

## An anionic chromogenic chemosensor based on 4-(4-nitrobenzylideneamine)-2,6-diphenylphenol for selective detection of cyanide in acetonitrile–water mixtures

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DOI: <http://dx.doi.org/10.3998/ark.5550190.0011.b13>

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### Abstract

4-(4-Nitrobenzylideneamine)-2,6-diphenylphenol **3a** was synthesized and studied as an anionic chromogenic chemosensor. Solutions of **3a** in acetonitrile are colorless but turn blue under deprotonation. From the various anions added to the solutions of **3a** only CN<sup>−</sup>, F<sup>−</sup>, and with less intensity CH<sub>3</sub>COO<sup>−</sup> and H<sub>2</sub>PO<sub>4</sub><sup>−</sup>, led to colored solutions. With the addition of up to 2.4% (v/v) of water, of all the anions used only CN<sup>−</sup> was able to act as a base and cause a change in the color of the solution. A model was used to explain the results, based on two **3a**:anion stoichiometries, 1:1 and 1:2.

**Keywords:** Anion sensing, chromogenic chemosensor, cyanide, naked-eye detection, preferential solvation, colorimetric assays

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### Introduction

The recognition and detection of anions is a field which has attracted increasing interest in recent years due to the fundamental importance of these species in many chemical and biological processes.<sup>1</sup> Therefore, many methodologies based on optical chemosensors have been designed to perform selective naked-eye and quantitative detection of anionic species.<sup>1</sup>

A very simple strategy used for the development of anionic chemosensors involves the study of molecules whose color in solution changes with the addition of a particular anion to the medium. The color change observed is due to an alteration in the molecular structure of the chemosensor, its selectivity toward an anionic species being related to the ability of the anions to

act as bases, which defines a differentiated level of interaction with the receptor site in the chemosensor, through hydrogen bonding (HB) or by means of proton transfer processes. This strategy has been used in the recent years to study chromogenic chemosensors for more basic anions, such as  $F^-$  and  $CN^-$ , in non-hydrogen bonding donor (non-HBD) solvents, being of particular importance in the case of  $F^-$ ,<sup>2</sup> which exhibits a greater effect compared with other anionic species due to its smaller size, higher charge density, and higher electron affinity. This makes the anion capable of forming strong interactions with, for instance, the hydroxylic group in phenols. The development of simple  $F^-$  chemosensors is of great interest considering the importance of this anion in the clinical treatment of osteoporosis, the role it plays as an environmental pollutant and the diseases related to its over-accumulation in bones.<sup>3</sup>

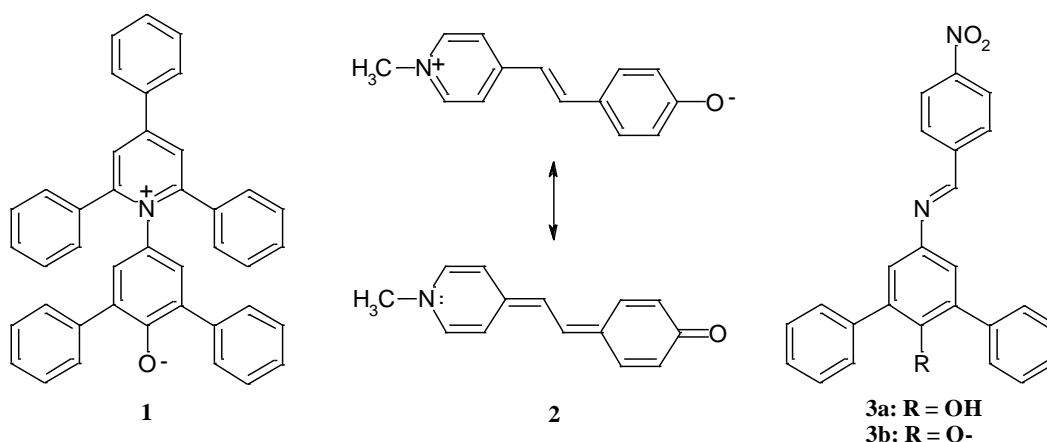
Another interesting anion in terms of detection is  $CN^-$ , which is lethal in very small amounts due to its ability to bind strongly to the active site of cytochrome-oxidase, leading to the inhibition of the mitochondrial electron transport chain, and to a decrease in the oxidative metabolism.<sup>4</sup>  $CN^-$  has many applications in metallurgy, fishing, mining, and the fabrication of polymers. Some fruit seeds and roots release  $CN^-$  through hydrolysis.<sup>4</sup> In addition, many chemical warfare compounds, such as sarin, soman, and tabun,<sup>5</sup> deliver  $F^-$  and  $CN^-$  through hydrolysis and this is important in relation to developing chemosensors for the detection of these neurotoxic agents.<sup>6</sup>

Phenol groups are used in the molecular structure of many chemosensors that have been studied.<sup>7-11</sup> The connection of the phenol donor group to an acceptor group by means of a conjugated bridge creates an interesting feature since the deprotonation of the compound generates a colored conjugated base. The acidity of the chemosensor can be, in principle, modulated through the effect of the medium polarity and modifications in the molecular structure of the compound.<sup>10,11</sup> Hong and co-workers used azophenols as  $F^-$ -selective chromogenic chemosensors, studying the ability of the anions to interact with the chemosensor in trichloromethane through HB according to their basicity.<sup>7</sup>

We have previously studied chemosensors with solvatochromic merocyanines, such as Reichardt's betaine, 2,6-diphenyl-4-(2,4,6-triphenylpyridinium-1-yl)phenolate **1**, and 4-[(1-methyl-4(1*H*)-pyridinylidene)-ethylidene]-2,5-cyclohexadien-1-one, known as Brooker's merocyanine **2**, as signaling units. Many studies have been carried out with these dyes due to the fact that they are solvatochromic, i.e., their UV-vis spectrum changes when the medium polarity is altered.<sup>12,13</sup> Besides the classical applications of these dyes, they have been recently used as signaling units in the development of chemosensors for anionic<sup>9-11,14</sup> and neutral<sup>15</sup> analytes. In this regard, we have shown that **1**, in chloroform, in its protonated form, acts as an anionic chemosensor for  $F^-$  and  $H_2PO_4^-$ .<sup>9</sup> These anions are able to abstract a proton from the phenol group of the protonated dye, leading to colored solutions. Protonated **1** was also able to signal the presence of  $I^-$  through the complexation of the anion with the pyridinium group.<sup>9</sup> A study was carried out in trichloromethane to find a protonated pyridiniophenolate able to operate in a biphasic system, aiming at the selective detection of  $CN^-$  in aqueous solution.<sup>11</sup>

Compounds **1** and **2**, both in their protonated form, were studied in acetonitrile and acetonitrile–water mixtures as anionic chemosensors.<sup>10</sup> Protonated **1** has potential for the recognition and visual detection of  $\text{CN}^-$ ,  $\text{F}^-$ , and  $\text{H}_2\text{PO}_4^-$  in acetonitrile.<sup>10</sup> The titration of the dye in its protonated form with the anions revealed two chemosensor:anion stoichiometries, one 1:1 and the other of the uncommon 1:3 type. The data suggested that the anion would be capable of forming firstly an ion pair with the pyridinium center in the chemosensor and after that a second equivalent of the anion would be needed to form a complex with the phenolic proton through HB. Finally, a third equivalent of the anion would be needed for the abstraction of the proton, with the formation of an  $[\text{HA}_2]^-$  complex.<sup>10</sup> With the addition of small amounts of water to the system, selectivity for  $\text{CN}^-$  was observed in relation to the other anions. Also, the addition of water to the system leads to a decrease in the magnitude of the binding constants related to the 1:3 stoichiometry, due to the action of water, which preferentially solvates the anion and inhibits its interaction with the pyridinium center.<sup>10</sup>

In order to obtain further experimental results to corroborate the proposal presented for the 1:3 chemosensor:anion stoichiometry observed for systems with **1** and **2**, as well as to obtain more efficient novel chromogenic systems with more simple molecular structures than **1**, this paper investigates the use of compound **3** as an anionic chemosensor. Compound **3**, 4-(4-nitrobenzylideneamine)-2,6-diphenylphenol, has a different acceptor group from that observed for compounds **1** and **2** and this should reflect in a change in the pattern of the chemosensor:anion stoichiometries, without altering in the efficiency of its anion sensing properties.

