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Chromogenic anionic chemosensors based on protonated merocyanine solvatochromic dyes in trichloromethane and in trichloromethane–water biphasic system

Lizandra Maria Zimmermann-Dimer, Dalci Clair Reis, Clodoaldo Machado, Vanderlei Gageiro Machado *

Departamento de Química, Universidade Regional de Blumenau, FURB, CP 1507, Blumenau, SC 89010-971, Brazil

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ABSTRACT

Three merocyanine dyes (two pyridiniophenolates and Brooker's merocyanine) were dissolved in trichloromethane in their protonated form, and their potential as anionic chromogenic chemosensors was explored by adding various anions. The experimental data collected were treated considering a model based on the proton transfer from the protonated dye to the anion. One of the dyes was used in the development of an anionic colorimetric assay based on a trichloromethane–water biphasic system, which was able to selectively detect cyanide among other anionic species.

1. Introduction

The recognition and detection of anions is a field that has received considerable interest due to the fact that these species play a very important role in many chemical and biological processes.[1] Therefore, many optical chemosensors have been developed to perform selective anion detection visually and, in addition, to allow the quantification of such species.[2–5] Of the strategies used for the development of anionic chemosensors, the most simple involves the design of molecules that change color following an alteration in their molecular structure due to their contact with anions. In this case, the selectivity of the chemosensor toward an anion is related to the fact that anionic species have differentiated capabilities to interact with the receptor site in the chemosensor, for instance, through hydrogen bonding (HB). In relation to this feature, the observation made by Steiner[6] should be considered, i.e., that all HB processes should be characterized as incipient proton-transfer processes and that for strong hydrogen bonds these reactions can be found in a very advanced state.

These aspects have been observed in the past few years in the development of anionic chromogenic chemosensors,[7–10] particularly for F^-. The greater effect of this anion in comparison with other anionic species is related to its smaller size, higher charge density, and higher electron affinity. These important properties make F^- capable of forming a stronger interaction with, for instance, phenol groups. The development of simple chemosensors for F^- is of great interest due to its importance in the clinical treatment of osteoporosis, the role it plays as an environmental pollutant, and the diseases related to its over-accumulation in the bones.[11] Another interesting target in terms of detection is CN^-, an extremely toxic anion, which is lethal in very small amounts due to its strong binding to the active site of cytochromo-oxidase, which inhibits the mitochondrial electron-transport chain, leading to a decrease in the oxidative metabolism due to the non-use of oxygen.[12] Besides its recognized toxicity, CN^- is used in the fishing, metallurgy, and mining industries as well as in the fabrication of polymers. Also, it is formed through hydrolysis in many foods, such as some fruit seeds and roots.[12] It is also important to emphasize that many chemical warfare nerve agents deliver F^- and CN^-, through hydrolysis, such as sarin, soman, and tabun,[13] and this represents an important feature in the development of chemosensors for detection of these compounds.[14]

Merocyanine dyes are heterocyclic compounds which have many applications[15] and, among these compounds, two examples are well known: Brooker's merocyanine,[16] 4-[(1-methyl-4(1*H*)-pyridinylidene)-ethylidene]-2,5-cyclohexadien-1-one (**1a**), and

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* Corresponding author. Tel.: +55 47 3221 6090; fax: +55 47 3321 0512.
E-mail address: gageiro@furb.br (V.G. Machado).

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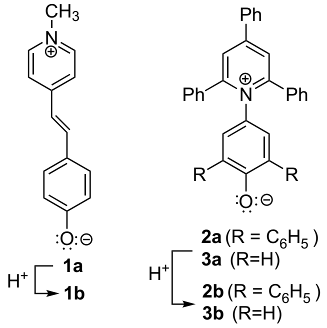
2,6-diphenyl-4-(2,4,6-triphenylpyridinium-1-yl)phenolate, known as Reichardt's betaine (**2a**).[17–19] Many studies have been carried out with these dyes due to the fact that they are solvatochromic, i.e., their UV–vis spectrum changes if the medium polarity is altered.[17–19] The maximum absorption of **2a** in different solvents represents the basis of the Reichardt polarity parameter $E_T(30)$, one of the most popular solvent polarity scales.[17–19] Besides the classical applications of dyes **1a**[20,21] and **2a**,[10,17–19,20,22] which are based on their perichromic properties, they have been recently used as signalizing units in the development of chemosensors for anionic[10,23] and neutral[24] analytes.

Hong and co-workers reported an F^--selective chromogenic chemosensor based on the structure of azophenols able to interact with anions through HB and on the basicity of the anions.[25] More recently, we have shown that pyridiniophenolate **2a** in trichloromethane, after protonation at the phenolate oxygen atom in trichloromethane, generates **2b**, which acts as an anionic chemosensor for F^- and $H_2PO_4^-$.[10] These anions are able to abstract a proton from the phenol group in the protonated dye, which makes its solutions colored. In its protonated form, it acts as an anionic chemosensor for F^- and $H_2PO_4^-$. Compound **2b** was also able to signal the presence of I^-, through the complexation of the anion with the pyridinium group.[10]

In this paper, compounds **1b** and **2b** were compared in the presence of various anions using trichloromethane as the solvent. Pyridiniophenolate **3a**, 4-(2,4,6-triphenylpyridinium-1-yl)phenolate, was also used in its protonated form with the same aim, as well as in the construction of a CN^--selective chemosensor based on a trichloromethane–water biphasic system.



2. Results and discussion

Dyes **1a**–**3a** were initially dissolved in trichloromethane which contained traces of water. A solution of **1a** in the same solvent is blue, displaying two absorption bands in the visible region, one with λ_{max} at 619 nm and a molar absorptivity of $2.28\times10^4\ dm^3\ mol^{-1}\ cm^{-1}$, and another band with λ_{max} at 579 nm and a molar absorptivity of $1.47\times10^4\ dm^3\ mol^{-1}\ cm^{-1}$. This pattern is typical for this dye in solvents with low polarity, as is the case of trichloromethane, and this aspect has been well discussed.[16f,26] A solution of **2a** in this medium is green, having a solvatochromic absorption band with λ_{max} at 725 nm and a molar absorptivity of $5.48\times10^3\ dm^3\ mol^{-1}\ cm^{-1}$. Trichloromethane solutions of compound **3a** are deep blue, because of a charge-transfer band exhibiting a λ_{max}=609 nm, with a corresponding molar absorptivity of $3.98\times10^3\ dm^3\ mol^{-1}\ cm^{-1}$. Protonation of these compounds makes solutions of **2b** and **3b** colorless and that of **1b** pale yellow. This is due to the disappearance of the solvatochromic band, having a charge-transfer nature involving the phenolate donor group and the pyridinium acceptor group in these dyes.

