 increases considerably reaching around 99%, suggesting the formation of the dye-CB7 complex. These results are in agreement with previous report where macrocycles like CB7 were used to prevent the aggregation of coumarin.[16] Similar behavior was observed for the **2** dye, where no variations in the lifetime components of free monomer were noted in water at pH=2.0 and 5.0 (Fig. S4). However, in these systems the population associated

with the free monomer reaches 99.8% and 97%, respectively. The dye **2** increases its fluorescence lifetime from 0.89 ns for the dye free to 1.16 ns inside the CB7 cavity. At pH=2.0 such lifetime rose from 0.89 ns to 1.05 ns. In both systems, the augmentation in the fluorescence lifetime points towards the formation of dye-CB7 complex. These results are in line with other photophysical studies on the interaction of CB7 and coumarin derivative containing a bulky julolidinyl moiety.[43] Nevertheless, considering the low quantum yield values observed for the dye **2** in the presence of CB7 and the little variation in its lifetime values, we suppose that this dye would present a little restriction in its rotational and/or vibrational motion included in the cavity or a different structure of the dye-CB7 complex.

3.2 Stoichiometry and Association Constants of CB7 Inclusion Complexes. To gain further understanding on the stoichiometry that follows the interaction between added CB7 and the tested dyes, we carried out experiments using mass spectrometry. The mass spectrum of an equimolar solution (25 μM) of **1** and CB7 (Fig. 3A) shows a charged ion at m/z 1527.3. This signal was assigned to [CB7+**1**], which is the 1:1 host/guest complex, while the signal at m/z 1163.9 is typically associated with the presence of the free single protonated CB7 host[47] and the signal at m/z 364.7 corresponds to dye **1** alone. In the case of adding an equimolar concentration of CB7 on a solution containing the dye **2**, the 1:1 host/guest complex is also formed, as suggested in Figure 3B given by the signal at m/z 1551.9. The other signal observed at m/z 389.3 corresponds to the free guest **2**.

