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**Fluorescence quenching of *p*-tert-buthylthiacalix[4]arene by
1-ethyl-3-methylimidazolium bromide**

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Abstract

The interaction between the ionic liquid 1-ethyl-3-methylimidazolium bromide (**EMIMBr**) and the macrocyclic compound *p*-tert-buthylthiacalix[4]arene (**TC4**) in solution ethanol/water 3/1 V/V was investigated by spectrophotometry and spectrofluorimetry. The UV-Vis spectra of the system (**EMIMBr** + **TC4**) were completely additive. The addition of **EMIMBr** decreases the fluorescence of the **TC4** significantly, excited at 325.0 nm. The Stern-Volmer quenching constants were determined at different temperatures (15.0, 25.0, and 35.0 °C), and the obtained values were the same, with an average value of $K_Q^{av} = (3.8 \pm 0.9) \cdot 10^3 \text{ M}^{-1}$ for the quenching constant. No changes in the emission spectra of **TC4** were obtained by the addition of inorganic salts, such as **NaCl**, **KBr**, **NH₄Cl**, and **NH₄PF₆** or the organic compound *N*-methylimidazole (**NMI**). More evidence of **EMIMBr-TC4** interaction was found with the determination of the fluorescence lifetime and the anisotropy of the mixture. Also, the slope of the quenching plot was the analytical sensitivity of calibration for **EMIMBr** using **TC4** as an indirect sensor, and a LOD = (26 ± 2) μM was obtained.

Keywords: ionic liquids; macrocycle; fluorescence; quenching; ethanol-water solution.

1. Introduction

Over the past few decades, molecular vessels have been the focal point of numerous studies due to their potential application in catalysis, stabilization of reactive intermediates, drug

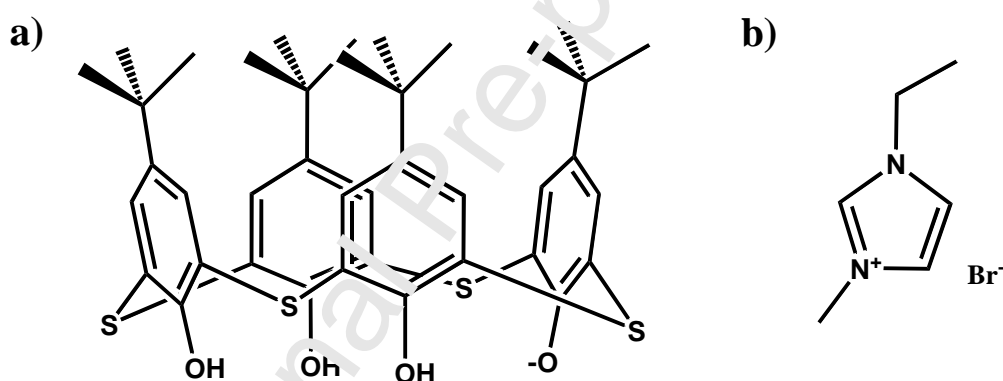
delivery, self-assembly properties, and sensing of ions and small molecules [1-3]. Alkylcalix[n]arenes (**CAn**) are basket-shaped metacyclophanes synthesized by condensation of *p*-alkyl-phenols (n units) and formaldehyde in the presence of alkalis [4,5]. Otherwise, the thiacalix[n]arenes (**TCn**) are **CAn** in which the methylene bridge has been replaced by a sulfur (S) bridge. An interesting macrocycle structure and electronic characteristics are shown due to the presence of the S atom, showing, for example, a different reactivity than their homolog **CAn** [6,7]. These macrocycles have a dual characteristic as receptors and polyprotic acid–base compounds [8,9]. Due to the hydrophobic character of the substituents, the solubility of *p*-alkyl-phenol-macrocycles in water is negligible [10,11]. The literature available of experimental results involving **CAn** in organic or mixture of organic solvents is extensive [12,13], but reports in aqueous–organic media (for example: in ethanol/water 95/5 V/V [8] are minimums. This fact is mainly attributable to the reduced solubility of the alkyl-macrocycles in these media. Also, an additional difficulty is the less precise pH measures in organic media that are necessary to establish the acid-base species present. Recently results of **TC4** in ethanol/water 75/25 V/V at different pH values have been informed by our lab [14].

Ionic liquids (**IL**) are considered as environmentally benign solvents, compared with the traditional volatile organic compounds (VOCs). They have high thermal stability (decomposition temperatures higher than 300 °C), negligible vapor pressure, non-flammable, excellent dissolution performance for organic, inorganic, and polymer materials, and easily tuneable properties [15-17]. Recent studies have shown that some IL have a wide range of toxicity, and the use of the term “green solvents” is starting to be questioned [18,19]. However, their multiple properties, especially their solubilization properties, make these compounds of great interest to explore their interaction with highly hydrophobic alkylated macrocycles.

Supramolecular complex formation involving **IL** and macrocyclic host molecules (e.g., calix[n]arenes, resorcin[n]arenes, pyrogallol[n]arenes, among others) represents an area of great potential interest for extractions, pharmaceuticals, chemosensing, chromatography, and

environment-responsive composites [20-22]. Until now, the research dealing with the interaction between **IL** and macrocycles as cyclodextrins, calixpyrroles, cucurbiturils, and calixarenes have been scarce [23-25].