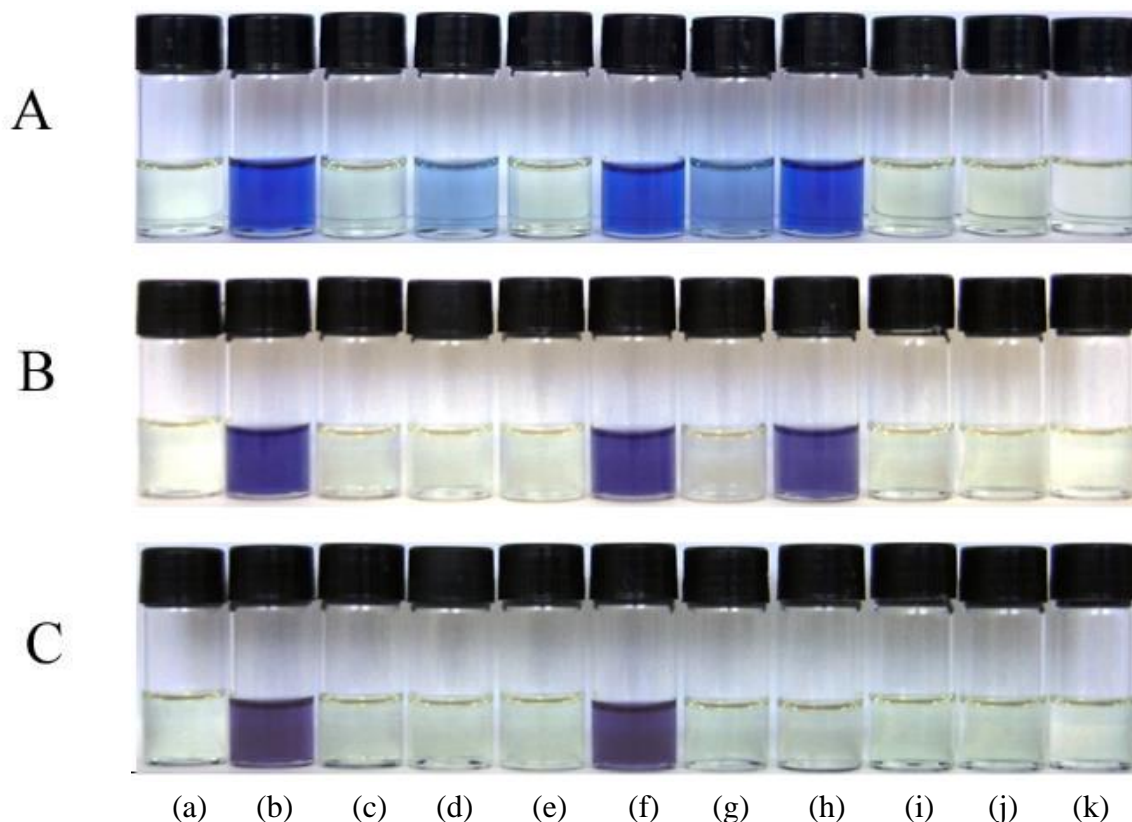


## Results and Discussion

Figure 1 shows the influence of various anions ( $\text{HSO}_4^-$ ,  $\text{H}_2\text{PO}_4^-$ ,  $\text{NO}_3^-$ ,  $\text{CN}^-$ ,  $\text{CH}_3\text{COO}^-$ ,  $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ , and  $\text{I}^-$ ) on the color of the solutions of **3a** in acetonitrile and acetonitrile–water mixtures. The solutions of compound **3a** in acetonitrile are pale yellow. With the addition of the anions, a

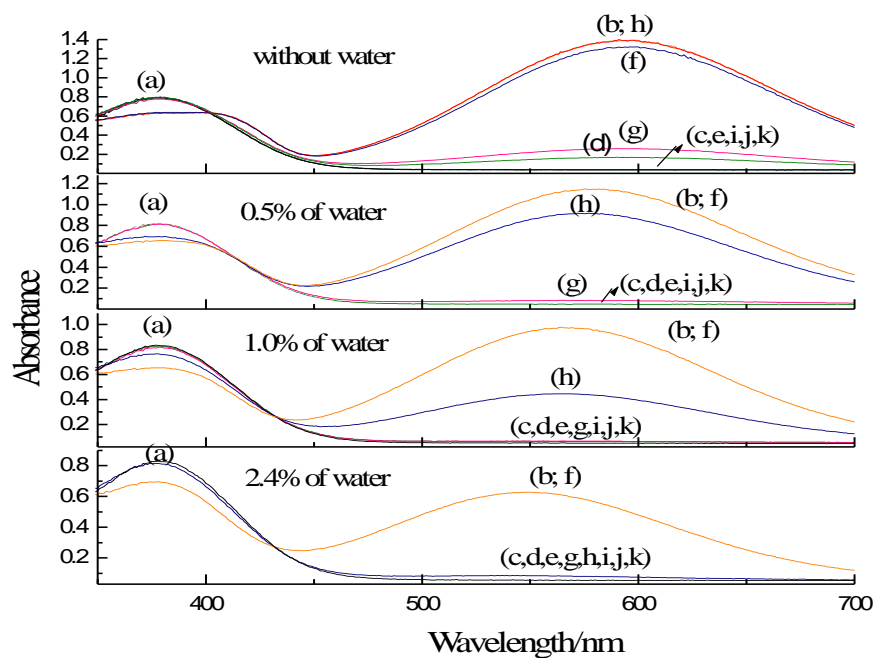
change in the color of the solutions, from pale yellow to blue, was observed for  $\text{CN}^-$  and  $\text{F}^-$ , and with less intensity for  $\text{CH}_3\text{COO}^-$  and  $\text{H}_2\text{PO}_4^-$  (Figure 1A). This is exactly the coloration of the solution obtained from the deprotonation of the compound with the use of tetra-*n*-butylammonium hydroxide, which demonstrates that these anions abstract the proton of the phenolic moiety in the chemosensor, according to their basicity, to generate its deprotonated form, i.e., **3b** species. It is still possible, with the naked-eye analysis of Figure 1A, to verify that, considering the same concentration, the less basic anions  $\text{CH}_3\text{COO}^-$  and  $\text{H}_2\text{PO}_4^-$  were less able to abstract the proton of **3a**, in comparison with the more basic anions  $\text{F}^-$  and  $\text{CN}^-$ . None of the other anions caused a change in the visual aspect of the **3a** solution.

Small amounts of water were added to the system, aiming to obtain selectivity towards one species of the anions studied. No changes in the color of the solutions of **3a** were observed in the system containing  $\text{H}_2\text{PO}_4^-$  and  $\text{CH}_3\text{COO}^-$  (Figure 1B). With 2.4% of water present in solution, only  $\text{CN}^-$  was found to be sufficiently basic to form **3b** (Figure 1C).