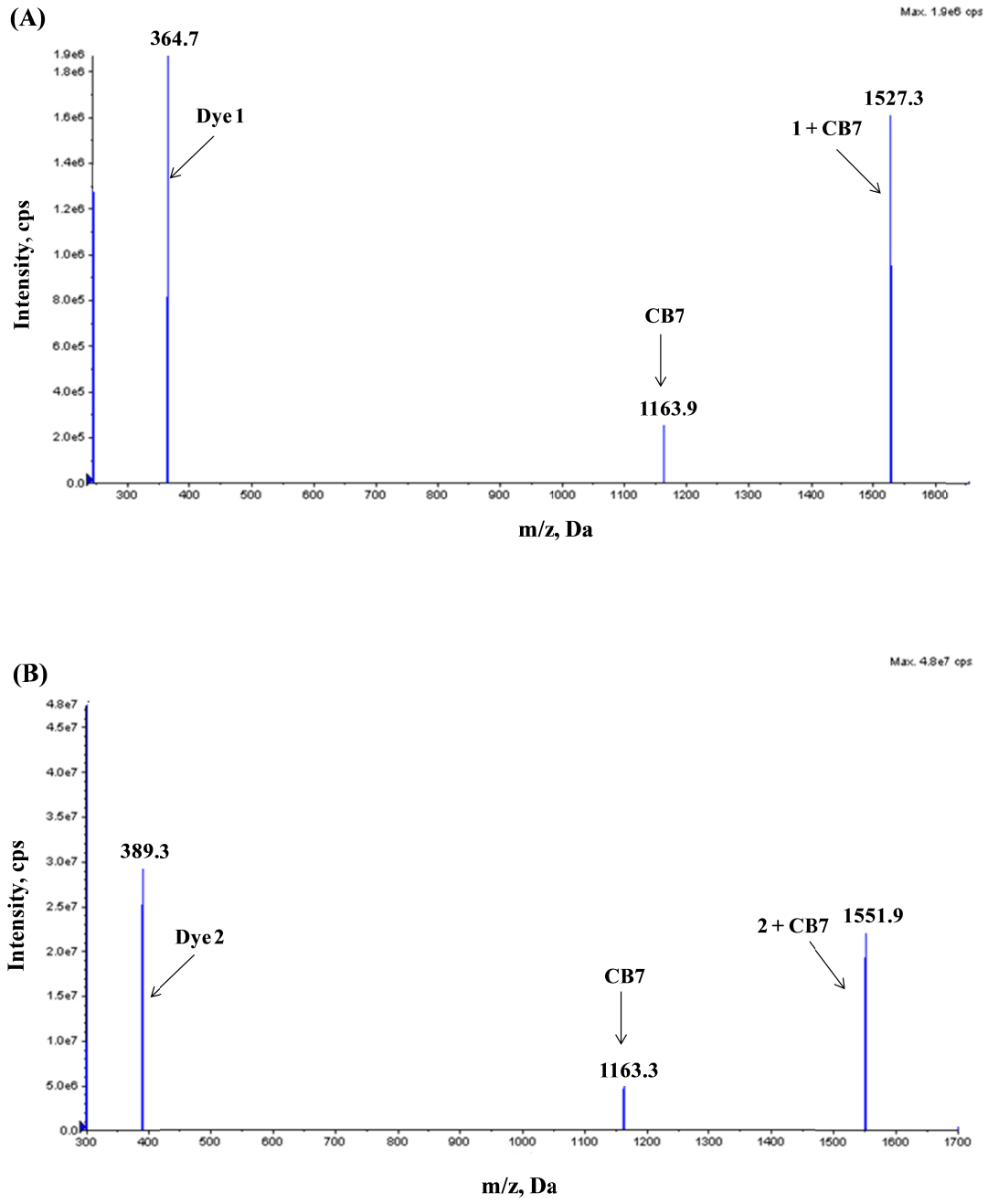


Figure 3. ESI mass spectra of a 1:1 molar ratio in aqueous solution of (A) coumarin-derivative **1** and CB7 and (B) coumarin-derivative **2** and CB7.

The association constant between tested dye **1** and CB7 was obtained by fluorescence titration experiments. In this case the concentration of **1** was maintained constant and the concentration of CB7 was increased between 0 and 50 μM. Inset to Figure 1(A) shows the variation of the fluorescence intensity of **1** with the concentration of CB7.

Fitting this data to a 1:1 binding model (see Supporting Information) an apparent equilibrium constant K_{1CB7} value of (3.3±0.4) $\times 10^5$ M^{-1} was determined (in aqueous solution). This value is higher than those reported for neutral coumarin[25] and it is comparable with other inclusion complexes containing guest like cationic amines.[27,43]

In the case of dye **2**, the stability constant (K_{2CB7}) for the inclusion of **2** in CB7 was obtained by competitive ^{1}H NMR experiments in D_2O, using tetrapropylammonium bromide (TPA) as competitor guest,[48] (representative spectra are shown in Supporting Information, Figs. S5 & S6). The value obtained by the competitive method was of (2.8±0.5) $\times 10^4$ M^{-1}, as described in Supporting Information.

3.3 ^{1}H-NMR Measurements. The interaction between CB7 and the tested dyes (**1-2**) were also studied by ^{1}H NMR spectroscopy, with the aim to obtain essential information about the structure of these complexes from the complexation-induced chemical shifts changes ($\Delta\delta=\delta_{bonded} - \delta_{free}$) of the proton resonances of the guest molecules. It is important to note that due to insolubility of **1** and **2** in D_2O, only the spectrum for the protonated form these dyes could be recorded in DCl(10%)-D_2O.