This work aims to give insight on the type of the interaction between 1-ethyl-3-methylimidazolium bromide (**EMIMBr**) and *p*-tert-butylthiacalix[4]arene (**TC4**) (Scheme 1) in solution as the first step for further studies in supramolecular and analytical chemistry. The changes in the absorption and the fluorescence of **TC4** by the addition of the **EMIMBr** were analyzed. Moreover, these results were differentiated of salinity and polarity effects determining the fluorescence changes of **TC4** produced by organic and inorganic compounds.



Scheme 1: a)

Structure of **TC4** at pH = 6.00 and b) **EMIMBr**.

2. Experimental

2.1. Reagents

p-tert-butylthiacalix[4]arene (**TC4**) was synthesized and characterized in our laboratory following previously reported methodology [6,14]. 1-ethyl-3-methylimidazolium bromide (**EMIMBr**, Aldrich) was dried by lyophilization in a high vacuum at (-52) °C for three days before use. Absolute ethanol HPLC grade (Sintorgan) was controlled by UV-Vis and fluorescence spectrophotometric measurements and used as received. All salts, NaCl (Taurus A.C.S.), KBr (Merck ≥99%), NH₄Cl (Cicarelli A.C.S.), and NH₄PF₆ (Aldrich ≥95%) were used as received. N-methylimidazole (**NMI**, Sigma 99%) was dried and kept under molecular sieves

4 Å. The buffers used for the calibration of the pH-meter, the basic, and the acid solutions were prepared from commercial reagents of analytical grade.

2.2. Instruments

The pH of the solutions and the solvent mixtures were determined with a Thermo Orion model 520 pH-meter with a Ross combination pH electrode. The pH-meter was calibrated using standard buffers (pH 4.008, 6.995, and 9.155) prepared according to the literature [26]. In all experiments, the temperature was maintained at (25.0 ± 0.1) °C with a thermostatic bath.

UV–Vis absorption spectra were recorded on a Shimadzu UV-1800 spectrophotometer with cell temperature control. Fluorescence spectra were acquired on an Agilent Cary Eclipse spectrofluorometer equipped with an Agilent Cary single-cell Peltier for cell temperature control. Fluorescence anisotropy spectra were recorded with a Cary Eclipse manual polarizer accessory. Time-resolved fluorescence measurements were taken with a Deltaflex Horiba Spectrofluorometer. The experimental data were analyzed by Origin 9.0 software.

2.3. Spectrophotometric measurements and spectra analysis

UV–Vis analysis was conducted with quartz cells of reduced volume and masked black walls with different path lengths (1 or 4 cm) depending on the absorbance (A) value. The spectra were collected from 200.0 to 400.0 nm.

The fluorescence spectra of **TC4** and **TC4+Salts** or **TC4+EMIMBr** were collected using 325.0 nm as excitation wavelength (λ_{ex}) collecting the emissions from 355.0 (to avoid the signal of the excitation peak) to 600.0 nm, with excitation and emission slits of 10 nm. Then, the area of the emission spectra in this interval was calculated and plotted with Origin 9.0 software.

2.4. Fluorescence lifetimes measurements

Time-resolved fluorescence measurements were taken using a diode laser available ($\lambda_{\text{exc}}=267.0$ nm) for the excitation of the samples and scanning at a $\lambda_{\text{em}}=340.0$ nm at 25.0 °C. Previously, the fluorescence emission spectra were recorded at this λ_{exc} showing the maximum emission at 340.0 nm. The best fits (ChiSq ~ 1) were obtained with 3rd order exponential calculated by the instrument software.

2.5. Steady-state fluorescence anisotropy measurements

Fluorescence anisotropy spectra were recorded at $\lambda_{\text{em}}=360.0$, $\lambda_{\text{exc}}=325.0$ nm and 25.0 °C. The anisotropy was calculated by the instrument software (eq 1).

$$\text{Eq. } r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}} \quad 1$$

I_{VV} and I_{VH} correspond to the vertically and horizontally polarized components of the emission after excitation by vertically polarized light. G is the instrumental correction factor and was determined according to the instrument instructions [27].

2.6. General procedures

The solvent mixture was prepared by mixing absolute ethanol HPLC grade with Milli-Q water in a volume proportion of 3/1 V/V, respectively. The pH of the mixture was carefully adjusted to 6.00 at 25.0 °C by using HCl and NaOH solutions (0.1 M) without altering the volume fraction of ethanol and water.

A concentrated solution of **TC4** in chloroform (10 mg/5 mL), were stored in the refrigerator (4 °C) sealed with a PTFE cap and Parafilm®, and entrapped with aluminum foil. The stability of the stock solutions was periodically checked by spectrophotometry before preparing the appropriate dilute solutions for the measurements. The dilute solutions were made

with no more than 1 % V/V of the concentrated solutions (chloroform) in ethanol/water 3/1 V/V. This is the optimal proportion of solvents supported for the high hydrophobicity of the macrocycle and the required stability of the pH meter measurements [14].

The final solution of **TC4** (0.5 μM) were done containing 1.6 μL of the concentrated solution of the macrocycle in a final volume of 10.00 mL (final percentage of CHCl_3 0.02%); completing the volume with ethanol/water 3/1 V/V after the addition or not of some other reagent. The final concentrations of **EMIMBr** (8 to 96 μM) were reached by the addition of an appropriate volume (13 to 160 μL) of a 6.0 mM solution prepared in the solvent mixture from a more concentrated solution (1.0 M) in the same medium.