Figure 1 shows the effect of addition of different anions (CN^-, Cl^-, Br^-, I^-, $H_2PO_4^-$, HSO_4^-, NO_3^-, and F^-) to the colorless solution of compounds **1b**–**3b**. It can be observed that the addition of F^- and CN^- caused the reappearance of the color characteristic of the dyes **1a**–**3a**, while $H_2PO_4^-$ caused the reappearance of the color in the solutions of **2b** and, although to a lesser extent, also of **3b**. None of the other anions caused the reappearance of the original color typical of deprotonated dyes, although some anions were responsible for the appearance of yellowish colors in solutions of **2b** (I^-) and **3b** (Cl^- and I^-) due to charge transfer or HB phenomena. Figure 2 shows UV–vis spectra of the compounds **1b**–**3b** in the absence and in the presence of the anions responsible for the alterations in the color of the solutions. It can be verified for **2b** that addition of CN^-, F^-, and $H_2PO_4^-$ caused the reappearance of the solvatochromic band of the deprotonated dye, and that using the same concentration of each anion, the effect of the anions follows the order: $CN^->F^->H_2PO_4^-$. For solutions of **1b**, only CN^- and to a lesser extent F^- were responsible for the reappearance of the solvatochromic band typical of the deprotonated dye. Finally, compound **3b** provided the same order observed for solutions of **2b**.

Figure 3 shows the relative absorbance values for solutions of **1b**–**3b** in the presence of F^-, CN^-, and $H_2PO_4^-$ anions. The highest absorbance values found for protonated dyes were in the presence of CN^-, followed by F^-. While $H_2PO_4^-$ caused a small increase in the solvatochromic band of the pyridiniophenolates, no effect was observed on addition of this anion to the solution of **1b**.

It has been observed that dye **2a** is preferentially solvated by the hydroxylic component in mixtures of trichloromethane with alcohols due to HB between the alcohol and the phenolate donor group in the dye.[27] In addition, another recent study has shown that the addition of methyl-β-cyclodextrin to a solution of dye **2a** in trichloromethane leads to a significant hypsochromic band shift due to a strong cyclodextrin–dye interaction by means of HB.[20b] Thus, our experimental results indicate that a full proton transfer from the protonated dye to the anion occurs, since a hydrogen-bonded interaction alone, involving the protonated dye and the anion, would lead to a visible band corresponding to a hypsochromic shift in relation to the deprotonated dye.

2.1. Titration of protonated dyes with the anionic species

Compounds **1b**–**3b** were titrated in trichloromethane with the anions able to change the color of the solutions. The absorbance values for solvatochromic bands of the deprotonated dyes were plotted as a function of the concentration of the anion added and the experimental data were fitted with the use of Eqs. 1–4,[28–30] which relate to the following situations according to different dye–anion stoichiometries:

Case 1. 1:1 stoichiometry

$$\mathrm{Abs} = \mathrm{Abs_o} + (\mathrm{Abs_{11}} - \mathrm{Abs_o})/2C_{\mathrm{dye}}\left\{C_{\mathrm{dye}} + C_{\mathrm{A^-}} + 1/K_{11} - \left[\left(C_{\mathrm{dye}} + C_{\mathrm{A^-}} + 1/K_{11}\right)^2 - 4C_{\mathrm{dye}}C_{\mathrm{A^-}}\right]^{1/2}\right\} \quad (1)$$

Case 2. 1:3 stoichiometry

$$\mathrm{Abs} = \left[\mathrm{Abs_o} + \mathrm{Abs_{13}}K_{13}C_{\mathrm{A^-}}^3\right]/\left[1 + K_{13}C_{\mathrm{A^-}}^3\right] \quad (2)$$

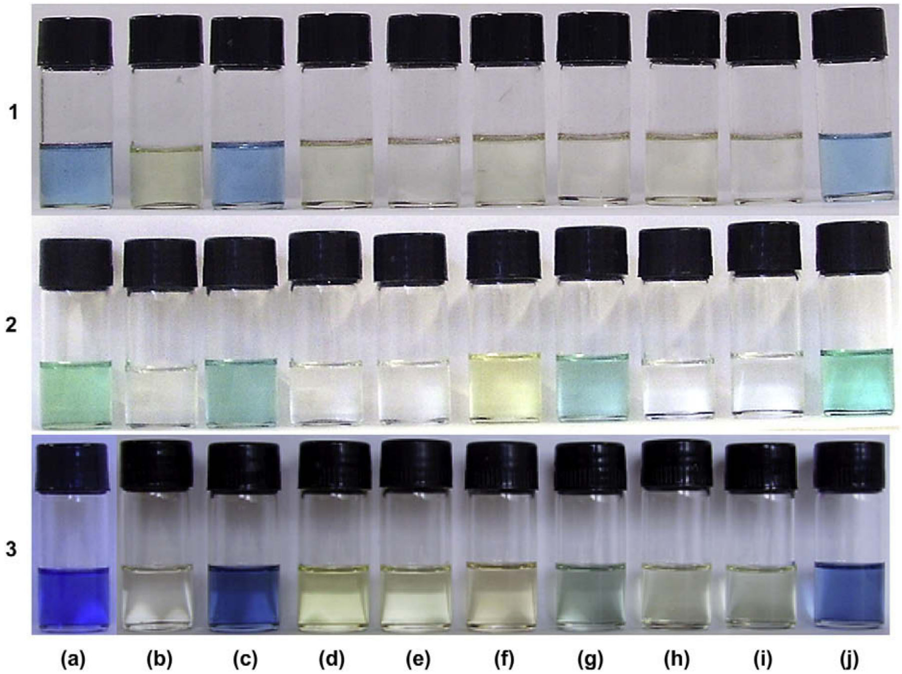


Figure 1. Solutions in trichloromethane of (a) dyes **1a–3a**, (b) **1b–3b**, and protonated dyes in the presence of (c) CN^-, (d) Cl^-, (e) Br^-, (f) I^-, (g) $H_2PO_4^-$, (h) HSO_4^-, (i) NO_3^-, and (j) F^-, as tetra-*n*-butylammonium salts. For concentrations of dyes and anions, see the text.

Case 3. 1:1 and 1:2 stoichiometries

$$\mathrm{Abs} = \left[\mathrm{Abs_o} + \mathrm{Abs}_{11}K_{11}C_{\mathrm{A}^-} + \mathrm{Abs}_{12}K_{11}K_{12}C^2_{\mathrm{A}^-}\right] / \left[1 + K_{11}C_{\mathrm{A}^-} + K_{11}K_{12}C^2_{\mathrm{A}^-}\right] \quad (3)$$

Case 4. 1:1 and 1:3 stoichiometries

$$\mathrm{Abs} = \left[\mathrm{Abs_o} + \mathrm{Abs}_{11}K_{11}C_{\mathrm{A}^-} + \mathrm{Abs}_{13}K_{11}K_{13}C^3_{\mathrm{A}^-}\right] / \left[1 + K_{11}C_{\mathrm{A}^-} + K_{11}K_{13}C^3_{\mathrm{A}^-}\right] \quad (4)$$

In these equations, Abs is the absorbance value after each addition of the anion, $\mathrm{Abs_o}$ is the initial absorbance without anion added, Abs_{11}, Abs_{12}, and Abs_{13} are the maximum absorbance values obtained by addition of the anion considering 1:1, 1:2, and 1:3 dye–anion stoichiometries, C_{dye} is the concentration of the dye, C_{A^-} is the concentration of the anion in each addition, and K_{11}, K_{12}, and K_{13} are the binding constants.[31] The results are given in Table 1 and show very good fits for all systems studied ($\mathrm{SD} < 6.0 \times 10^{-4}$).