It is known that upfield shifts ($\Delta\delta<0$) are observed for protons located within the hydrophobic cavity, whereas deshielding and subsequent downfield shifts ($\Delta\delta>0$) of the guest protons are associated with the proximity of the host carbonyl oxygen atoms.[49,50]

As shown in Figure 4, the ^{1}H-NMR spectra of **1** in the presence of CB7 (3 eq.) depicts an upfield shifts of -0.7 ppm and -0.6 ppm for the $–CH_2$ and $–CH_3$ protons with respect to their original position, respectively, indicating that the $–NEt_2$ group is buried inside the hydrophobic cavity of CB7. Additionally, the aromatic protons (Figure S8) of coumarin scaffold H_{a1} (at δ 8.89, s) and H_{b1} (at δ 8.13, d, J = 7 Hz) showed an upfield shift, appearing as broad unresolved signals lower than 8.75 ppm in the presence of CB7.

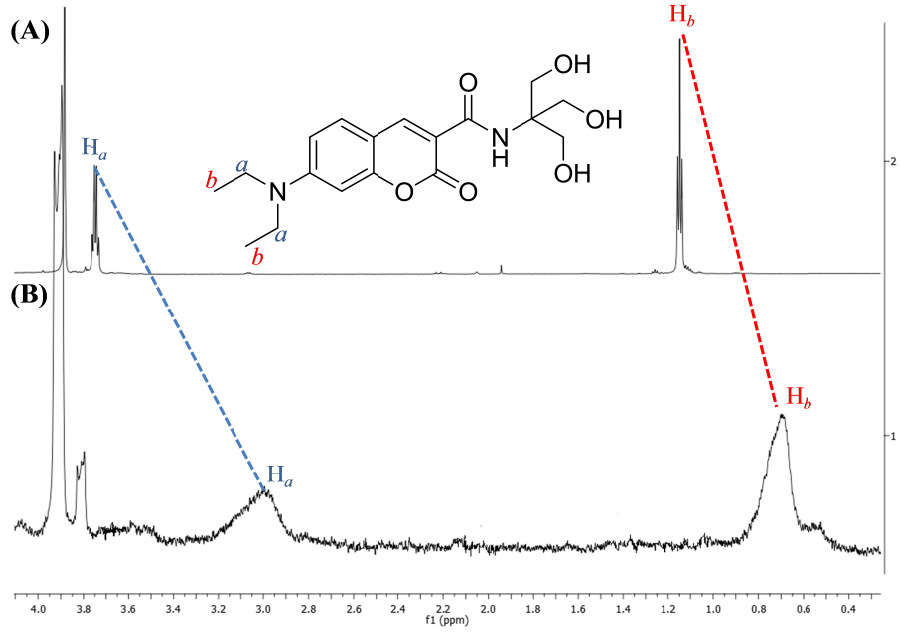


Figure 4. Partial ^{1}H NMR spectra (750 MHz) comparison for: (A) **1** alone in DCl (10%)−D_2O and (B) **1** and CB7 (3 eq.).

In contrast, the dye **2**, in the presence of ~3 equiv of CB7, displayed considerable complexation induced upfield shifts in the $–CH_2$ of 2-amino-2-(hydroxymethyl) propane-1,3-diol (tris) (δ 3.73-3.52) demonstrating that these protons reside in the hydrophobic shielding region of the cavity as shown in Figure 5. Other protons in the N,N′-diethylaminomethyl group and aromatic positions no were shifted, thus these results suggest that the site of interaction between **2** and CB7 is only the $-CH_2$ of Tris moiety. The latter could be a consequence of the steric effect, toward the inclusion, arising from the presence of bridged julolidinylaminocoumarin and it is in accordance with our results related to the absence of changes in the emission spectra for **2** (Fig. 2) and in its fluorescence quantum yield. In fact, other authors[43] have reported that the inclusion in the cavity of CB7 of coumarin derivatives is unlikely to contain a bulky julolidinyl moiety. Therefore, they suggest the formation of an exclusion complex between CB7 and the tested coumarin (2,3,5,6-1H,4H-tetrahydroquinolizino[9,9a,1-gh]coumarin) mainly through the interaction at the portal region of the host. Nonetheless, in our study the presence of Tris moiety in the structure of the dye **2** imply that the macrocycle only has the possibility to access to dye **2** *via* this group and to be able to form an inclusion complex.