**Figure 1.** Solutions of (a) **3a**, (b) **3b**, and **3a** in the presence of (c)  $\text{HSO}_4^-$ , (d)  $\text{H}_2\text{PO}_4^-$ , (e)  $\text{NO}_3^-$ , (f)  $\text{CN}^-$ , (g)  $\text{CH}_3\text{COO}^-$ , (h)  $\text{F}^-$ , (i)  $\text{Cl}^-$ , (j)  $\text{Br}^-$ , and (k)  $\text{I}^-$  as tetra-*n*-butylammonium salts in (A) acetonitrile and acetonitrile with (B) 1.0% (v/v) and (C) 2.4% (v/v) of water. The concentrations of the anions and **3a** were  $6.0 \times 10^{-4} \text{ mol dm}^{-3}$  and  $5.9 \times 10^{-5} \text{ mol dm}^{-3}$ , respectively.

Figure 2 shows the UV-vis spectra for compound **3a** in acetonitrile and in acetonitrile with small amounts of water. This compound in its deprotonated form **3b** exhibits a Vis band in acetonitrile with a  $\lambda_{\text{max}}$  of 592 nm. The effect of the addition of anionic species on the UV-vis spectra of **3a** in acetonitrile was then studied. The UV-vis spectrum for the solution of **3a** with  $\text{F}^-$  added shows a Vis band with the maximum absorbance coinciding with that obtained for the phenolate **3b**, which was obtained with an excess of tetra-*n*-butylammonium hydroxide. A similar observation can be made with the solution of **3a** in the presence of  $\text{CN}^-$ , but with a slightly less intense effect, followed by  $\text{CH}_3\text{COO}^-$  and, with least intensity,  $\text{H}_2\text{PO}_4^-$ . In other words, the data demonstrate that in acetonitrile the ability of the anions to abstract the proton of **3a** and generate **3b** observes the following increasing order:  $\text{H}_2\text{PO}_4^- < \text{CH}_3\text{COO}^- < \text{CN}^- < \text{F}^-$ . None of the other anions were able to deprotonate the compound in solution, nor did they show the appearance of a Vis band in a region that would indicate the formation of species formed by HB.

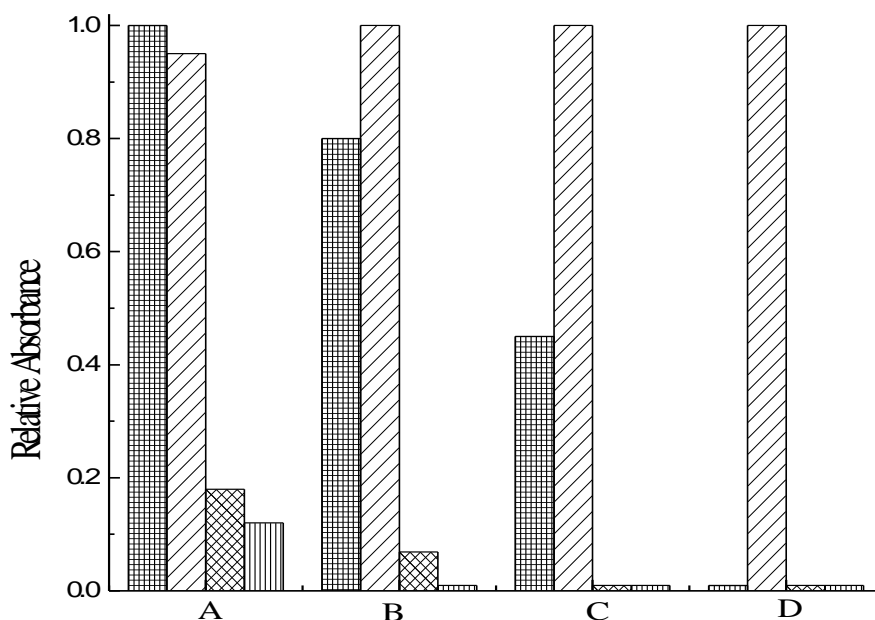


**Figure 2.** UV-vis spectra for solutions of (a) **3a**, (b) **3b**, and **3a** in the presence of (c)  $\text{HSO}_4^-$ , (d)  $\text{H}_2\text{PO}_4^-$ , (e)  $\text{NO}_3^-$ , (f)  $\text{CN}^-$ , (g)  $\text{CH}_3\text{COO}^-$ , (h)  $\text{F}^-$ , (i)  $\text{Cl}^-$ , (j)  $\text{Br}^-$ , and (k)  $\text{I}^-$  in acetonitrile and acetonitrile with 0.5%, 1.0%, and 2.4% (v/v) of water. The concentrations of **3a** and of the anions are given in Figure 1.

Figure 2 also shows the influence of water on the UV-vis spectra of **3a** in the presence of the anions. With the addition of only 0.5 % (v/v) of water  $\text{H}_2\text{PO}_4^-$  does not cause any spectral effect while under the same conditions  $\text{CN}^-$  becomes a more efficient base than  $\text{F}^-$ . It can be also observed that in the presence of 1.0% (v/v) of water  $\text{CH}_3\text{COO}^-$  is no longer detected. With the

addition of 2.4% (v/v) of water to the solution, selectivity for  $\text{CN}^-$  in relation to the other anions was achieved, due to the strong preferential solvation of  $\text{F}^-$ ,  $\text{CH}_3\text{COO}^-$ , and  $\text{H}_2\text{PO}_4^-$  for water through HB, inhibiting them from acting efficiently as bases. In addition, the presence of 0.5%, 1.0%, and 2.4% (v/v) of water in the solutions of **3b** in acetonitrile led to a hypsochromic shift of 15, 27, and 43 nm, respectively, in the position of the Vis band. This indicates that **3b** exhibits a very important negative solvatochromism and that in mixtures of acetonitrile with very small amounts of water the dye is preferentially solvated by water, more specifically by means of HB involving water molecules and the phenolate moiety of **3b**. This kind of interaction results in a hypsochromic shift of the solvatochromic band of the compound in comparison with the position of the band in pure acetonitrile. These results are consistent with data reported in the literature for dyes with a phenolate group in their structure, such as compounds **1** and **2**.<sup>16</sup>

The results discussed in the previous paragraph can be better visualized in Figure 3, which shows that the selectivity for  $\text{CN}^-$ , in relation to the other anions, is gradually reached with the addition of water to the system. The highest value for the absorbance corresponding to the  $\lambda_{\text{max}}$  value was verified for  $\text{F}^-$  in acetonitrile, but  $\text{CN}^-$  gave the most important spectral effect with the addition of only 0.5% (v/v) of water, while with the presence of 2.4% (v/v) of water only  $\text{CN}^-$  was able to modify the spectrum of the chemosensor in solution.



**Figure 3.** Relative absorbance for  $\text{F}^-$  (solid black),  $\text{CN}^-$  (diagonal lines),  $\text{CH}_3\text{COO}^-$  (cross-hatch), and  $\text{H}_2\text{PO}_4^-$  (vertical lines), added to the solutions of **3a** in (A) acetonitrile and in acetonitrile with (B) 0.5%, (C) 1.2%, and (D) 2.4% of water. The concentrations of **3a** and of the anions are given in Figure 1.