For the experiments of **TC4** with a variable concentration of **EMIMBr**, the solutions were prepared at least 30 minutes before the spectroscopic measured. For the experiments at variable temperature, each solution at a determined **EMIMBr** concentration was maintained at 15.0 $^{\circ}\text{C}$ for 20 minutes before taking the spectrum. Then, the same solution was maintained at 25.0 $^{\circ}\text{C}$ for 20 min before taking the spectrum, and this procedure was repeated at 35.0 $^{\circ}\text{C}$. The times informed are the average times that all the solutions of a series were completed and thermostated. However, changes in fluorescence were not observed in shorter times for individual solutions.

For the experiments in which the concentration of salts or **NMI** was varied while **TC4** concentration has remained constant, 3 mL of 0.5 μM solution of **TC4** in the solvent mixture was added to the quartz cell with a 3 mL ball pipette. Then, small volume additions (a few μL) of 6 mM solutions of salts or **NMI** in the solvent mixture were added. Consecutive volume addition of salts or **NMI** solutions was aggregated to achieve the final concentrations to be examined. The total volume added for the higher concentration was less than the 1.5 % of the total volume, so dilution of the **TC4** solutions was not considered.

All the experiences were done at least by triplicate, stabilizing the temperature of the mixture before the spectroscopic measured. The fluorescent values informed (F) are relative values

(F_i/F_r) determined as the ratio for a particular solution (F_i) respect to the selected reference (F_r), to avoid instrumental errors. The variation of (F_i/F_r) triplicates is around 10%. So we consider changes above this value as possible real effects.

3. RESULTS AND DISCUSSION

3.1. Spectrophotometric and spectrofluorimetric study

3.1.1. General Considerations

Considering the pK_a value for the first phenol dissociation of **TC4**, $pK_{a1} = 5.10$ [14], it is assumed that at $pH = 6.00$, the majority of the macrocycle molecules have one phenolate ion, and three not ionized phenol groups. This fact was explored because the substrates used are very polar compounds, and the negative charge could improve the interaction between these compounds and the macrocycle. The conditions of solvent mixture and pH were previously optimized in our laboratory [14] to solubilize **TC4** with environmentally friendly solvents no so contaminant as chloroform or other halogenated compounds.

3.1.2. Effect of the addition of **EMIMBr** on the absorbance of **TC4**

Firstly, the study of the effect on the absorbance and fluorescence of **TC4** induced by the addition of **EMIMBr** was examined. It was not possible to observe changes on UV-Vis spectroscopy, even at a final concentration of **EMIMBr** of $112 \mu M$. As can be seen in Figure 1, the spectra of the mixture **TC4+EMIMBr** was additive.

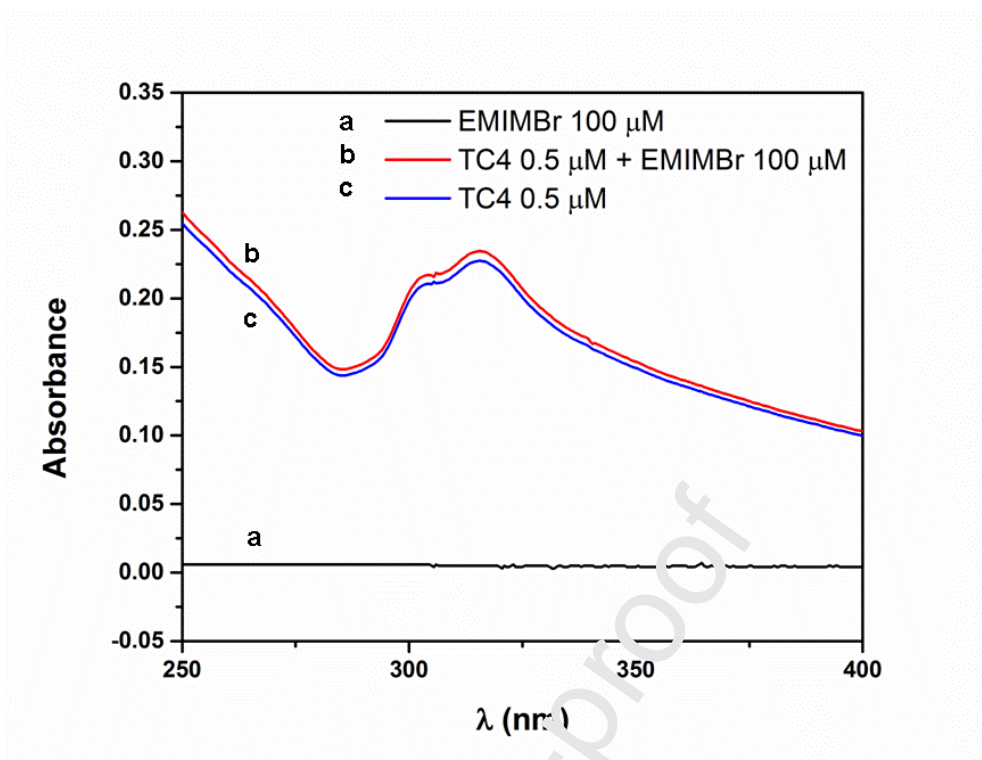


Figure 1. UV-Vis spectra of (a) **EMIMBr** = 100 μ M, black line; (b) mixture of (**TC4** = 0.5 μ M + **EMIMBr** = 100 μ M, red line; and (c) **TC4** = 0.5 μ M, blue line; in solution ethanol/water 3/1 V/V at pH = 6.00 and 25.0 $^{\circ}$ C with optical length = 4 cm.