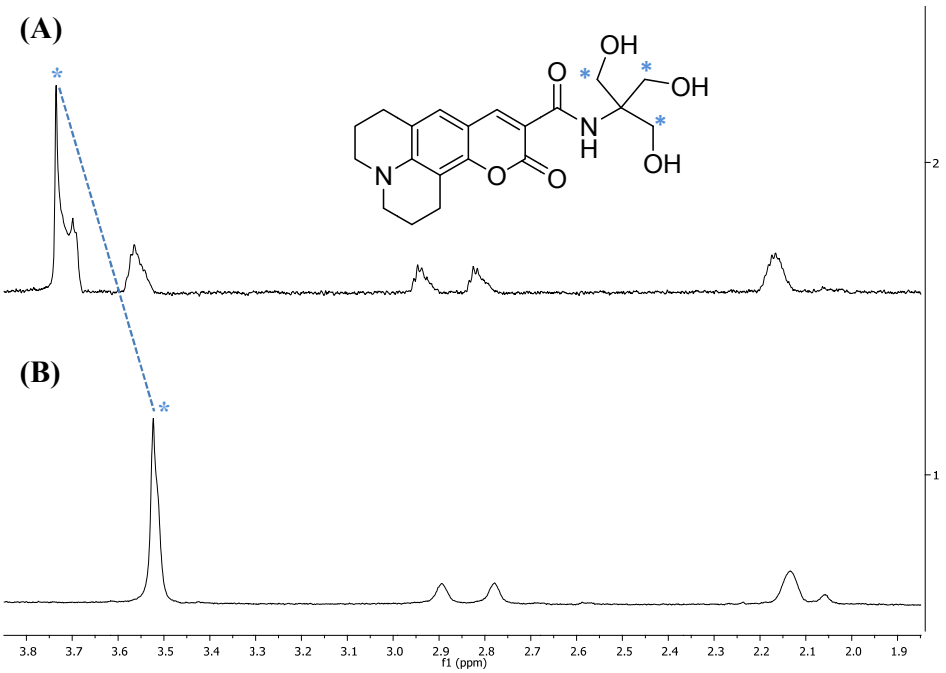


Figure 5. Partial ^{1}H NMR spectra (750 MHz) comparison for: (A) **2** alone in DCl (10%)-D_2O and (B) **2** and CB7 (3 eq.).

Thus, the results obtained from NMR spectra allow us to propose the positioning of CB7 on dyes **1** and **2** as is represented in Figure 6.

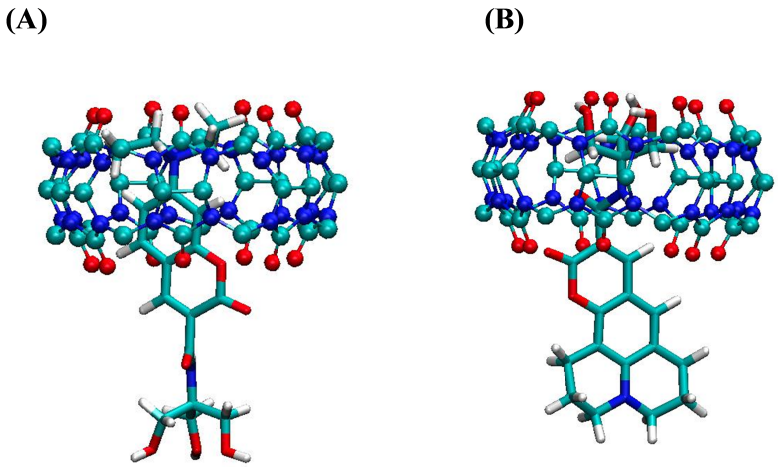


Figure 6. Schematic representation of the coumarin-derivatives tested and CB7: (A) dye **1** and (B) dye **2**.