### Titration of **3a** with the anions

Compound **3a** was titrated with the anionic species able to change the color of the solutions in acetonitrile and in acetonitrile–water mixtures. The absorbance values for the maximum of the Vis band of **3b** in acetonitrile and in each mixture were plotted as a function of the anion concentration added. The experimental data were fitted with the use of Eqs. 1–3,<sup>10,11,17</sup> which are related to the following situations according to different chemosensor:anion stoichiometries:

**Case 1.** 1:1 stoichiometry

$$Abs = [Abs_0 + Abs_{11}K_{11}C_{A^-}] / [1 + K_{11}C_{A^-}] \quad (1)$$

**Case 2.** 1:1 and 1:2 stoichiometries

$$Abs = [Abs_0 + Abs_{11}K_{11}C_{A^-} + Abs_{12}K_{11}K_{12}C_{A^-}^2] / [1 + K_{11}C_{A^-} + K_{11}K_{12}C_{A^-}^2] \quad (2)$$

**Case 3.** 1:2 stoichiometry

$$Abs = [Abs_0 + Abs_{12}K_{12}C_{A^-}^2] / [1 + K_{12}C_{A^-}^2] \quad (3)$$

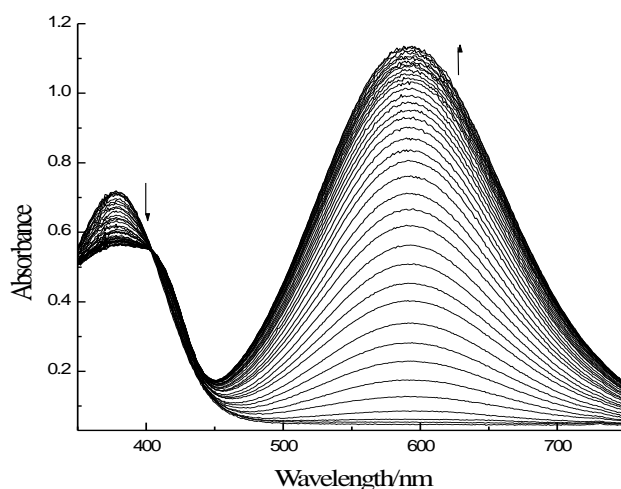
In these equations, *Abs* is the absorbance value after each addition of the anion, *Abs*<sub>0</sub> is the initial absorbance without anion added, *Abs*<sub>11</sub> and *Abs*<sub>12</sub> are the maximum absorbance values obtained by addition of the anion considering 1:1 and 1:2 **3a**:anion stoichiometries, *C*<sub>A<sup>−</sup></sub> is the anion concentration in each addition, and *K*<sub>11</sub> and *K*<sub>12</sub> are the binding constants. The results are given in Table 1 and show very good fits for all systems studied (S.D. < 1.0×10<sup>−4</sup>).

**Table 1.** Binding constants at 25 °C of **3a** with the anions in acetonitrile and acetonitrile with small amounts of water

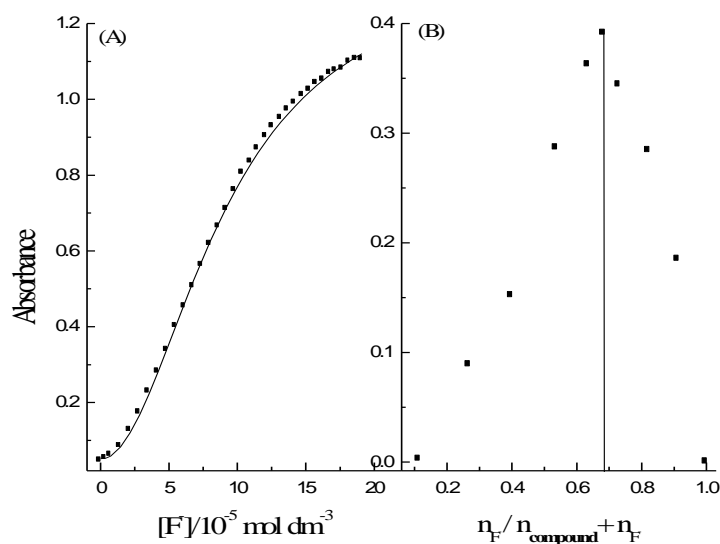
Experimental conditions	Anion	<i>K</i> <sub>11</sub> / dm <sup>3</sup> mol <sup>−1</sup>	<i>K</i> <sub>12</sub> /dm <sup>6</sup> mol <sup>−2</sup>	S.D.
Acetonitrile	F <sup>−</sup>	–	(1.27±0.03)×10 <sup>8</sup>	9×10 <sup>−5</sup>
0.5 % of water	F <sup>−</sup>	(5.12±1.76)×10 <sup>4</sup>	(4.18±0.08)×10 <sup>3</sup>	4×10 <sup>−5</sup>
1.0 % of water	F <sup>−</sup>	(1.25±0.24)×10 <sup>4</sup>	(3.53±0.02)×10 <sup>3</sup>	8×10 <sup>−6</sup>
Acetonitrile	CN <sup>−</sup>	(9.88±1.33)×10 <sup>3</sup>	(7.58±0.63)×10 <sup>4</sup>	2×10 <sup>−5</sup>
0.5 % of water	CN <sup>−</sup>	(2.24±0.18)×10 <sup>4</sup>	(2.44±0.13)×10 <sup>4</sup>	1×10 <sup>−4</sup>
1.0 % of water	CN <sup>−</sup>	(3.08±0.41)×10 <sup>4</sup>	(1.35±0.03)×10 <sup>4</sup>	2×10 <sup>−5</sup>
1.5 % of water	CN <sup>−</sup>	(5.10±0.13)×10 <sup>4</sup>	(9.04±0.22)×10 <sup>3</sup>	2×10 <sup>−5</sup>
2.0 % of water	CN <sup>−</sup>	(8.23±0.52)×10 <sup>4</sup>	(8.73±0.17)×10 <sup>3</sup>	7×10 <sup>−5</sup>
2.5 % of water	CN <sup>−</sup>	(1.24±0.35)×10 <sup>5</sup>	(8.44±0.10)×10 <sup>3</sup>	9×10 <sup>−6</sup>
3.0 % of water	CN <sup>−</sup>	(1.04±0.31)×10 <sup>5</sup>	(4.45±0.12)×10 <sup>3</sup>	1×10 <sup>−5</sup>
4.0 % of water	CN <sup>−</sup>	(1.07±0.02)×10 <sup>4</sup>	(3.00±2.30)×10 <sup>1</sup>	2×10 <sup>−5</sup>
4.5 % of water	CN <sup>−</sup>	(2.84±0.99)×10 <sup>3</sup>	–	6×10 <sup>−5</sup>
4.7 % of water	CN <sup>−</sup>	(2.56±0.08)×10 <sup>3</sup>	–	4×10 <sup>−5</sup>
4.9 % of water	CN <sup>−</sup>	(1.59±0.07)×10 <sup>3</sup>	–	1×10 <sup>−5</sup>
5.0 % of water	CN <sup>−</sup>	(1.29±0.08)×10 <sup>3</sup>	–	4×10 <sup>−5</sup>
6.0 % of water	CN <sup>−</sup>	(9.41±0.40)×10 <sup>2</sup>	–	3×10 <sup>−5</sup>

Acetonitrile	$\text{CH}_3\text{COO}^-$	$(1.76 \pm 0.72) \times 10^4$	$(5.72 \pm 0.18) \times 10^2$	$5 \times 10^{-6}$
0.5 % of water	$\text{CH}_3\text{COO}^-$	$(9.40 \pm 1.60) \times 10^1$	—	$2 \times 10^{-5}$
Acetonitrile	$\text{H}_2\text{PO}_4^-$	$(3.27 \pm 0.17) \times 10^2$	$(7.60 \pm 3.40) \times 10^1$	$2 \times 10^{-5}$

Figure 4 shows the titration of **3a** with  $\text{F}^-$  in acetonitrile. It can be seen that with the addition of the anion a band with maximum absorbance at 592 nm appears, relating to **3b**. A plot of the absorbance values at 592 nm as a function of the concentration of  $\text{F}^-$  (Figure 5A) showed a sigmoidal shape, with a behavior typical of a 1:2 chemosensor:anion stoichiometry and the fitting of the experimental data led to a binding constant of  $K_{12} = (1.27 \pm 0.03) \times 10^8 \text{ dm}^6 \text{ mol}^{-2}$  (Table 1). In Figure 5B the Job's plot is shown, which confirms 1:2 **3a**:anion stoichiometry.