3.1.3. Effect of the addition of **EMIMBr** on the Fluorescence of **TC4**

Changes in the spectral behavior of **TC4** in the presence **EMIMBr** by fluorescence spectroscopy were observed (Figure 2). The fluorescence of **TC4** ($\lambda_{\text{exc}} = 325$ nm) was quenched by the addition of **EMIMBr**.

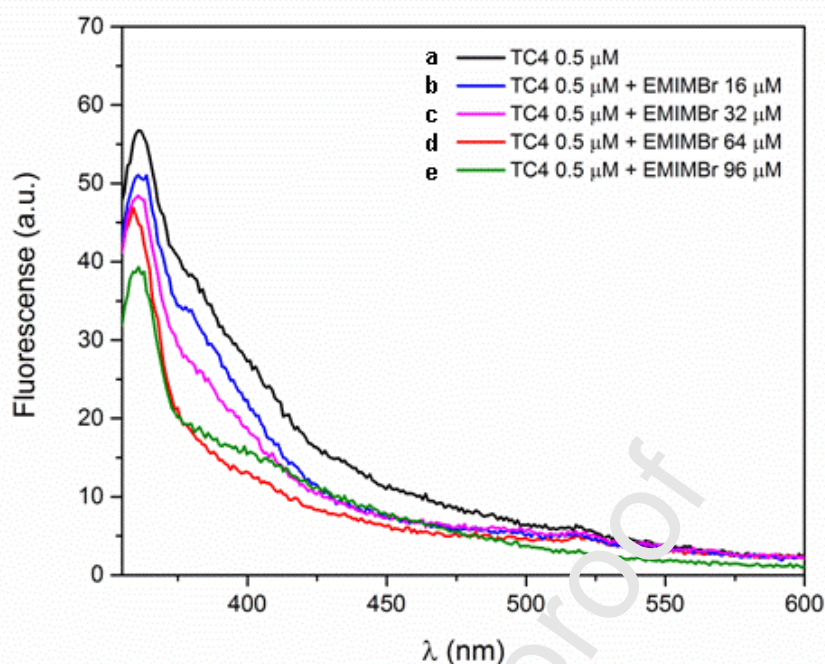


Figure 2. Fluorescence spectra of (a) **TC4** = 0.5 μM , black line; (b) to (e) mixtures of **TC4** = 0.5 μM with increasing concentrations of **EMIMBr** in the order that the F intensity at 363.0 nm decreases; in solution ethanol/water 3/1 V/V at pH= 6.0 and 25.0 $^{\circ}\text{C}$ with optical length = 1 cm.

These experiments were carried out at three different temperatures (15.0, 25.0, and 35.0 $^{\circ}\text{C}$) to understand if the quenching is of dynamic or static type (see Figure 2). The expected behavior for a static type of quenching is that temperature must not significantly affect the value of K_Q ; on the contrary, if quenching is of a dynamic type, variations in K_Q would be observed [27]. The dynamic quenching is a process in the excited state produced by the collision between the quencher and the substrate and $K_Q = (k_q \tau_0)$; at higher T, the k_q (collisional rate) increases and the τ_0 (the lifetime of the the unquenched substrate) is not modified [27].

Different amounts of **EMIMBr** were added to a solution 0.5 μM of **TC4**. Each solution mixture (0.5 μM **TC4** + x μM **EMIMBr**) was assayed at the three temperatures, letting a 20 min interval of

time to reach the correct temperature. The area ratios of the fluorescence spectrum of **TC4** (F_0) and the mixtures of **TC4** with **EMIMBr** (F) were plotted against **EMIMBr** concentrations (Figure 3).

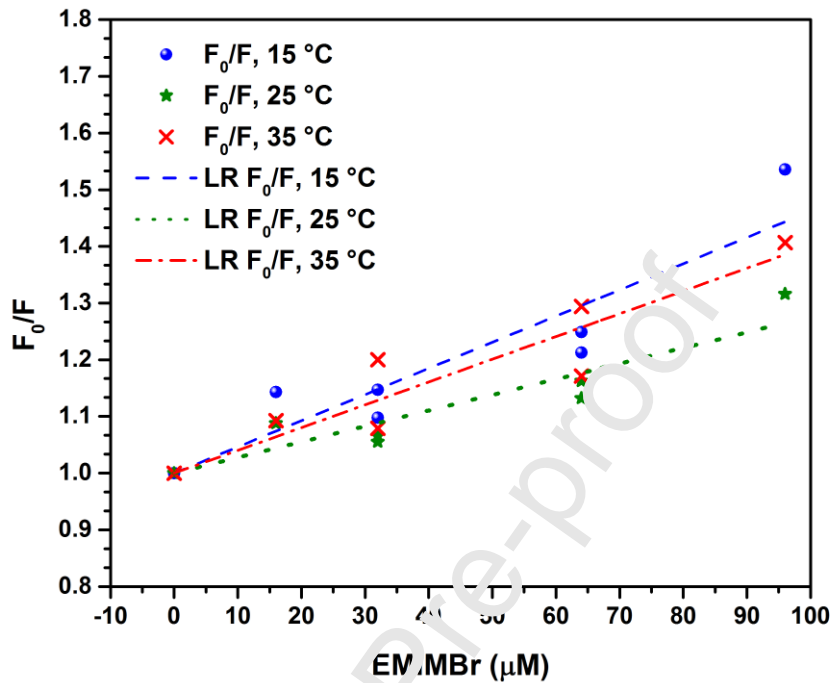


Figure 3. Quenching plot for the interaction between **TC4** and **EMIMBr** at 15.0 °C, blue circles; 25.0 °C, green stars; and 35.0 °C, red crosses; in solution ethanol/water 3/1 V/V at pH = 6.00 with optical length = 1 cm.