3.4 Effect of mercuric ions. We took advantage of the above demonstrated complexation ability of the coumarin-derivatives (**1** and **2**) toward CB7 and of the various coordinating sites to metal ions present in them to develop novel assemblies

containing coumarin-derivatives as compounds capable to interact with mercuric ions. The last mention is based on our previously reported capacity to bind mercuric ions (Hg^{2+}) by a coumarin-derivative also containing a Tris moiety.[38]

Thus, the photophysical behavior of the tested dyes in the absence and presence of CB7 was examined upon addition of mercuric ions in aqueous solution and in acidic media. As shown in Figure 7, the emissions intensities of **1** and **2** alone at 480 and 490 nm, respectively, is accompanied by a gradual fluorescence quenching upon addition of increasing concentration of Hg^{2+} ions. The quantum yield obtained for a solution containing **1** (Φ = 0.24) decreased after the addition of mercuric ions (Φ = 0.13). No shifts of the fluorescence band were observed in the presence of this ion (not shown).

The association constants between tested dyes with mercuric ions were calculated for a 1:1 stoichiometry using fluorescence changes through the Benesi-Hildebrand equation.[51] It is important to note that at low concentration of the tested dyes, this equation can be used.[52-53]

$$\frac{1}{P - P_0} = \frac{1}{K_n(P_n - P_0)}\frac{1}{[Hg(II)]^n} + \frac{1}{P_n - P_0} \qquad \text{(Eq. 1)}$$

In equation 1, P_0 and P_n are the fluorescence intensities or absorbencies for the free and bound dye, respectively and K_n represents the binding constants of the 1:*n* (mercuric ions:dye) complexes. The Benesi-Hildebrand type double reciprocal equation should give a straight line when a proper value of *n* is applied in equation 1.

As seen in Figure S9 a plot $1/(P - P_0)$ vs $1/[Hg^{2+}]_0$ is linear ($R > 0.98$). This implies that the 1:1 complexes are formed between mercuric ions and the tested coumarin derivatives (**1** and **2**). The binding constants toward mercury ions ($K_{1:1}$) obtained are presented in Table S1. In our experimental conditions (*i.e.* in aqueous solution and at pH=2) and in the absence of CB7 no significant differences were found for the binding constant values.

On the other hand, the response of the host-guest supramolecular assembly **1**-CB7 to Hg^{2+} was evaluated by addition of 10 equiv. of CB7 in aqueous solution to **1**, which ensures all molecules of **1** are incorporated into the host. As shown in Figure 7, the fluorescence signal induced by dye **1** is quenched after addition of 50 equiv. of Hg^{2+} (at pH=2). However, in the presence of **1**-CB7 only 20 equiv. of Hg^{2+} ion were required to quench a similar level of the signal. These results suggest that the CB7 host would exhibit a beneficial cooperative effect of **1** toward mercuric ions mainly at pH=2. This

effect could be explained considering that the dye **1** in acidic conditions would be protonated in its 7-diethylamino group; thus, there is a high affinity towards CB7 that would lead to an efficient response of the **1**-CB7 complex to interact with the metal ion. In fact, an apparent binding constant of (1.6±0.6) × 10^7 M^{-1} was determined for **1**-CB7 plus mercuric ions (Fig. 7). This constant value is almost three orders of magnitude higher than the one obtained for 1-Hg^{2+} in the absence of CB7 and for **1**-CB7 alone. Therefore these results support the concept of a positive cooperative effect between them and is in line with a previous study conducted by Nau *et al*. (2011)[36] in which the ligation affinity of other metal ions to a complexed azoalkane by CB7 considerably increase the binding constants as a consequence of the added ion–dipole interactions to the host. This cooperative effect, in our study, would be responsible for annuling the possibility of a shift of coumarin **1** from the CB7 cavity.