**Figure 4.** UV-vis spectra at 25°C for the behavior of **3a** ( $5.9 \times 10^{-5} \text{ mol dm}^{-3}$ ) in acetonitrile with the addition of increasing amounts of  $\text{F}^-$ . The final concentration of  $\text{F}^-$  was  $2.0 \times 10^{-4} \text{ mol dm}^{-3}$ .





**Figure 5.** (A) Titration curve for compound **3a** with  $F^-$ . The final concentration of  $F^-$  was  $2.0 \times 10^{-4} \text{ mol dm}^{-3}$  and the absorbance values were collected at 592 nm. (B) Job's plot for compound **3a** with  $F^-$  in acetonitrile.

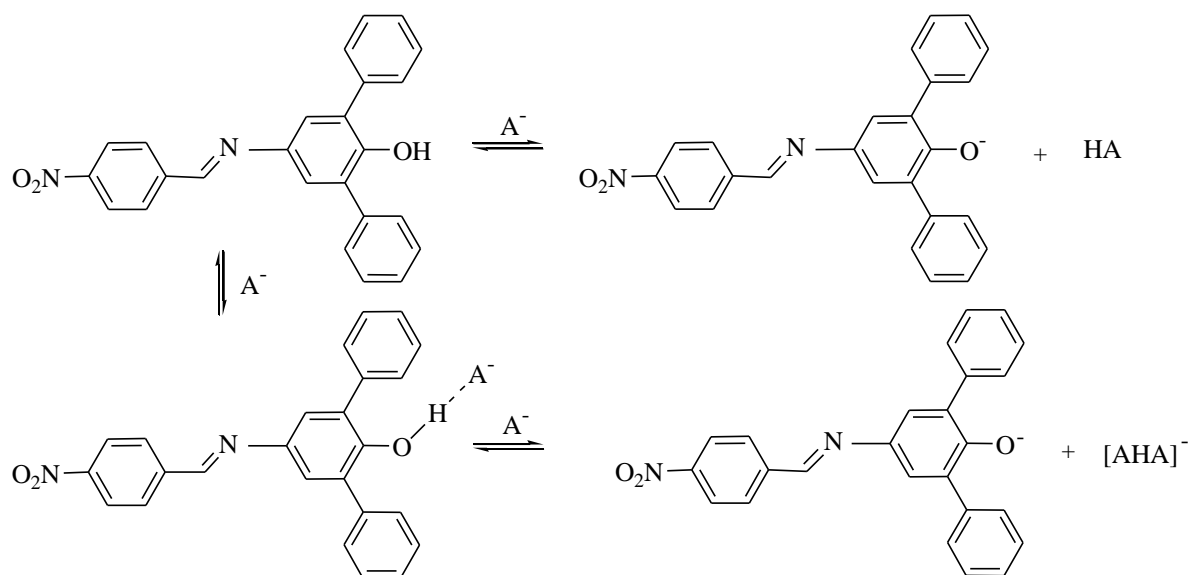
The addition of very small amounts of water led to a change in the profile of the titration with  $F^-$ , and this is due to a strong interaction of water with the anion, hindering its interactions as a base. A change in the **3a**:anion stoichiometry occurred with the addition of 1.0% (v/v) of water to the system containing **3a** from 1:2 to both 1:1 and 1:2 (Table 1).

The titration of **3a** in acetonitrile with increasing amounts of  $CN^-$  was made. It could be observed that the band with a  $\lambda_{\text{max}}$  value of 378 nm related to compound **3a** shows a reduction in the absorbance with the addition of the anion, and simultaneously a band with maximum absorbance at 592 nm, due to the appearance of **3b**, occurs with an isosbestic point at 403 nm. The corresponding titration curve was obtained with experimental data for the absorbances at 592 nm and these data were fitted using Eq. 3, giving the values of  $K_{11} = (9.88 \pm 1.33) \times 10^3 \text{ dm}^3 \text{ mol}^{-1}$  and  $K_{12} = (7.58 \pm 0.63) \times 10^4 \text{ dm}^6 \text{ mol}^{-2}$  (Table 1). The addition of water to the system had a considerable effect not only on the magnitude of the binding constants but also on the profile of the process: with the addition of 5% (v/v) of water the stoichiometry observed was 1:1 **3a**:anion, with  $K_{11} = (1.29 \pm 0.08) \times 10^3 \text{ dm}^3 \text{ mol}^{-1}$ .

The titrations with  $CH_3COO^-$  in acetonitrile had a behavior similar to those observed for  $CN^-$ , with 1:1 and 1:2 **3a**:anion stoichiometries, changing to 1:1 with the addition of 0.5% (v/v) of water to the system. No effect of  $H_2PO_4^-$  was observed on the solution containing **3a** in acetonitrile with 0.5% (v/v) of water, although the behavior for this anion in acetonitrile without water added is similar to that observed for  $CN^-$  and  $CH_3COO^-$ . The results are clearly due to the effect of water solvating the species in solution (Table 1).

### The role of water on the behavior of **3a** in the presence of the anions

The titrations of **3a** with the anions show two possible **3a**:anion stoichiometries, 1:1 and 1:2, according to Scheme 1. If the stoichiometry is 1:1, the proton can be transferred directly from **3a** to the anion. The other possibility involves firstly the interaction of the anion with the phenol moiety of the chemosensor through HB, and this interaction weakens the O–H bond. After this, a second equivalent of the anion is needed to abstract the proton, with the formation of complex species of the type  $[HA_2]^-$ . This has been reported in other papers<sup>2i,10,18</sup> and the formation and the stability of  $[HA_2]^-$  complexes, such as  $[HF_2]^-$ , have been studied by means of theoretical calculations.<sup>19</sup>



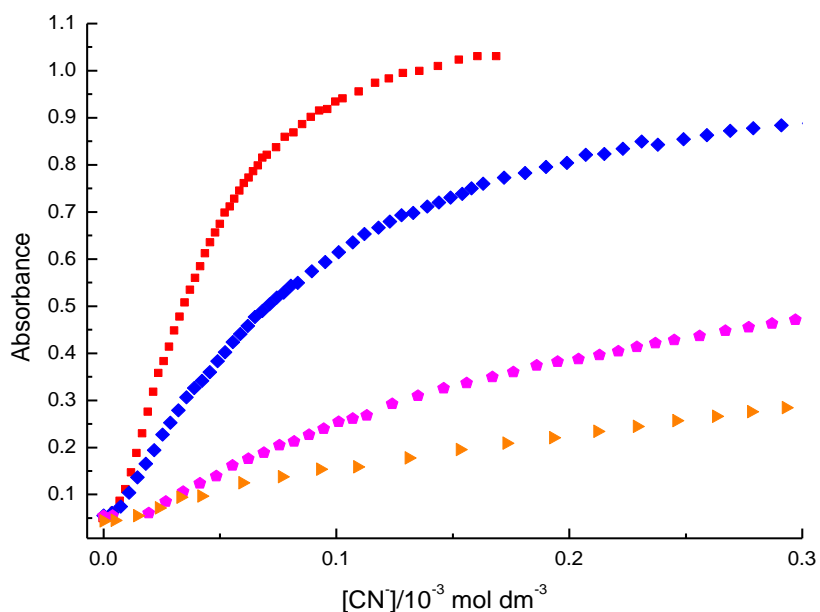
**Scheme 1.** Possible interactions of **3a** with the anions in acetonitrile.