Figure 4 shows a sequence of UV–vis spectra for a titration of **1b** with F^-. It can be noted that after the addition of this anion the solvatochromic bands of the dye appear at 579 nm and at 619 nm, with the simultaneous disappearance of the band at 396 nm. An isosbestic point at 462 nm can also be observed, suggesting an equilibrium between protonated and deprotonated dyes. A plot of the absorbance values at 619 nm as a function of the F^- concentration displayed a behavior typical of a 1:1 protonated dye–anion stoichiometry (inset in Fig. 4). A

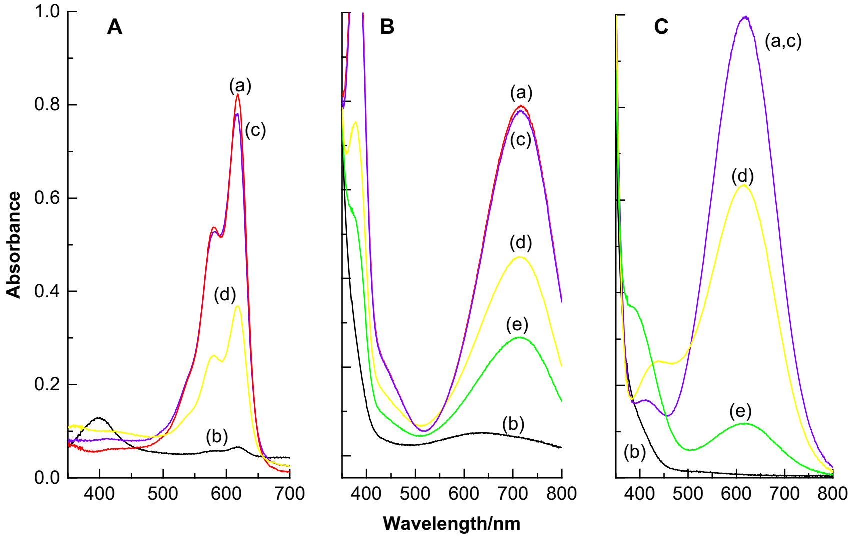


Figure 2. UV–vis spectra for trichloromethane solutions of dyes **1a–3a** at 25 °C. (A) dye **1a** (a), **1b** (b), and **1b** in the presence of CN^- (c) and F^- (d). (B) dye **2a** (a), **2b** (b), and **2b** in the presence of CN^- (c), F^- (d), and $H_2PO_4^-$ (e). (C) dye **3a** (a), **3b** (b), and **3b** in the presence of CN^- (c), F^- (d), and $H_2PO_4^-$ (e). For concentrations of dyes and anions, see the text.

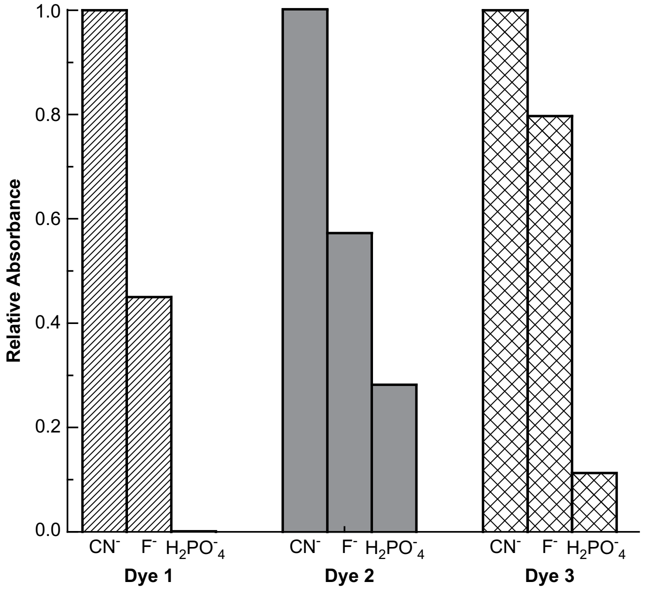


Figure 3. Relative absorbances for compounds **1b** (▨; 1.8×10^{-5} mol dm^{-3}), **2b** (■; 1.3×10^{-4} mol dm^{-3}), and **3b** (▩; 2.5×10^{-4} mol dm^{-3}) in trichloromethane, with the addition of each anion in the concentration of 1.5×10^{-4} mol dm^{-3} for **1b**, 1.5×10^{-3} mol dm^{-3} for **2b**, and 1.0×10^{-3} mol dm^{-3} for **3b**.

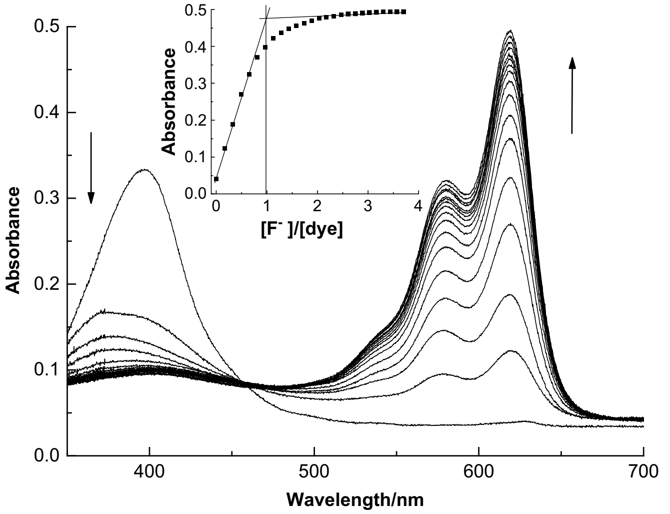


Figure 4. Influence of the addition of increasing amounts of F$^-$ on the UV–vis spectra of **1b** (1.8×10^{-5} mol dm^{-3}) in trichloromethane at 25 °C. The final concentration of F$^-$ was 1.1×10^{-4} mol dm^{-3}. The inset displays a mole ratio plot for the interaction of **1b** with F$^-$, which clearly indicates a 1:1 dye–anion stoichiometry.

fitting of the experimental data based on the use of a treatment described by Valeur and co-workers[28] gave a binding constant of $(7.51\pm0.66)\times10^4$ dm^3 mol^{-1}. Compound **1b** was also titrated under the same conditions with CN$^-$, and it was observed that the first part of the experiment was similar to that observed for the titration with F$^-$, with the appearance of the bands typical of a deprotonated dye. A maximum in absorbance at 619 nm was verified with addition of the anion for a concentration nearly 5 times in excess of the dye concentration. Thereafter, the addition of CN$^-$ led to a decrease in the maximum absorbance, probably due to a reaction between the deprotonated dye and the anion.[32]

The UV–vis spectra in Figure 5 show the behavior of **2b** dissolved in trichloromethane during the addition of CN$^-$. It can be seen that the addition of the anion led to the appearance of a band with a maximum at 380 nm and the simultaneous appearance of the solvatochromic band of the deprotonated dye at 725 nm. An increase in absorbance occurs, but the anion concentration required for the full deprotonation of the dye is nearly 15 times greater than the dye concentration, suggesting that other events occur before the abstraction of the phenolic proton. A plot of the absorbance values at 725 nm as a function of CN$^-$ concentration (see inset in Fig. 5) shows a behavior typical of a 1:3 protonated dye–anion stoichiometry and a fitting of the experimental data gave a binding constant of $(1.81\pm0.91)\times10^9$ dm^9 mol^{-3}.