On the other hand, similar quenching effects induced by Hg^{2+} and apparent binding constant values (Table S1) were obtained when dye **2** instead of **1,** was evaluated in aqueous solution and at pH=2, by comparison between the absence and presence of CB7. These results rule out a dissociation of the host–guest complex induced by mercuric ions and suggest a cooperative action of these ions *via* complexation between CB7 and the dye.

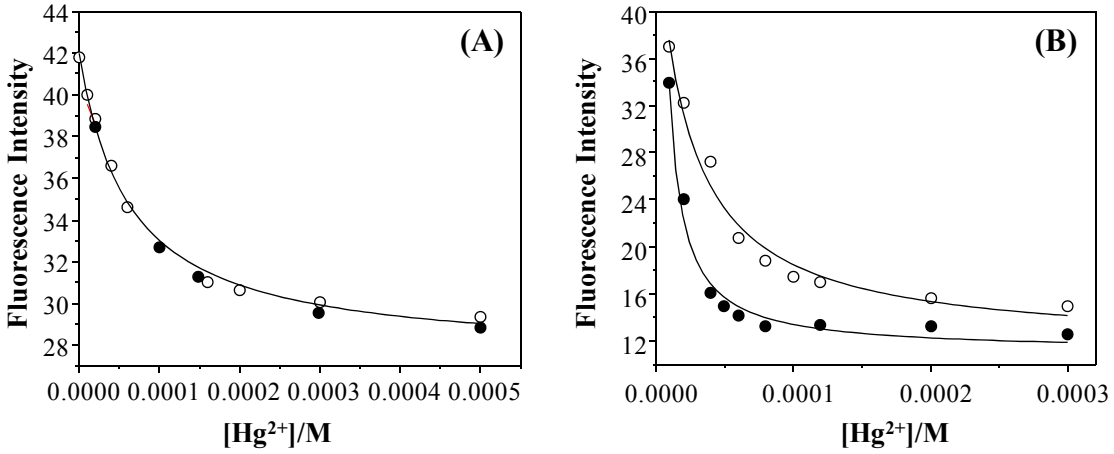


Figure 7. Fluorescence response of **1** (2 μM) to Hg^{2+} ion, under the form of $HgCl_2$, in the presence (black circles) and absence (open circles) of 20 μM of CB[7] in aqueous solution (A) and at pH = 2 (B). Excitation and emission slits of 2.5 (nm) were used.

Control experiments using **1** and mercury (II) acetate demonstrated the same quenching effect.

To elucidate, in each case, the possible chemical nature of the Hg(II)-containing complexes, we conducted ^{1}H-NMR experiments. Figure 8 depicts the partial ^{1}H NMR spectra of **1** upon the addition of Hg^{2+} ions in the absence and in the presence of CB7. Notably, when 1 equiv of Hg^{2+} was added, the signal of H* (Δδ, 0.1; Fig. 8) of the CH_2 protons of the Tris moiety present in dye **1** was shifted downfield however, the coumarin signals are practically unchanged. These results were in accordance with those of a previous study where other coumarin-derivative containing a Tris moiety was asses in the presence of mercuric ions.[38] Thus, we propose that this shift would be a consequence of the deshielding effect created by the binding of dyes **1** with the metal ion.