The ratio of F_0/F vs. **EMIMBr** concentration responds to a linear behavior, consistent with a Stern-Volmer fluorescence quenching (eq.2), and a linear regression was used to fit the experimental points to obtain the quenching constant K_Q [27].

$$\frac{F_0}{F} = 1 + K_Q[\text{EMIMBr}] \quad \text{Eq.2}$$

The values of the K_Q for **TC4-EMIMBr** at three different temperatures are summarized in Table 1.

Table 1: K_Q of **TC4-EMIMBr** determined by fluorescence at different temperatures

in solution ethanol/water 3/1 V/V at pH = 6.00

K_Q (10^3 M^{-1})	Temperature ($^{\circ}\text{C}$)
4.6 ± 0.5	15.0
2.8 ± 0.3	25.0
4.0 ± 0.4	35.0

As it can be seen in **Table 1**, there was no difference in the values of K_Q (**TC4-EMIMBr**) with an average value (K_Q^{av}) of $(3.8 \pm 0.9) 10^3 \text{ M}^{-1}$, indicating that the phenomenon observed is a static quenching due to the formation of a complex in the basal state [27]. Also, K_Q^{av} represents the average association equilibrium constants (K_A^{av}) for the complex **EMIMBr-TC4** in a 1:1 stoichiometric ratio [27].

Even though the experimental conditions (macrocycle, solvent, concentrations, techniques) assayed in the present work were very different compared with those employed by other authors, the K_A determined were very similar. For example, a K_A value of $6.6 10^4 \text{ M}^{-1}$ was determined for 4-sulfonatocalix[4]arene (**SCA4**) and 1-ethyl-3-methylimidazolium chloride (**EMIMCl**) and a lower K_A value of $1.7 10^3 \text{ M}^{-1}$ for 4-sulfonatocalix[6]arene (**SCA6**) and **EMIMCl** at pH = 2. The K_A was determined, in both cases, by competitive binding using the fluorescence decrease of the **berberine-SCA_n** complex as an indirect fluorescent probe, corroborating a 1:1 stoichiometric ratio [28]. On the other hand, K_A values of $1.2 10^5 \text{ M}^{-1}$ and $8.5 10^3 \text{ M}^{-1}$ for **SCA4** and **SCA6** with **EMIMCl**, respectively, were determined at pH = 6.5 by ITC determinations [29]. As can be seen, the K_A^{av} informed in the present work is of the same magnitude to those determined for **EMIMCl** with **SCA6** and lower than those determined with **SCA4**. In all these cases, the macrocycles support negative charges. In the case of **TC4**, a phenol group is dissociated at pH = 6, and in the cases of **SCA4** and **SCA6**, all the sulphonic acid groups are dissociated at pH = 2. Moreover, one or two phenol groups are deprotonated at pH = 6.5

for **SCA4** and **SCA6**, respectively [30]. The electrostatic interactions between the macrocycles and the **IL** are one of the driving forces for the complex formation [29]. Also, the tight fit between the guest and the host increases the binding stability. The ring of **TC4** is 15% bigger than **CA4** due to the longer bond C-S than C-C [7]. In consequence, the distance between the hydroxyls increases as is revealed by the increase in the OH stretching band in IR and a lower chemical shift ^1H NMR [14]. Regardless, as was previously mentioned, the experimental conditions are very different, and straight comparisons are not suitable between **TC4** and **SCA_n**.

3.1.4. Salt effects on the fluorescence of TC4

Because **EMIMBr** is an ionic compound, the quenching observed was distinguished from salinity and polarity effects carrying out experiments with the addition of salts to **TC4**. The salts employed were some of the most typical ones, such as **NaCl** and **KBr**, and other more complex salts that are commonly used as a counter ion in other ionic liquids like **NH₄Cl** and **NH₄PF₆**.

NaCl was the first obvious choice of compounds to test because **HCl** and **NaOH** were used to adjust the pH of the solvent mixture. As can be seen in Figure 4, the changes induced by the addition of **NaCl** (Figure 4, blue stars) were considered almost insignificant because there is a dispersion of around 5%. Nevertheless, **NH₄Cl** produced a minimum exaltation of fluorescence of **TC4** below 10% (Figure 4, red circles). Thus, **Cl⁻** presence was not the driving force for the changes observed with **NH₄Cl**. Instead, the presence of a bigger and more polarizable cation (**NH₄⁺**) produced the smallest changes observed in this case.