Figure 6 shows the UV–vis spectra of the titration of **3b** with CN$^-$. It can be observed that in the first step of the experiment there is a significant increase in the absorbance values in the range of 400–441 nm, with the occurrence of a bathochromic band shift up to 441 nm. Absorbance at this wavelength then decreased, with the simultaneous appearance of the solvatochromic band of **3a** with a maximum at 609 nm. The occurrence of an isosbestic point at 497 nm was also detected, which suggests an equilibrium situation. Figure 6B represents these two main features of the titration, by means of two data sets on the influence of CN$^-$ addition on the absorbance at 441 and 609 nm. From this latter curve it was possible to calculate the value of $K_{13}=(1.47\pm0.04)\times10^{10}$ dm^9 mol^{-3}.

Titration of **3b** with F$^-$ (not shown) is very similar to that with CN$^-$, giving a slightly larger K_{13} of $(4.68\pm0.10)\times10^{10}$ dm^9 mol^{-3}. According to the titration profiles obtained with these two anions it is possible to note three distinct trends, and thus to separate the UV–vis spectra set into three steps, as shown in Figure 7 with F$^-$ as the anionic species. In the first step (Fig. 7A), the absorbance increase occurs close to 400 nm, with small bathochromic band shifts, while in the second step (Fig. 7B) a bathochromic band shift occurs up to 441 nm with a significant increase in absorbance in this region. Finally, in the last step of the process (Fig. 7C), the absorbance decreases at 441 nm with a simultaneous increase at 609 nm and with an isosbestic point at 497 nm.

Table 1

Calculated binding constants for the interaction of compounds **1b**–**3b** with F$^-$, CN$^-$, and $H_2PO_4^-$

Compound	Anion	K_{11}/dm^3 mol^{-1}	K_{12}/dm^6 mol^{-2}	K_{13}/dm^9 mol^{-3}	SD
1b	F$^-$	$(7.51\pm0.66)\times10^4$	—	—	2×10^{-5}
2b	F$^-$	$(7.08\pm4.03)\times10^7$ [a]	—	—	5×10^{-5}
2b	$H_2PO_4^-$	$(5.61\pm0.14)\times10^4$ [a]	—	—	7×10^{-5}
2b	CN$^-$	—	—	$(1.81\pm0.91)\times10^9$	2×10^{-4}
3b	F$^-$	—	—	$(4.68\pm0.10)\times10^{10}$	4×10^{-5}
3b	F$^-$ [b]	$(5.92\pm1.42)\times10^3$	—	$(1.06\pm0.07)\times10^7$	3×10^{-5}
3b	F$^-$ [c]	$(7.69\pm4.15)\times10^3$	$(8.27\pm0.27)\times10^2$	—	1×10^{-5}
3b	CN$^-$	—	—	$(1.47\pm0.04)\times10^{10}$	5×10^{-5}
3b	CN$^-$(biphasic system)	$(2.15\pm0.21)\times10^3$	—	$(3.46\pm1.54)\times10^4$	2×10^{-4}

[a] From Ref. 10.

[b] With 6 times excess of iodide.

[c] Experiment made in presence of 2% (v/v) methanol.

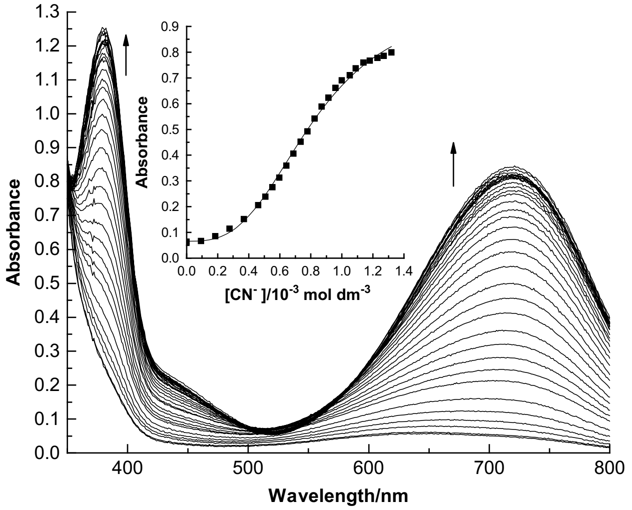


Figure 5. UV–vis spectra of **2b** (1.3×10^{-4} mol dm^{-3}) in trichloromethane at 25 °C after addition of increasing amounts of CN$^-$. The final concentration of CN$^-$ was 3.3×10^{-3} mol dm^{-3}. The inset shows the corresponding titration curve and experimental data at 725 nm were fitted with Eq. 2.

2.2. Interaction of 3b with CN$^-$ in a biphasic system

Dyes **1a**–**3a** have low solubility in water which, in principle, could be advantageous in the development of an anionic chromogenic sensing set based on a trichloromethane–water biphasic system. The rationale here is based on the fact that basic anions in the aqueous phase would abstract a proton of the protonated dye in the organic phase, leading to a change in the color of this phase. Thus, some preliminary experiments were performed with protonated dyes in trichloromethane–water in the absence of anions. Under these conditions, it was observed that **3b** remained protonated, while **1b** was partially transferred toward the aqueous phase and compound **2b** underwent deprotonation in the absence of the anions. Addition of tetra-*n*-butylammonium hydroxide to the aqueous phase of the biphasic system containing **3b**, followed by stirring, led to the appearance of color in the organic phase corresponding to the deprotonated dye. A UV–vis spectrum was taken and shows a solvatochromic absorption band with a maximum at 575 nm. Comparison with the data obtained in pure trichloromethane indicates a hypsochromic band shift of $\Delta\lambda=34$ nm, which can be explained by the fact that the organic phase in the biphasic system is saturated with water,[33] and the hydroxylic component preferentially solvates the deprotonated dye. The presence of color in the organic phase was then observed with **3b** by adding anions to the aqueous phase. Addition of NaCN caused the appearance of the solvatochromic absorption band of **3a** (Fig. 8). It was also verified that, after stirring the phases, only CN$^-$ led to a colored organic phase, while F$^-$ and $H_2PO_4^-$ did not cause any alteration in the system (see inset in Fig. 8).

Figure 9 shows a plot of the absorbance values at 575 nm as a function of the CN$^-$ concentration, taken from the spectra of Figure 8. The data were fitted by means of Eq. 4, which is used for 1:1 and 1:3 dye–anion stoichiometries, giving $K_{11}=(2.15\pm0.21)\times10^3$ dm^3 mol^{-1} and $K_{13}=(3.46\pm1.54)\times10^4$ dm^9 mol^{-3}. In order to check the functionality and reproducibility of the experimental conditions of this calibration curve, tests were carried out by adding CN$^-$ to systems where other anions were absent as well as in the presence of F$^-$ and $H_2PO_4^-$ at the same concentrations. It was observed that the effect of the presence of F$^-$ is minimal in relation to the presence of only CN$^-$ in the medium, while the presence of $H_2PO_4^-$ caused a reduction in the absorbance value at 575 nm, hindering the quantitative analysis (see inset in Fig. 9). This latter result is due to the fact that $H_2PO_4^-$ and HPO_4^{2-} coexist in equivalent proportions at pH 7.23,[34] and these species are sufficiently acidic to protonate a fraction of CN$^-$ anions in solution. Finally, a biphasic system was prepared again mixing **3b** in trichloromethane, at the same concentration used to construct the calibration curve, with an aqueous NaCN solution in a concentration of 5.0×10^{-4} mol dm^{-3}. The mixture was stirred and the UV–vis spectrum was then obtained for the organic phase. The absorbance value collected at 575 nm was used to calculate the CN$^-$ concentration, using the equation from the calibration curve, and it confirmed the concentration of this anion in the system.