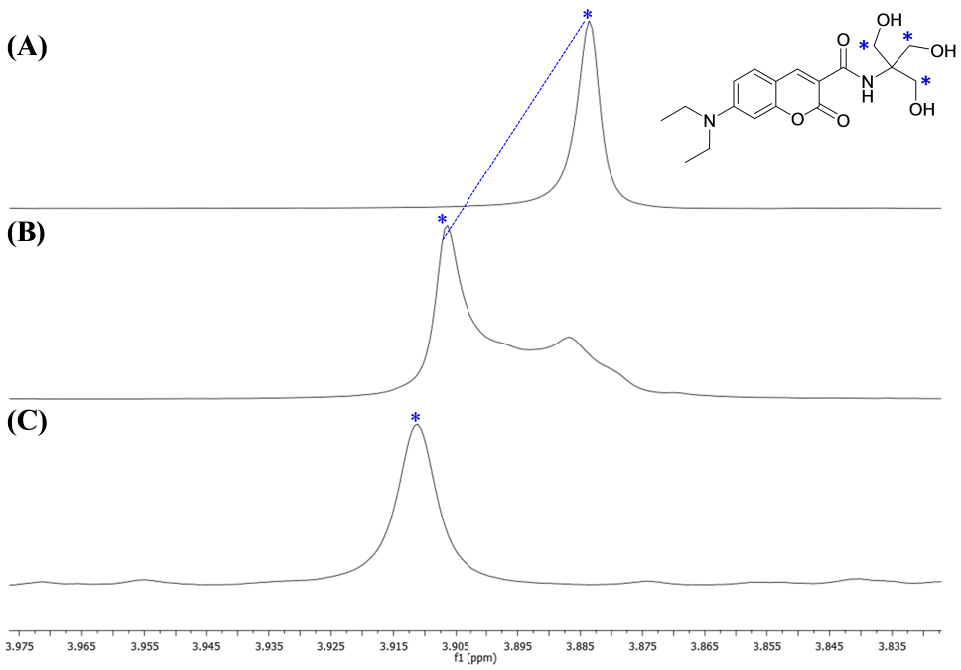


Figure 8. Partial ^{1}H NMR spectra (750 MHz) comparison for: (A) **1** alone in DCl (10%)-D_2O, (B) **1** and $HgCl_2$ (5 eq.) and (C) **1**, CB7 and $HgCl_2$.

In the case of this dye when CB7 is present, the position of the carbonyl portal from host would increase the negative charge density around this guest and influences the binding to the mercuric ions. Almost certainly the complexation of Hg(II) ions only occurs at the ureido rims and the oxygen present in the Tris moiety and, as a

consequence, a ternary supramolecular complex composed of host, included guest, and associated metal ion would be forming. It is important to note that the changes depicted in the NMR spectra of Figure 4, for the $–CH_2$ and $–CH_3$ protons of 7-diethylamino group after the inclusion in CB7, remain unaltered in the presence of mercuric ions. Thus, the interaction between the macrocycle and the dye exists even when the metal was added.