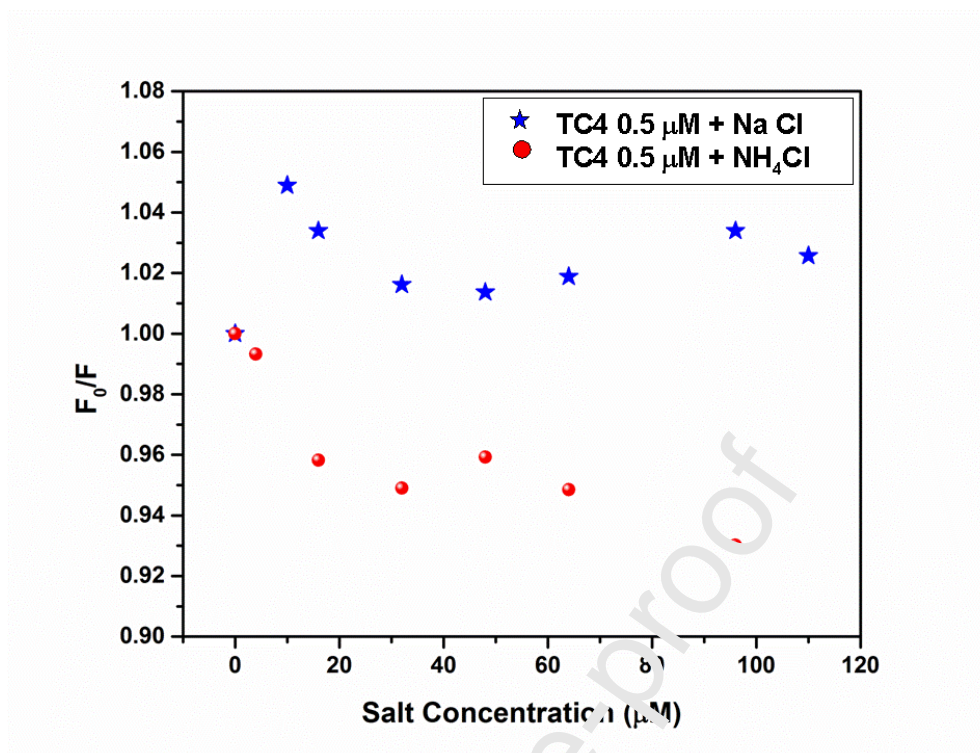


Figure 4: Effect on the fluorescence of TC4 of the addition of NaCl, blue stars; or NH₄Cl, red circles; in solution ethanol/water 3/1 V/V at pH = 6.00 and 25.0 °C with optical length = 1 cm

Consequently, a comparison between the changes in fluorescence of TC4 due to the presence of NH₄PF₆ (Figure 5, red circles) or NH₄Cl (Figure 4, red circles) was considered, since the common factor was the cation. Although the effects were minimal, these show a contrary behavior. We could conclude that NH₄PF₆ and NH₄Cl produced negligible changes in fluorescence (less than 10%).

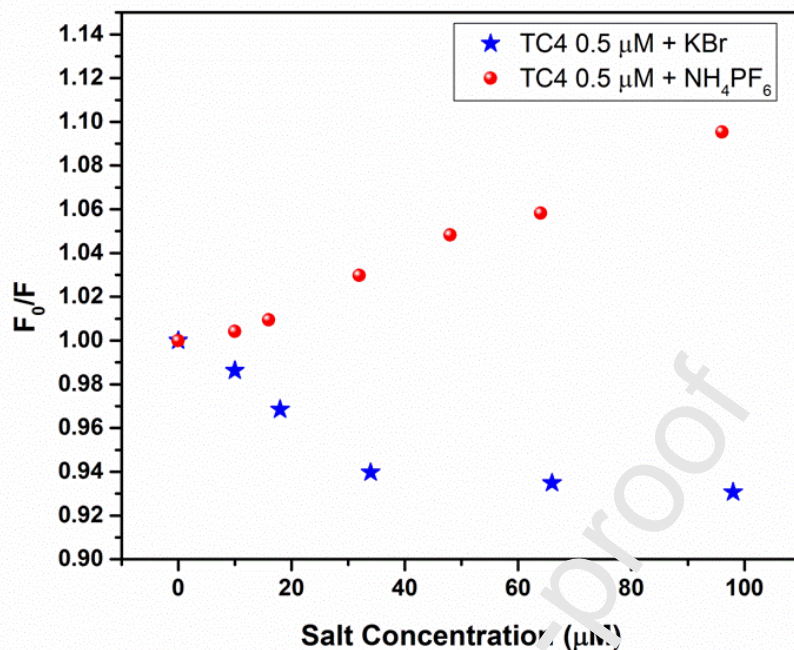


Figure 5: Effect on the fluorescence of **TC4** of the addition of **NH₄PF₆**, red circles; or **KBr** blue stars; in solution ethanol/water 3/1 V/V at pH = 5.00 and 25.0 °C with optical length = 1 cm.

In the case of **KBr** (Figure 5, blue stars), the choice was founded in visualizing if the changes observed for **EMIMBr** was due to the presence of bromide counter-ion. That assumption can be neglected because the difference was also below 10%, but also the change was more like an incipient exaltation of the fluorescence of **TC4**. The major shift was observed at 36 μM of **KBr**, and the ratio F_0/F remained unalterable without change up to 96 μM.

The insensitivity to the anion was also observed in the cases of the complexes alkyl-methylimidazolium and **SCA6** [28] and with cucurbit[7]uril [31]. In contrast, the anion dependence observed in the cases of alkyl-methylimidazolium and β-cyclodextrin was attributed to ion pair formation inside the cavity of the β-cyclodextrin [32]. But, it is neither possible in the case of cucurbit[n]uril, because of the negative charge density of the carbonyl portals, nor in the cases of the

negatively charged macrocycles **SCA_n** and **TC4**, since the interactions with the anion were avoided. Therefore, considering that bromide addition was not the driving force for the observed quenching of **TC4** with **EMIMBr**, the 1-ethyl-3-methylimidazole cation must be it.

3.1.5. Alkyl versus imidazole effects on the fluorescence of TC4

Additionally, to discern if the effect observed in the system **TC4-EMIMBr** was due to the imidazole core or if the aliphatic substituent plays a more critical role in the interaction with **TC4**, it was of interest to test what effect produces the addition of **NMI** on the fluorescence spectrum of **TC4**.