2.3. Behavior of protonated dyes in the presence of the anions

Table 1 compares the binding constants obtained for the interaction of the protonated dyes with the anions. The experimental data show that $H_2PO_4^-$ is sufficiently basic to abstract a proton from **2b** but was unable to deprotonate **1b** and had little effect in the deprotonation of **3b**.[35] In addition, comparison between the K_{11}

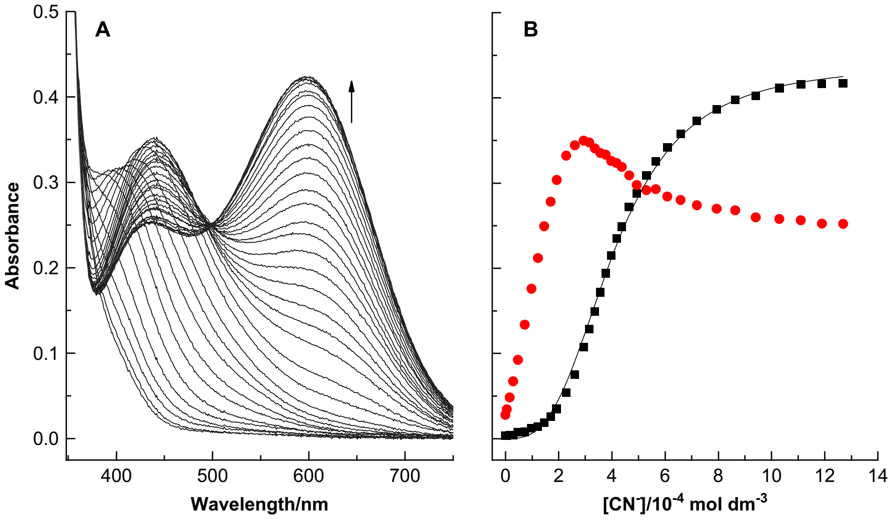


Figure 6. (A) UV–vis spectra of **3b** (2.5×10^{-4} mol dm^{-3}) in trichloromethane at 25 °C after addition of increasing amounts of CN$^-$. (B) Variation in the absorbance at 609 nm (■) and 441 nm (●) of **3b** with the addition of increasing amounts of CN$^-$. The final concentration of CN$^-$ was 1.4×10^{-3} mol dm^{-3} and experimental data at 609 nm were fitted with Eq. 2.

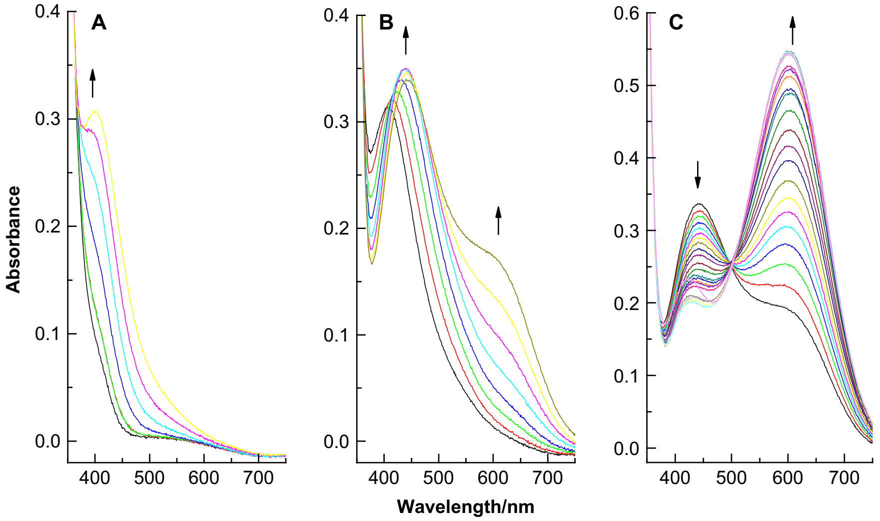


Figure 7. UV–vis spectra of **3b** (2.5×10^{-4} mol dm^{-3}) in trichloromethane at 25 °C after addition of increasing amounts of F^-. (A) UV–vis spectra of the first part of the titration, with the concentration of F^- varying between zero and 1.0×10^{-4} mol dm^{-3}. (B) UV–vis spectra of the second part of the titration, with the concentration of F^- varying between 1.2×10^{-4} mol dm^{-3} and 2.4×10^{-4} mol dm^{-3}. (C) UV–vis spectra of the latter part of the titration, with the concentration of F^- varying between 2.6×10^{-4} mol dm^{-3} and 2.6×10^{-3} mol dm^{-3}.

values for **1b** and **2b** shows a larger K_{11} value for **2b**. This suggests that **2b** is more acidic than the other protonated merocyanines. In the literature the pK_a value for compound **1a** in water is reported as being 8.37[36] or 8.54.[37] For compound **2b**, a similar pK_a value of 8.6 was obtained in aqueous solution.[38] Transferring these compounds from water, a hydrogen-bond donating (HBD) solvent, to a non-HBD medium or to a solvent with a low ability to act as an HBD will cause a change in their pK_a values because, while water is able to solvate the phenolate moiety through HB, these latter interactions are weaker or absent in the other media. Thus, in these non-aqueous media, the molecular structure of the conjugated base is crucial in order to stabilize its negative charge. Hajdu and Smith discussed this aspect with some classical examples[39,40] and the effect of the medium and also of the molecular structure on the pK_a values of phenols was shown in a recent paper.[41] With respect to compounds **1b**–**3b**, the only pK_a value available in the literature for a non-HBD solvent was collected in acetonitrile by Coleman and Murray for compound **2b** (pK_a=22.1±0.2).[42] Thus, an experiment was performed by titrating with F^- a mixture of compounds **2b** and **3b**, in trichloromethane, both in the same concentration (2.0×10^{-4} mol dm^{-3}). It was verified that the addition of the anion in the concentration range between zero and 5.2×10^{-3} mol dm^{-3} caused only the deprotonation of **2b**, which was evidenced by the appearance of a band at λ_{max}=709 nm, turning the solution green. Successive additions of the anion in concentrations of 5.3×10^{-3} mol dm^{-3} up to 0.1 mol dm^{-3} caused a hypsochromic shift from λ_{max}=709 nm to λ_{max}=652 nm with formation of a large band, forming a blue colored solution, typical of dye **3a**. This band with λ_{max}=652 nm corresponds to the merging of the bands of dye