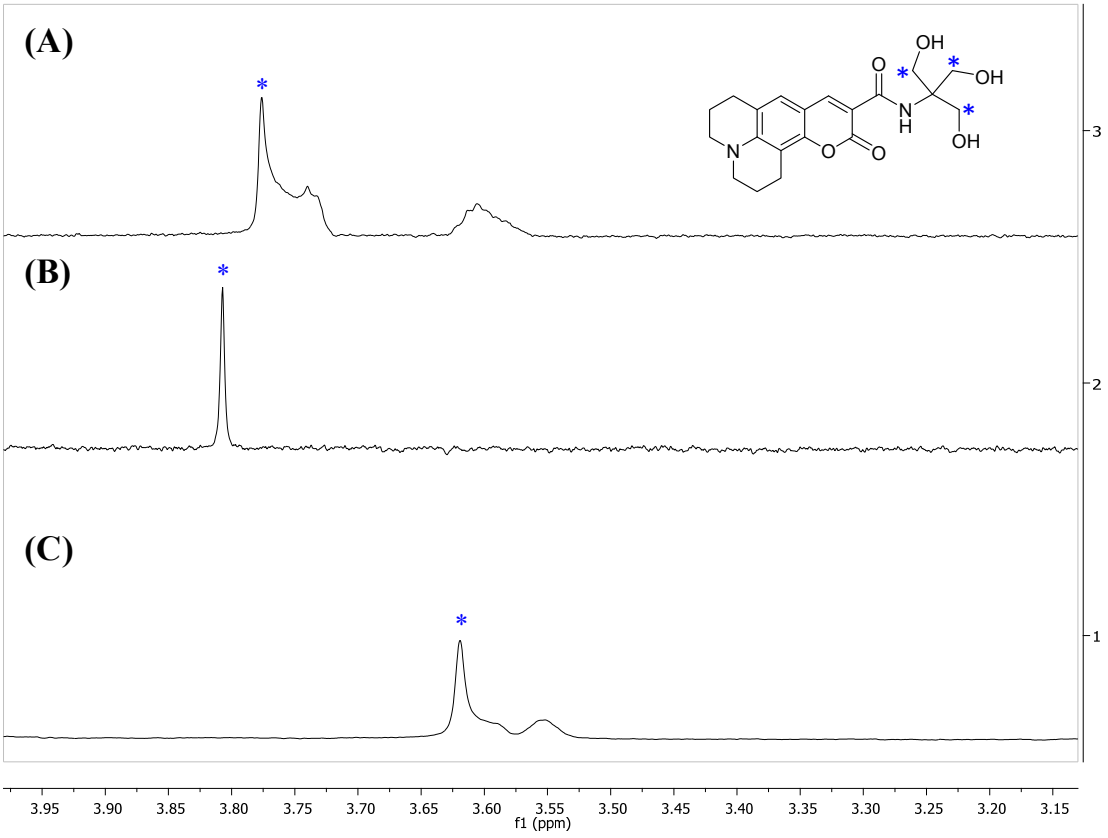
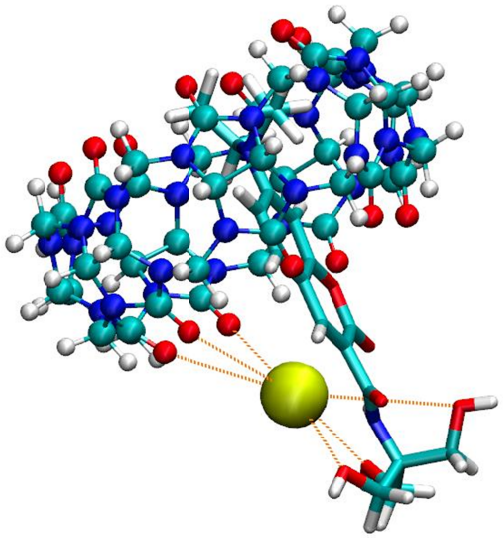


Figure 9. Partial ^{1}H NMR spectra (750 MHz) comparison for: (A) **2** alone in DCl (10%)−D_2O, (B) **2** and $HgCl_2$ (5 eq.) and (C) **2**, CB7 and $HgCl_2$.

Considering the previously mentioned, the probable structural arrangement for the ternary complexes is presented for dye **1** in Scheme 2.



Scheme 2. Schematic representation of the ternary 1-Hg^{2+}-CB7 complex.

According to the results depicted from Figures 5 and 9, in the case of the **2**, its Tris moiety is able to enter the cavity of CB7 and also of coordinate the metal. Thus the terminal portals keep the partial coordination from the dye toward mercuric ion.

Based on all above-mentioned results, our future work is aimed to develop novel components of more complex sensing systems toward mercury ions, which might potentially function in biologically relevant aqueous media.

4. CONCLUSION

Supramolecular interactions of the macrocycle, CB7 with the coumarin-derivatives dyes **1** and **2**, have been investigated in aqueous solution using ground state absorption, steady-state and time-resolved fluorescence measurements. In the presence of CB7, distinct differences have been observed in the photophysical characteristics of **1**, suggesting the formation of host–guest complexes with 1:1 stoichiometry. However, for the dye **2** in the presence of CB7, no differences were observed as a consequence of the moiety involved in the formation of its inclusion complex. In both cases, CB7 is able to prevent the formation of dimeric aggregates of coumarin.

Interestingly, this study also demonstrates a cooperative novel response of metal ion binding with tested CB7-coumarin derivative complexes, forming ternary supramolecular complexes. The last mention became feasible due to the stoichiometry and the structural arrangement of the host-guest complex with the CB7 portal due to its

strong negative charge density over the metal ions therefore sealing the complexes and protecting the incorporated dye.

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