The pK_a of **NMI** is approximately 7 in this solvent mixture (6.993 in water) [33], so the majority of **NMI** must be as the protonated form at the pH of the solutions (pH = 6.00). Thus, **NMI** is a cation similar to **EMIM**, but with a subtle difference of an ethyl pendant group.

In Figure 6 can be seen that the addition of **NMI** produced minimum and different changes on the **TC4** fluorescence (enhancements lowers than 10%) in comparison with **EMIMBr** that produced quenching of **TC4** fluorescence (Fig. 3).

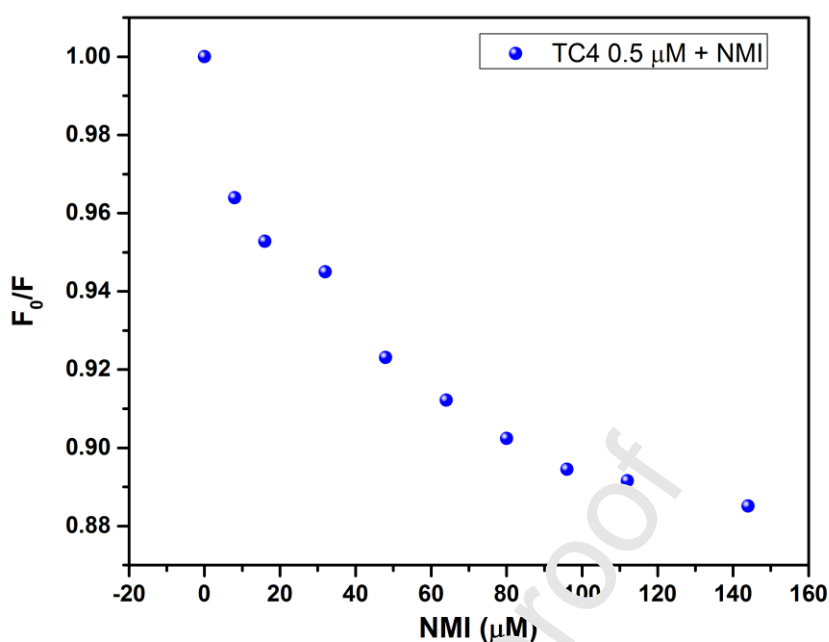


Figure 6: Effect on the fluorescence of **TC4** of the addition of **NMI** in solution ethanol/water 3/1 V/V at pH = 6.00 and 25.0 °C with optical length = 1 cm.

The chemical structures of the two substrates were compared to interpret these behaviors. Considering that **EMIMBr** has a more hydrophobic character, the effect of the ethyl group makes it possible that **EMIMBr** interacts probably more inside the cavity of **TC4**. The ethyl hydrocarbon moiety of **EMIMBr** could fit inside the **TC4** cavity due to the good fit between the hydrocarbon rest (diameter CH_2/CH_3 4.2 Å) [23] and the **TC4** cavity (~ 7 Å) [23]. In contrast, **NMI** has a more hydrophilic character and thus could interact better with the endo edge of **TC4**, forming stronger interactions with the OH groups without affecting the fluorescence of the macrocycle because of the superficial interaction.

The concepts of the hydrophobic interactions with the alkylic chain and the guest-host fit have been mentioned in the literature in a carefully revised study of the complex formation between cyclodextrins and alkyl-methylimidazolium [23], among other cations and anions. Although, in

previous papers, the inclusion of only the long alkylic chain [34], or the aromatic together with the short alkylic parts of the alkyl-methylimidazolium [24], has been proposed by NMR evidence.

Moreover, the hydrophobicity has been mentioned as the driven force in the case of the complexes of alkyl-methylimidazolium with cucurbit[7]uril for the inclusion of the alkylic chain with a less partial penetration of the methylimidazolium head and an increase of K_A for the alkylic chain from one carbon to six carbons [31] by NMR studies for ethyl- and butyl-methylimidazolium with cucurbit[6]uril [25].

The increase of the K_A with the length of the alkylic chain has also been observed in the case of the complexes between alkyl-methylimidazolium with **SCA6**. Moreover, the interactions cation- π and π - π between the imidazolium nucleus and the aromatic macrocycle have been mentioned as the dominant binding forces [29]. Nevertheless, upfield shifts in ^1H NMR were observed for all the protons in the complex for hexyl-methylimidazolium with **SCA6** [28].

In the examples mentioned previously the concentrations employed for the macrocycles and the **IL** were in the mM order, appropriated for NMR studies that give more experimental evidence for the mode of the inclusion. However, in the case presented in this study, the concentrations of **EMIMBr** and **TC4** were in the μM order due to the scarce solubility of the macrocycle. This level of concentration was optimal for the fluorimetric study but not for NMR experiments. In consequence, the structures of the complexes were proposed on the base of the results presented here and the interpretations from literature.

In addition, to reinforce these interpretations, measurements of fluorescence lifetimes and steady-state anisotropy were performed as follows.

3.2. Fluorescence lifetimes

As the quenching observed of **TC4** by the addition of **EMIMBr** was attributed to the formation of a ground-state complex (static quenching), the only fluorescent specie in the medium is the uncomplexed macrocycle. To confirm this hypothesis, fluorescence lifetimes of **TC4** in the absence and presence of the quencher, **EMIMBr**, were measured at conditions of maximum fluorescence quenching (**TC4** 0.5 μM + **EMIMBr** 90 μM). The results are shown in Table 2.