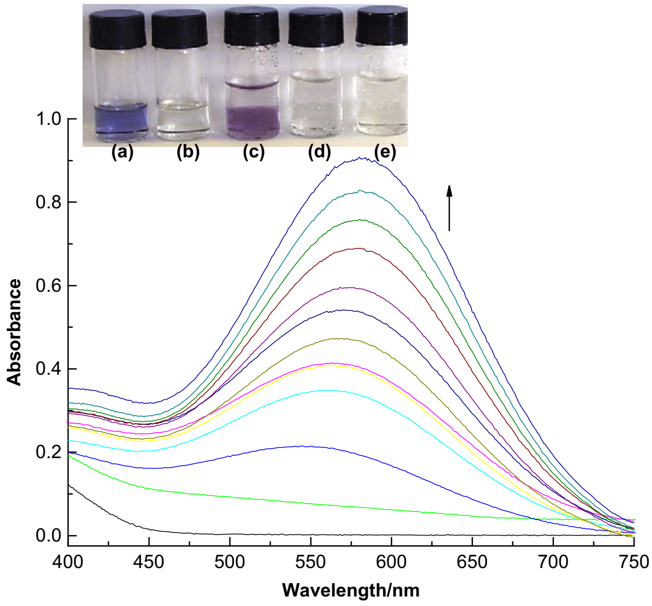


Figure 8. UV–vis spectra of **3b** (2.5×10^{-4} mol dm^{-3}) in trichloromethane at 25 °C, in a trichloromethane–water biphasic system, after addition of increasing amounts of NaCN in water, with a final concentration of the salt of 5.7×10^{-3} mol dm^{-3}. The inset shows solutions of deprotonated (a) and protonated (b) dyes in the absence of CN^- and biphasic systems with protonated dye in trichloromethane in the presence of NaCN (c), NaF (b), and KH_2PO_4 in the aqueous phase, in a concentration of 1.0×10^{-3} mol dm^{-3}.

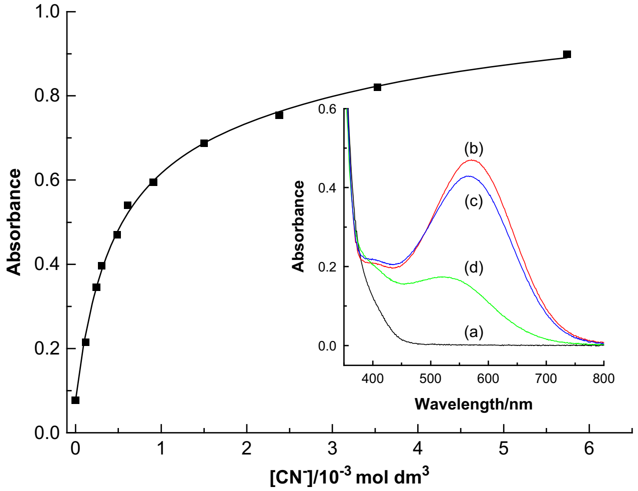
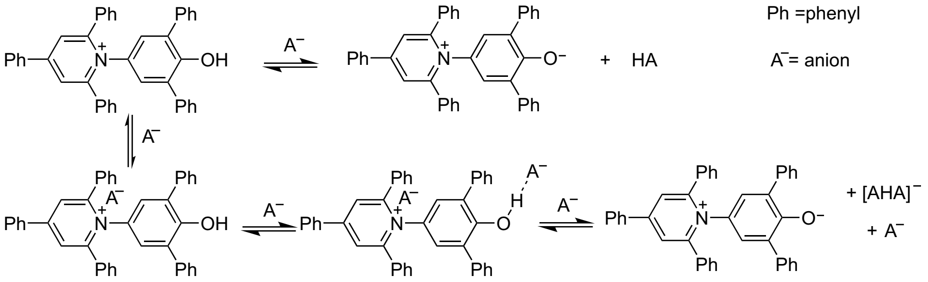


Figure 9. Calibration curve for the titration of **3b** (2.5×10^{-4} mol dm^{-3}) in a trichloromethane–water biphasic system at 25 °C with the addition of increasing amounts of NaCN in the aqueous phase. Experimental data were collected at 575 nm. The inset shows UV–vis spectra at 25 °C of the organic phase in the biphasic system for: (a) **3b**; (b) NaCN; (c) NaCN and NaF; and (d) NaCN, NaF, and KH_2PO_4. All salts were added to the aqueous phase in a concentration of 5.0×10^{-4} mol dm^{-3}.



Scheme 1.

2a (λ_{max}=725 nm) and of dye **3a** (λ_{max}=609 nm) in the same concentration. Thus, this experiment demonstrates that, in trichloromethane, **2b** is more acidic than **3b**.

The preference of the protonated dyes for CN^-, F^-, and to a lesser extent, $H_2PO_4^-$ (in the case of **2b** and **3b**) over other studied anions is due to the high basicity of these species, enabling them to strongly interact with the phenol group in the chemosensors. This explanation has been put forward in other studies on anion phenol-based chromo- and fluorogenic chemosensors exhibiting very high selectivity toward F^- in comparison to other anions.[7–10,23,25]

The results show that various stoichiometries can be obtained for the studied systems depending on the protonated dye, the medium, and the anion used. Scheme 1 shows different behaviors of the compound **2b**, which can be generalized and applied to the other dyes. If the dye–anion stoichiometry is 1:1, the anion is sufficiently basic to completely abstract the proton and generate the deprotonated dye. The experimental conditions that favor this profile are a very acidic dye, a basic anion, and a medium able to solvate both the anion (to a lesser extent) and the deprotonated dye (to a greater extent). Another possible stoichiometry requires two anion species for each protonated molecule to generate the deprotonated dye. In this situation, which is discussed in some studies in the literature,[8,9,43,44] firstly the anion forms a hydrogen-bonded complex with the protonated dye, weakening the H–O bond in the compound. The addition of the second anion then leads to abstraction of the proton, with formation of an $[HA_2]^-$ complex, such as the $[HF_2]^-$ species whose formation and stability have been verified by theoretical studies.[45] As a new feature in relation to other chromogenic systems based on proton transfer found in the literature, in our study we obtained not a 1:2 but a 1:3 dye–anion stoichiometry for the experimental conditions less favorable to proton transfer. Recently, we have observed that compounds **1b**–**3b** showed a similar behavior in acetonitrile.[46] Thus, the data suggest that the anion would firstly form an ion pair with the pyridinium group and after that a second equivalent of the anion would be used to form a hydrogen-bonded complex. Finally, a third equivalent of the anion would be needed for the abstraction of the proton, with formation of an $[HA_2]^-$ complex. In order to obtain further experimental evidence for this proposal, two experiments were carried out. In the first experiment, a solution of compound **3b** in trichloromethane with a 6-fold excess of I^- was titrated with F^-. It is well known that *N*-alkyl pyridinium cations can form ion-pair charge-transfer complexes with I^- in solution.[10,47] Thus, it would be expected that the presence of I^- would compete with F^- for the pyridinium center, promoting a change in the stoichiometry of the process. The experiment revealed that this effectively occurred, with the dye–anion stoichiometry of the process changing from 1:3 to both 1:1 and 1:3 (Table 1). In addition, a reduction in the magnitude of the K_{13} value was observed with the addition of I^-.