The studies carried out show the significant role of water in determining the selectivity of the system studied for  $\text{CN}^-$  in relation to the other anions. The reason for this is that the hydration energies of  $\text{F}^-$  ( $-465 \text{ kJ mol}^{-1}$ ),  $\text{CH}_3\text{COO}^-$  ( $-365 \text{ kJ mol}^{-1}$ ), and  $\text{H}_2\text{PO}_4^-$  ( $-465 \text{ kJ mol}^{-1}$ ) are high if they are compared to that determined for  $\text{CN}^-$  ( $-295 \text{ kJ mol}^{-1}$ ).<sup>20</sup> The strong solvation of the anions by water makes the hydrated species less able to act as a base. Since  $\text{CN}^-$  is less hydrated with the addition of water, this anion becomes more ‘naked’ in relation to its ability to act as a base, being more efficient in the abstraction of the proton of **3a**. The effectiveness of the use of water as a strategy to increase the selectivity of the chemosensor for  $\text{CN}^-$  over other more hydrated anions has been reported in the recent literature.<sup>2i,10,11,21</sup>

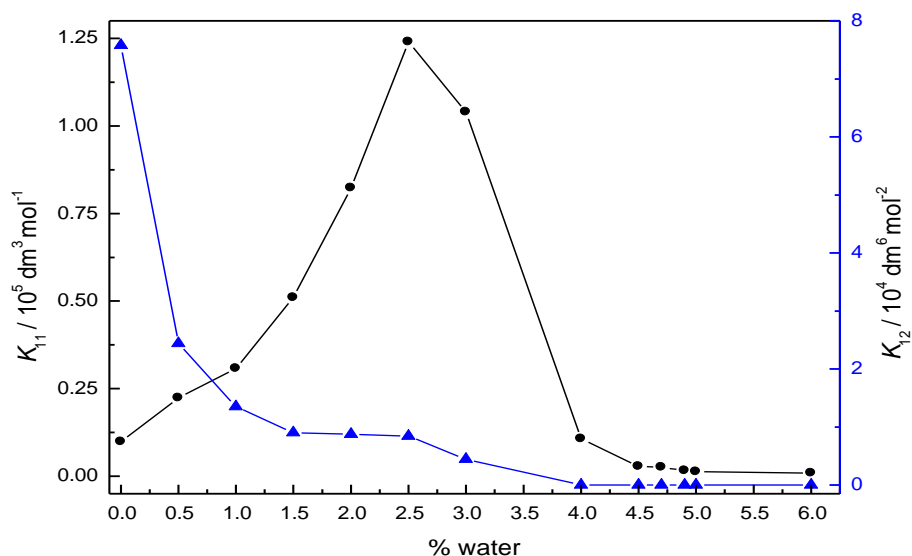
The addition of water has another important role, which is to promote the stabilization of the conjugated base **3b** through HB, making **3a** more acidic. This explains the change observed in the **3a**: anion stoichiometry (Table 1), which can be observed through the change in the shape of the titration curves, as showed in Figure 6 for the titrations of **3a** using  $\text{CN}^-$  in acetonitrile and in its mixtures with small amounts of water. The titration curve of **3a** with  $\text{CN}^-$  in acetonitrile has a sigmoidal shape, typical of 1:2 stoichiometry, which is gradually replaced by a curve with a shape typical of 1:1 stoichiometry when up to 6% (v/v) of water is added to the system.

Figure 7 shows the influence of water on the binding constants obtained from the titration curves of **3a** with  $\text{CN}^-$  (Table 1). The addition of water caused a very important decrease in the  $K_{12}$  values and, curiously, the  $K_{11}$  values increase with the addition of up to 2.5% (v/v) of water and then decrease above this water content. Since the presence of small amounts of water to the medium makes **3a** more acidic, this explains the increase in the  $K_{11}$  values with the addition of up to 2.5% (v/v) of water in the system containing **3a** and  $\text{CN}^-$ . Another important aspect is that with the addition of water to the system the polarity of the medium increases and the selectivity for  $\text{CN}^-$  is achieved due to its lower hydration energy in comparison with the other anions.

Furthermore, water is able to solvate the anion and **3b**, hindering the interaction between the compound and the anion, being responsible for a strong solvation of the anion, causing a considerable weakening in its action as a base and consequently lowering the values of binding constants. This makes the design of chemosensors for  $F^-$  in aqueous medium a challenging and very difficult task.



**Figure 6.** Variations in the absorbance values for the  $\lambda_{\max}$  values of **3a** ( $5.9 \times 10^{-5} \text{ mol dm}^{-3}$ ) with the addition of increasing amounts of  $CN^-$  in acetonitrile (■) and in acetonitrile with 1.0% (◆), 3.0% (◀), and 5.0% (▶) of water.



**Figure 7.** Influence of water on the values of binding constants  $K_{11}$  (●) and  $K_{12}$  (▲) of **3a** with  $\text{CN}^-$  in acetonitrile at 25 °C.

## Conclusions

Compound **3a** was demonstrated to be an efficient anionic chromogenic chemosensor based on a simple acid–base strategy. In acetonitrile, the following increasing order was obtained:  $\text{H}_2\text{PO}_4^- < \text{CH}_3\text{COO}^- < \text{CN}^- < \text{F}^-$ , which matches the increasing order of basicity for these anions, and for the other anions studied no spectral changes were verified. The selectivity for  $\text{CN}^-$  in relation to all other anions studied was achieved with the use of only 2.4% (v/v) of water due to the fact that this solvent, or hydrogen–bonded acetonitrile–water complexes, can preferentially solvate anions such as  $\text{H}_2\text{PO}_4^-$ ,  $\text{CH}_3\text{COO}^-$ , and  $\text{F}^-$  through HB, leading  $\text{CN}^-$  freer to interact with the chemosensor.

The titrations of **3a** with the anions that caused alterations in its UV–vis spectrum revealed the occurrence of two chemosensor:anion stoichiometries. The anion can abstract the proton from the chemosensor in one step, in a 1:1 stoichiometry, or in two steps, with the formation of  $[\text{HA}_2]^-$  complexes, in a 1:2 stoichiometry. The addition of water causes a decrease in the  $K_{12}$  values because the hydroxylic solvent interacts with both the anion and the phenol moiety of **3a**, making the interaction between the anion and the compound more difficult. In addition, water is able to stabilize through HB the conjugated base of the chemosensor, making it more acidic, and making the anion able to abstract the proton in one step.

For the chemosensors with a pyridinium cation as an acceptor center in their molecular structure, as is the case of the merocyanines **1** and **2**, titrations with basic anions in acetonitrile lead to a 1:3 chemosensor:anion stoichiometry, due to an electrostatic interaction between the pyridinium group and the anion prior to the interaction of the anion with the phenol moiety in the compound.<sup>10</sup> For a compound with a more simple molecular structure, without a cationic center in its structure, such as **3a** in acetonitrile with  $\text{CN}^-$ , 1:1 and 1:2 **3a**:anion stoichiometries are observed, while for  $\text{F}^-$  the stoichiometry observed is only of type 1:2. Therefore, the stoichiometry obtained is dependent on the molecular structure of the chemosensor, of the medium, and of the anion used.

## Experimental Section

**General.** Acetonitrile was purified according to a procedure described in the literature and then stored in molecular sieves (4Å, Aldrich).<sup>22</sup> Karl–Fischer titrations were performed with this solvent and demonstrated the presence of water in a concentration of  $7.11 \times 10^{-3} \text{ mol dm}^{-3}$  (0.0286%). Deionized water was used in all measurements. This solvent was boiled and bubbled with nitrogen and kept under a nitrogen atmosphere to avoid the presence of carbon dioxide. All anions ( $\text{HSO}_4^-$ ,  $\text{H}_2\text{PO}_4^-$ ,  $\text{NO}_3^-$ ,  $\text{CN}^-$ ,  $\text{CH}_3\text{COO}^-$ ,  $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ , and  $\text{I}^-$ ) were used as tetra-*n*-butylammonium salts with purity greater than 97–99%. The anions were purchased from Fluka ( $\text{F}^-$ , >97%;  $\text{Cl}^-$ , >98%;  $\text{NO}_3^-$ , >97%; and  $\text{H}_2\text{PO}_4^-$ , >97%), Vetec ( $\text{Br}^-$ , >99%;  $\text{I}^-$ , >99%; and  $\text{HSO}_4^-$ , >99%) and Sigma–Aldrich ( $\text{CH}_3\text{COO}^-$ , >97%). They were dried over phosphorous pentoxide under vacuum before use. Karl–Fischer experiments were performed for the following tetra-*n*-butylammonium salts in order to determine the content of water in each salt:  $\text{CN}^-$  (0.116% water),  $\text{F}^-$  (1.125% water),  $\text{H}_2\text{PO}_4^-$  (0.111% water), and  $\text{CH}_3\text{COO}^-$  (0.067% water).