Table 2. Fluorescence lifetimes (ns) of **TC4** 0.5 μM and (**TC4** 0.5 μM + **EMIMBr** 90 μM).

Sample	$\tau_1 \pm s^\dagger$ (ns)	$\tau_2 \pm s^\dagger$ (ns)	$\tau_3 \pm s^\dagger$ (ns)
TC4	2.7 ± 0.2	17 ± 2	0.243 ± 0.009
TC4+EMIMBr	2.8 ± 0.1	18 ± 1	0.264 ± 0.003

† (s) is the standard deviation from triplicate measurements

Three fluorescence lifetimes were obtained for **TC4** and **TC4+EMIMBr**, and no significant differences were observed between them confirming the presence of the same emissive species and the formation of a non-fluorescent complex. Since there is not a new fluorescent lifetime, the mechanism of fluorescence quenching is interpreted as static [27,35].

Comparable results have been reported, since the addition of phenylalaninol did not modify the fluorescence lifetimes of propranolol-amide-calix[4]arene from a fluorescence static quenching phenomenon [36]. In this paper, the values of lifetimes obtained were of 9.4 ns and 4.6 ns in methanol. The different lifetimes were attributed to the conformations that can be adopted by the calixarene macrocycle since the naphthalene groups could be oriented in different positions depending on the calixarene conformer [36]. Also, an average fluorescence lifetime of 0.49 ns was determined for a calix[4]arene di methoxycarbonyl methyl ester, also owing to the fluorescence of the aryl moieties of the monomers [37].

In conclusion, the three fluorescence lifetimes obtained here correspond to the different conformers of **TC4**, as reported by some authors for other macrocycles, since this is the only emissive species existing in the medium.

3.3. Steady-state fluorescence anisotropy

Fluorescence anisotropy is a useful methodology that provides information about the changes in the mobility or flexibility of a fluorophore owing to the interaction or binding with a specific molecule [38].

The value of fluorescence anisotropy for **TC4** was 0.199 and 0.299 for (**TC4+EMIMBr**) at the **TC4** maximum of emission. The anisotropy of the fluorescent compound, **TC4**, is changing owing to the changes in the mobility or flexibility of either the fluorophore or the surrounding media, based on literature interpretations, as mentioned following.

For example, it has been reported an increase in the anisotropy of (9-*O*-(*n*-decyl)-berberrubine chloride) from 0.075 to 0.110 after the addition of 2 μM bovine serum albumin. This enhancement was attributed to the binding of the sensor to the protein that reduces fluorophore mobility [38]. Furthermore, the variations of anisotropy of α -(2'-hydroxyphenyl)-4-methyloxazole in fluid media and in the presence of β -cyclodextrin were zero and 0.115, respectively, as a consequence of a complex formation [39]. Moreover, the interaction between 1-pyrenesulfonic acid and human serum albumin protein was proposed through the changes in the fluorophore anisotropy from 0.01 to 0.10 after the addition of 6 μM of the protein [40].

3.4. Analytical concern

Besides, the plot of F_0/F vs. **EMIMBr** concentrations corresponds to the calibration curve for the determination of **EMIMBr** by an indirect fluorimetric method using **TC4** as the sensor. The analytical sensitivity of calibration (m) is the slope of the plot $(3.8 \pm 0.9) 10^3 \text{ M}^{-1}$. The standard deviation of the regression (s_r) was used as the standard deviation of the blank (s_B). In the base of these parameters, the LOD was calculated as $(26 \pm 2) \mu\text{M}$ as recommended for the IUPAC $(3.29 \times s_r / m)$ [41]. The selectivity

of the method has been demonstrated by the small effect (below 10%) on the fluorescence of **TC4** by the addition of inorganic salts and **NMI** in the previous subsections. Since this **IL** has not owned UV-vis absorption or fluorescence, the quenching of fluorescence produced on **TC4** represents an analytical application.

Finally, up to our knowledge, this work is the first report that evidences the supramolecular complex formation between **TC4** and an ionic liquid by direct spectrofluorimetry and represents a great starting point to further studies and applications.

4. Conclusions

The formation of a complex **EMIMBr-TC4** in the ground state is proposed in the base of the static Stern-Volmer fluorescence quenching observed, since the K_Q is the same at different temperatures.

The formation of this complex has been differentiated of salinity or polarity effects since **NaCl**, **NH₄Cl**, **NH₄PF₆**, and **KBr** did not produce changes (between the experimental errors) in the fluorescence of **TC4**.

Also, it is proposed the possible inclusion of the ethyl moiety of **EMIMBr** in the cavity of **TC4** since **NMI** did not produce changes (statistically significant) in the fluorescence signal of **TC4**.

Fluorescence lifetimes and fluorescence anisotropy data reinforce that **TC4** is the unique fluorescent specie in the system (**TC4** + **EMIMBr**) since the no fluorescent complex **TC4-EMIMBr** is formed in the ground state.

The fluorescence quenching curve for **TC4** produced for **EMIMBr** also represents the calibration curve for this **IL** concentration given the possibility of its indirect determination by fluorescence that is not possible by direct UV-Vis or fluorescence.

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Conflicts of interest

There are no conflicts to declare.

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GRAPHICAL ABSTRACT**Highlights**

- 1-ethyl-3-methylimidazolium quenched the fluorescence of *p-t*-butylthiacalix[4]arene.
- An average fluorescence quenching constant were determined at different temperatures.
- A static quenching indicates a no fluorescent complex in the ground state.
- Measures of lifetime and anisotropy of fluorescence reinforce the interpretation.