Another experiment was carried out to investigate the possibility of the interaction of the anion with the pyridinium center of the compound involved in the titration of **3b** with F^- in trichloromethane in the presence of 2% (v/v) of methanol. It was observed here that addition of the anion resulted in an increase in the solvatochromic band of **3a** with λ_{max}=547 nm (Fig. 10A).[48] Absorbance values were taken at this wavelength and plotted as a function of

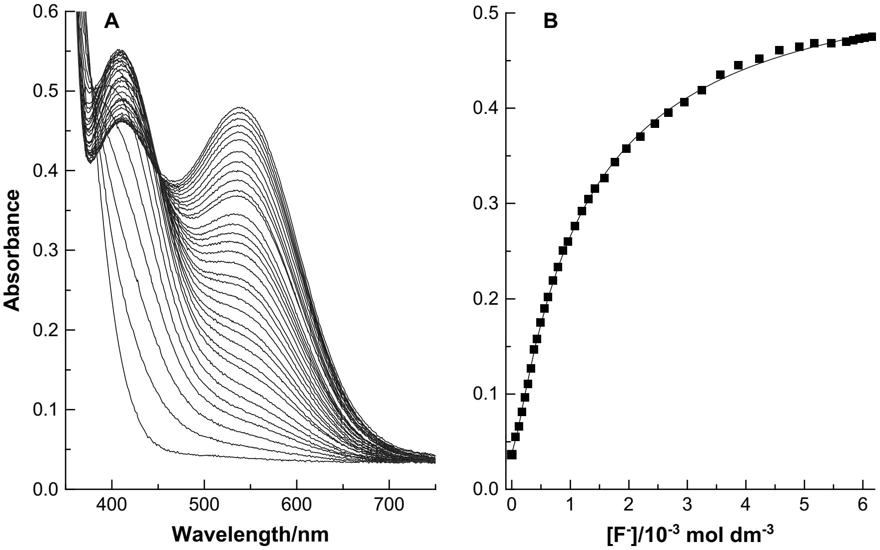


Figure 10. (A) UV–vis spectra of **3b** (2.5×10^{-4} mol dm^{-3}) in trichloromethane with methanol (2% v/v) at 25 °C after addition of increasing amounts of F^-. (B) Variation in the absorbance at 547 nm (■) of **3b** with the addition of increasing amounts of F^-. The final concentration of F^- was 6.1×10^{-3} mol dm^{-3} and experimental data at 547 nm were fitted with Eq. 3.

the anion concentration (Fig. 10B). A change in the shape of the titration curve was verified, corresponding to a change in the dye–anion stoichiometry from 1:3 in pure trichloromethane to 1:1 and 1:2 in the trichloromethane–methanol mixture. This occurred because the alcohol preferentially solvates the charged species inhibiting the electrostatic interactions between the pyridinium moiety and the anion.

The experimental data demonstrate that although **3b** in trichloromethane is selective toward CN^-, F^-, and $H_2PO_4^-$ in relation to the other anions employed, the trichloromethane–water biphasic set with the same protonated dye in the organic phase makes the system very selective toward CN^-. The reason for this selectivity is related to the fact that the free energies of hydration for $H_2PO_4^-$ (-465 kJ mol^{-1}) and F^- (-465 kJ mol^{-1}) are high in comparison to that observed for CN^- (-295 kJ mol^{-1}).[34,49] Thus, water strongly preferentially solvates $H_2PO_4^-$ and F^-, and these hydrated species become less capable of acting as bases. Since CN^- is less hydrated in the presence of water, this anion is more ‘naked’ and therefore acts more readily as a base, promoting the abstraction of the proton from the protonated dye in the organic phase. The influence of water in reducing the action of F^- as a base is also known.[50] The contact of trichloromethane with water makes the protonated compound more acidic, since water stabilizes the deprotonated dye through HB. This explains the fact that, in the mixtures of **3b** with CN^- in the biphasic system, the presence of water causes a change in the dye–anion stoichiometry from 1:3 to 1:1 and 1:3.

3. Conclusions

The ability of the compound **1b** to recognize CN^- and F^- more readily than $H_2PO_4^-$, as compared with the system assembled with **2b**, and to a lesser extent with **3b**, suggests that **1b** is less acidic than the protonated pyridiniophenolates. This may be related to the fact that dye **1a** and compounds **2a** and **3a** are structurally different, although with the same donor and acceptor groups in their structure. In a solvent such as trichloromethane, the role of the structure of the conjugate base is crucial in order to promote the stabilization of the negative charge on the phenolate group. This observation can help to explain the differences verified for these systems with respect to the addition of the anionic species.

An understanding of these systems in trichloromethane allowed the study of a simple chromogenic chemosensor based on a biphasic system with **3b** in the organic phase. This set was able to detect selectively CN^- in aqueous media and the experimental data obtained are in agreement with the results recently described by Raymo and co-workers.[51]

An aspect common to the chromogenic chemosensors presented here, and others recently used in several studies,[7–10,44] is the design of molecules with an acidic site for recognizing more basic anions. It is important to observe that the effects observed with these systems are not restricted to a certain anion, but can also be caused by species acting as sufficiently strong bases, hindering the qualitative analysis of such species. Therefore, many structural modifications in dyes **1a**, **2a**, and other merocyanines can be envisioned aiming to promote a change in the acidity of these dyes in order to make more efficient anionic chromogenic and also fluorogenic chemosensors.

4. Experimental

4.1. General methods

Trichloromethane was purified according to a procedure described in the literature.[52] Karl Fischer titrations were performed with this solvent and have demonstrated the presence of water in a concentration of 3.56×10^{-3} mol dm^{-3}. 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 (F^-, Cl^-, Br^-, I^-, CN^-, NO_3^-, HSO_4^-, and $H_2PO_4^-$) were used as tetra-*n*-butylammonium salts, and were purchased from Fluka (F^- (>97%), CN^- (>95%), Cl^- (>98%), NO_3^- (>97%), and $H_2PO_4^-$ (>97%) salts) and Vetec (Br^- (>99%), I^- (>99%), and HSO_4^- (>99%) salts). They were dried over phosphorus pentoxide under vacuum before use. Karl Fischer experiments were performed for the following tetra-*n*-butylammonium salts in order to determine the water content of each salt: CN^- (0.116% water), F^- (1.125% water), and $H_2PO_4^-$ (0.111% water). The protonated form of dye **1a** was synthesized as an iodide salt, as described previously.[53] Dye **2a** was synthesized according to a method described in the literature,[54] recrystallized three times from hot water and dried under vacuum. Very small amounts of acetic acid stock solution (3.5×10^{-3} mol dm^{-3}) in trichloromethane were added with a microsyringe to the solutions of the dye until its protonation. Compound **3b** was prepared through the reaction of 2,4,6-triphenyl pyrylium perchlorate with 4-aminophenol.[55,56]

4.2. UV–vis studies of the protonated dyes 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 on the UV–vis spectra (λ_{max}) were calculated from the first derivative of the absorption spectrum.

The experiments were performed as follows: a stock solution of the dye (1.8×10^{-5} mol dm^{-3} for dye **1a**, 1.3×10^{-4} mol dm^{-3} for dye **2a**, and 2.5×10^{-4} mol dm^{-3} for dye **3a**) was prepared in trichloromethane in its protonated form. Protonation was performed cautiously, because an excess of the acid in the medium would adversely affect an analysis of the titration results. Thus, in all experiments, protonated solutions were slightly colored, presenting always a very small fraction of the dye in its deprotonated form. The protonated dye solution was then used to prepare the stock solution of each anion in a concentration of 1.5×10^{-4} mol dm^{-3} for dye **1a**, 1.5×10^{-3} mol dm^{-3} for dye **2a**, and 1.0×10^{-3} mol dm^{-3} for dye **3a**. These solutions were prepared and transferred using volumetric flasks and cuvettes hermetically closed with rubber stoppers in order to minimize problems with the evaporation of the trichloromethane. All experiments were carried out at 25 °C.