**Compound 3a** was prepared in two steps. Firstly, 4-amino-2,6-diphenylphenol was obtained according to the procedure described in the literature,<sup>23</sup> for which 2,6-diphenylphenol (Aldrich) was stirred with an excess of sodium nitrite followed by the reduction of the product with granulated tin and concentrated HCl (yield: 59.7%; p.f. obs. 138 °C (p.f. lit.<sup>23</sup> 135–138 °C); IR  $\nu_{\text{max}}/\text{cm}^{-1}$  (KBr): 3510 (O–H, m), 3427 ( $\text{NH}_2$ , m), 3060, 2872 (=C–H, m), 1423, 1470, 1595 (C=C, m), and 1342 (C–N, s). This amine was used to prepare **3a** through the following procedure: 4-nitrobenzaldehyde (0.24 g, 1.6 mmol), 4-amino-2,6-diphenylphenol (0.50 g, 1.9 mmol), and ethanol (15 mL) were added to an Erlenmeyer flask. The contents of the flask were then heated slowly with stirring until the complete solubilization of the reactants. One drop of acetic acid was then added and the reaction mixture was stirred for four hours. The mixture was then left to stand in a freezer overnight. The mixture was poured into cold water with stirring and the solid obtained was filtered under vacuum, washed with iced ethanol and recrystallized three times from methanol. The product, after drying, was an amorphous yellow solid (yield of 16.0 %), with a melting point of 134.7 °C. IR (KBr,  $\nu_{\text{max}}/\text{cm}^{-1}$ ): 3539 (O–H, m), 1588 (C=N, m),

1475, 1425 (C=C, m), 1518, 1344 (N=O, vs), and 1227 (C–O, s).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 5.518 (1H, s), 7.322 (2H, s), 7.429 (4H, t,  $J=7.2$  Hz), 7.514 (2H, t, 7.2 Hz), 7.607 (4H, d, 7.2 Hz), 8.067 (2H, d, 8.8 Hz), 8.325 (2H, d, 8.4 Hz), 8.665 (1H, s).  $^{13}\text{C}$  NMR (100.6 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 123.12, 124.28, 128.27, 129.22, 129.38, 129.51, 129.83, 137.23, 142.08, 143.71, 149.24, 149.31, 155.54. Anal. Calcd. for  $\text{C}_{25}\text{H}_{18}\text{N}_2\text{O}_3$ : C, 76.13; H, 4.60; N, 7.10. Found: C, 75.82; H, 4.62; N, 7.07.

### UV–vis studies of **3a** with the anions

UV–vis measurements were performed with a Varian Cary Bio 50 spectrophotometer at 25 °C, using a 1 cm quartz square cuvette. The maxima of the UV–vis spectra ( $\lambda_{\text{max}}$ ) were calculated from the first derivative of the absorption spectrum.

A solution of **3a** was prepared in acetonitrile in a concentration of  $5.9 \times 10^{-5}$  mol  $\text{dm}^{-3}$ . This solution was then used to prepare the solution of each anion in a concentration of  $6.0 \times 10^{-4}$  mol  $\text{dm}^{-3}$ , using 5  $\text{cm}^3$  volumetric flasks. Subsequently, these solutions were transferred to cuvettes hermetically closed with rubber stoppers in order to minimize the evaporation of the solvent and to avoid the entrance of water to the system and the UV–vis spectra were taken. All experiments were carried out at 25 °C.

The experiments involving acetonitrile–water mixtures were carried out using the following procedure. Solutions of **3a** containing each anion were prepared in acetonitrile, as described in the previous paragraph. Small volumes of water were then added to each system that gave a positive response, that is, a change in the color of the acetonitrile, and UV–vis spectra were taken in order to evaluate whether this amount was sufficient to allow selective detection with the naked-eye. The absorbance values were collected for the  $\lambda_{\text{max}}$  values verified in each mixture.

### Titration experiments

Titration experiments in acetonitrile were performed with the preparation of the solution of **3a** as described previously. This solution was used to prepare the stock anion solutions in flasks closed with rubber stoppers and the titrations were carried out by adding small amounts (2–10  $\mu\text{L}$ ) of the salt solution with a microsyringe to closed quartz cuvettes containing the solution of **3a**. The UV–vis spectra were taken after each addition and the absorbance values were collected at 592 nm. Titration experiments were also performed in acetonitrile–water systems, using the minimal water content which allowed selective detection of the anion, defined in the previous UV–vis studies.

### Stoichiometry determinations

The experiment for the stoichiometry determination was performed in acetonitrile with the method of continuous variations (Job's method).<sup>17a</sup> These plots were obtained considering the formation of **3b** produced from **3a** and the anionic species. Thus, these studies were performed at 25 °C using stock solutions in the experiment with concentrations of  $6.0 \times 10^{-5}$  mol  $\text{dm}^{-3}$  for **3a** and  $7.0 \times 10^{-5}$  mol  $\text{dm}^{-3}$  for  $\text{F}^-$  and the absorbance values were read at  $\lambda_{\text{max}}=592$  nm.

## Calculations

The binding constants were calculated through the fitting of least-squares regression curves using the ORIGIN 6.1 program.

## Acknowledgements

The financial support of the Brazilian Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), INCT-Catálise, FAPESC, and FURB is gratefully acknowledged.

## References

1. See for instance: (a) Martínez-Mañez, R.; Sancenón, F. *Chem. Rev.* **2003**, *103*, 4419. (b) Suksai, C.; Tuntulani, T. *Chem. Soc. Rev.* **2003**, *32*, 192. (c) Kubik, S.; Reyheller, C.; Stüwe, S. *J. Inclusion Phenom. Macrocyclic Chem.* **2005**, *52*, 137. (d) Gunnlaugsson, T.; Glynn, M.; Tocci, G. M.; Kruger, P. E.; Pfeffer, F. M. *Coord. Chem. Rev.* **2006**, *250*, 3094. (e) Nguyen, B. T.; Anslyn, E. V. *Coord. Chem. Rev.* **2006**, *250*, 3118. (f) Anslyn, E. V. *J. Org. Chem.* **2007**, *72*, 687. (g) Gale, P. A.; García-Garrido, S. E.; Garric, J. *Chem. Soc. Rev.* **2008**, *37*, 151. (h) Zimmermann-Dimer, L. M.; Machado, V. G. *Quim. Nova* **2008**, *31*, 2134. (i) Caltagirone, C.; Gale, P. A. *Chem. Soc. Rev.* **2009**, *38*, 520. (j) Cho, D.-G.; Sessler, J. L. *Chem. Soc. Rev.* **2009**, *38*, 1647.
2. See for instance: (a) Lee, D. H.; Lee, K. H.; Hong, J. *Org. Lett.* **2001**, *3*, 5. (b) Piatek, P.; Jurczak, J. *Chem. Commun.* **2002**, 2450. (c) Mizuno, T.; Wei, W.-H.; Eller, L. R.; Sessler, J. L. *J. Am. Chem. Soc.* **2002**, *124*, 1134. (d) Cho, E. J.; Moon, J. W.; Ko, S. W.; Lee, J. Y.; Kim, S. K.; Yoon, J.; Nam, K. C. *J. Am. Chem. Soc.* **2003**, *125*, 12376. (e) Zhou, G.; Cheng, Y.; Wang, L.; Jing, X.; Wang, F. *Macromolecules* **2005**, *38*, 2148. (f) Bonizzoni, M.; Fabbri, L.; Taglietti, A.; Tiengo, F. *Eur. J. Org. Chem.* **2006**, 3567. (g) Yu, M.; Lin, H.; Zhao, G. H.; Lin, H. K. *J. Mol. Recognit.* **2007**, *20*, 69. (h) Li, J. W.; Lin, H.; Cai, Z. S.; Lin, H. K.; *Spectrochim. Acta A* **2009**, *72*, 1062. (i) Marini, V. G.; Zimmermann, L. M.; Machado, V. G. *Spectrochim. Acta, Part A* **2010**, *75*, 799.
3. (a) Faibish, D.; Ott, S. M.; Boskey, A. L. *Clin. Orthop. Relat. Res.* **2006**, *28*. (b) Ayoob, S.; Gupta, A. K. *Crit. Rev. Environ. Sci. Technol.* **2006**, *36*, 433.
4. (a) Lv, J.; Zhang, Z. J.; Li, J. D.; Luo, L. R. *Forensic Sci. Int.* **2005**, *148*, 15. (b) Nelson, L. J. *Emerg. Nurs.* **2006**, *32*, S8. (c) Zelder, F. H.; Männel-Croisé, C. *Chimia* **2009**, *63*, 58.
5. Royo, S.; Martínez-Mañez, R.; Sancenón, F.; Costero, A. M.; Parra, M.; Gil, S. *Chem. Commun.* **2007**, 4839.
6. He, S.; Iacono, S. T.; Budy, S. M.; Dennis, A. E.; Smith, Jr., D. W.; Smith, R. C. *J. Mater. Chem.* **2008**, *18*, 1970.
7. Lee, D. H.; Lee, K. H.; Hong, J. *Org. Lett.* **2001**, *3*, 5.

8. (a) Tong, H.; Zhou, G.; Wang, L.; Jing, X.; Wang, F.; Zhang, J.; *Tetrahedron Lett.* **2003**, 44, 131. (b) Zhang, X.; Guo, L.; Wu, F.-Y.; Jiang, Y.-B.; *Org. Lett.* **2003**, 5, 2667.
9. Reis, D. C.; Machado, C.; Machado, V. G. *Tetrahedron Lett.* **2006**, 47, 9339.
10. Zimmermann-Dimer, L. M.; Machado, V. G. *Dyes Pigments* **2009**, 82, 187.
11. Zimmermann-Dimer, L. M.; Reis, D. C.; Machado, C.; Machado, V. G. *Tetrahedron* **2009**, 65, 4239.
12. (a) Reichardt, C. *Solvents and solvent effects in organic chemistry*, 3rd Edn.; VCH: Weinheim; 2003; pp 329–452. (b) Reichardt, C. *Chem. Rev.* **1994**, 94, 2319. (c) Reichardt, C. *Pure Appl. Chem.* **2008**, 80, 1415.
13. Testoni, F. M.; Ribeiro, E. A.; Giusti, L. A.; Machado, V. G. *Spectrochim. Acta, Part A* **2009**, 71, 1704.
14. (a) Nicolini, J.; Testoni, F. M.; Schuhmacher, S. M.; Machado, V. G. *Tetrahedron Lett.* **2007**, 48, 3467. (b) Linn, M. M.; Poncio, D. C.; Machado, V. G. *Tetrahedron Lett.* **2007**, 48, 4547. (c) García-Acosta, B.; Martínez-Máñez, R.; Ros-Lis, J. V.; Sancenón, F.; Soto, J. *Tetrahedron Lett.* **2008**, 49, 1997. (d) Bae, J. S.; Gwon, S. Y.; Matsumoto, S.; Son, Y. A.; Kim, S. H. *Fibers and Polymers* **2009**, 10, 858.
15. (a) Crowther, D.; Liu, X. M. *J. Chem. Soc., Chem. Commun.* **1995**, 2445; (b) Suslick, K. S.; Rakow, N. A.; Sen, A. *Tetrahedron* **2004**, 60, 11133; (c) Onida, B.; Fiorilli, S.; Borello, L.; Viscardi, G.; Macquarrie, D.; Garrone, E. *J. Phys. Chem. B* **2004**, 108, 16617; (d) Fiorilli, S.; Onida, B.; Macquarrie, D.; Garrone, E. *Sens. Actuators, B* **2004**, 100, 103; (e) Janzen, M. C.; Ponder, J. B.; Bailey, D. P.; Ingison, C. K.; Suslick, K. S. *Anal. Chem.* **2006**, 78, 3591; (f) Budag, R.; Giusti, L. A.; Machado, V. G.; Machado, C. *Fuel* **2006**, 85, 1494.
16. (a) Ortega, J.; Ràfols, C.; Bosch, E.; Rosés, M. J. *Chem. Soc., Perkin Trans. 2* **1996**, 1497. (b) Bosch, E.; Rosés, M.; Herodes, K.; Koppel, I.; Leito, I.; Koppel, I.; Taal, V. *Phys. Org. Chem.* **1996**, 9, 403. (c) da Silva, D. C.; Ricken, I.; Silva, M. A. R.; Machado, V. G. *J. Phys. Org. Chem.* **2002**, 15, 420.
17. (a) Connors, K. A. *Binding Constants*; Wiley Interscience: New York, 1987, p 21. (b) Chen, Y.-L.; Xu, T.-K.; Shen, X.-H.; Gao, H.-C. *J. Photochem. Photobiol., A* **2005**, 173, 42.
18. (a) Boiocchi, M.; Del Boca, L.; Gómez, D. E.; Fabbrizzi, L.; Licchelli, M.; Monzani, E. *J. Am. Chem. Soc.* **2004**, 126, 16507. (b) Wu, Y.; Peng, X.; Fan, J.; Gao, S.; Tian, M.; Zhao, J.; Sun, S. *J. Org. Chem.* **2007**, 72, 62. (c) Chen, C.-Y.; Lin, T.-P.; Chen, C.-K.; Lin, S.-C.; Tseng, M.-C.; Wen, Y.-S.; Sun, S.-S. *J. Org. Chem.* **2008**, 73, 900.
19. Gronert, S. *J. Am. Chem. Soc.* **1993**, 115, 10258.
20. (a) Marcus, Y. *J. Chem. Soc., Faraday Trans.* **1991**, 87, 2995. (b) Dickins, R. S.; Parker, D. In: Gloe, K. (Ed.), *Macrocyclic Chemistry: Current Trends and Future Perspectives*; Springer: Dordrecht, 2005, p 121.
21. (a) Ros-Lis, J. V.; Martínez-Máñez, R.; Soto, J. *Chem. Commun.* **2002**, 2248. (b) Tomasulo, M.; Raymo, F. M. *Org. Lett.* **2005**, 7, 4633. (c) Ros-Lis, J. V.; Martínez-Máñez, R.; Soto, J. *Chem. Commun.* **2005**, 5260. (d) Sun, Y.; Wang, G.; Guo, W. *Tetrahedron* **2009**, 65, 3480.



22. Furniss B. S.; Hannaford A. J.; Smith P. W. G.; Tatchell, A. R. *Vogel's Textbook of Practical Organic Chemistry*, 5th Edn.; Wiley: New York, 1989.
23. Rezende, M. C.; Radetski, C. M. *Quim. Nova* **1988**, *11*, 353; *Chem. Abstr.* **1989**, *111*, 8876w.