4.3. UV–vis titrations

Titration experiments were performed with the preparation of the solutions of protonated dyes as described previously. Stock anion solutions were prepared in flasks closed with rubber stoppers and the titrations were carried out by adding small amounts (2–10 μL) of the salt stock solution with a microsyringe to closed quartz cuvettes containing the protonated dye solution. After each addition the UV–vis spectra were taken and the absorbance values were collected at 619 nm for dye **1a**, 725 nm for dye **2a**, and 609 nm for dye **3a**. Absorbance values were collected at 547 nm in the titration of **3b** with F^- in trichloromethane with 2% (v/v) of methanol. A titration of **3b** in trichloromethane with F^- was also carried out with an excess of 6 times of tetra-*n*-butylammonium iodide in relation to the concentration of the dye (2.5×10^{-4} mol dm^{-3}).

The titration of the solution with compounds **2b** and **3b**, both in a concentration of 2.0×10^{-4} mol dm^{-3}, was carried out as follows. From the solution containing the protonated dyes, a volume of 2 cm^3 was used to prepare a stock solution of F^- (1.0×10^{-2} mol dm^{-3}). This latter solution was utilized to titrate, by adding increasing amounts with a microsyringe, a solution containing **2b** and **3b**, as described in the previous paragraph. UV–vis spectra were collected after each addition of the anion.

4.4. Anion detection in the biphasic system

A biphasic system was studied comprising 1.5 cm^3 of an organic phase with **3b** (2.5×10^{-4} mol dm^{-3}) and 1.5 cm^3 of an aqueous phase containing the salt (NaCN, NaF, and KH_2PO_4, each in a concentration of 1.0×10^{-3} mol dm^{-3}). In all experiments, the pH of the aqueous phase was 7.1 and the ionic strength was kept constant with NaCl at a concentration of 5.0×10^{-4} mol dm^{-3}. Firstly, the following systems were prepared to give a set for naked-eye detection: (a) **3a** in the organic phase, without the salt in the aqueous phase; (b) **3b** in the organic phase, without the salt in the aqueous phase; (c) **3b** in the organic phase, NaCN in the aqueous phase; (d) **3b** in the organic phase, NaF in the aqueous phase; and (e) **3b** in the organic phase, KH_2PO_4 in the aqueous phase. Then, the systems were stirred to observe whether the anion would be able to abstract the proton from the protonated dye and UV–vis spectra were performed for the organic phase.

4.5. Calibration curve for the quantitative determination of CN^- in aqueous systems

A calibration curve was obtained through a titration in the biphasic system, containing the organic phase comprising 1.6 cm^3 of **3b** (2.5×10^{-4} mol dm^{-3}) in trichloromethane and 1.6 cm^3 of aqueous phase. An aqueous titrating stock solution containing NaCN (9.2×10^{-2} mol dm^{-3}) was prepared. The first reading of the UV–vis spectrophotometer was taken without the anion and the following readings were performed after each anion addition, stirring the system, and waiting for the separation of the phases. Absorbance values were then collected for the organic phase at 575 nm.

The next step was the evaluation of the calibration curve in the quantitative detection of CN^- in aqueous media. Therefore, a biphasic system was prepared again mixing **3b** in trichloromethane, in the same concentration used to make the calibration curve, with an NaCN aqueous solution in a concentration of 5.0×10^{-4} mol dm^{-3}. The mixture was stirred and the UV–vis spectrum was then obtained for the organic phase.

4.6. Calculations

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

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References and notes

1. For reviews, see: (a) Dietrich, B. *Pure Appl. Chem.* **1993**, *65*, 1457; (b) Seel, C.; Galán, A.; de Mendoza, J. *Top. Curr. Chem.* **1995**, *175*, 101; (c) Schmidtchen, F. P.; Berger, M. *Chem. Rev.* **1997**, *97*, 1609; (d) Bianchi, A.; Bowman-James, K.; Garcia-España, E. *Supramolecular Chemistry of Anions*; Wiley-VCH: New York, NY, 1997; (e) Antonisse, M. M. G.; Reinhoudt, D. N. *Chem. Commun.* **1998**, 443; (f) Gale, P. A. *Coord. Chem. Rev.* **2000**, *199*, 181; (g) Beer, P. D.; Gale, P. A. *Angew. Chem.* **2001**, *113*, 502; *Angew. Chem., Int. Ed.* **2001**, *40*, 486; (h) Gale, P. A. *Coord. Chem. Rev.* **2001**, *213*, 79; (i) Special issue on anionic recognition: *Coord. Chem. Rev.* **2003**, *240*, 1; (j) Kubik, S.; Reyheller, C.; Stüwe, S. *J. Inclusion Phenom. Macrocycl. Chem.* **2005**, *52*, 137; (k) Gunnlaugsson, T.; Glynn, M.; Tocci, G. M.; Kruger, P. E.; Pfeffer, F. M. *Coord. Chem. Rev.* **2006**, *250*, 3094; (l) Zimmermann-Dimer, L. M.; Machado, V. G. *Quim. Nova* **2008**, *31*, 2134.
2. (a) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515; (b) Callan, J. F.; de Silva, A. P.; Magri, D. C. *Tetrahedron* **2005**, *61*, 8551.
3. (a) Wiskur, S. L.; Ait-Haddou, H.; Lavigne, J. J.; Anslyn, E. V. *Acc. Chem. Res.* **2001**, *34*, 963; (b) Nguyen, B. T.; Anslyn, E. V. *Coord. Chem. Rev.* **2006**, *250*, 3118.
4. Martínez-Mañez, R.; Sancenón, F. *Chem. Rev.* **2003**, *103*, 4419.
5. (a) Suksai, C.; Tuntulani, T. *Chem. Soc. Rev.* **2003**, *32*, 192; (b) Suksai, C.; Tuntulani, T. *Top. Curr. Chem.* **2005**, *255*, 163.
6. Steiner, T. *Angew. Chem.* **2002**, *114*, 50; *Angew. Chem., Int. Ed.* **2002**, *41*, 48.
7. 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) Zhang, X.; Guo, L.; Wu, F.; Jiang, Y. *Org. Lett.* **2003**, *5*, 2667; (e) 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; (f) Zhou, G.; Cheng, Y.; Wang, L.; Jing, X.; Wang, F. *Macromolecules* **2005**, *38*, 2148; (g) Wu, J.-L.; He, Y.-B.; Wei, L. H.; Meng, L.-Z.; Yang, T.-X.; Liu, X. *Aust. J. Chem.* **2005**, *58*, 53; (h) Costero, A. M.; Bañuls, M. J.; Aurell, M. J.; Ochando, L. E.; Doménech, A. *Tetrahedron* **2005**, *61*, 10309; (i) Hill, J. P.; Schumacher, A. L.; D'Souza, F.; Labuta, J.; Redshaw, C.; Elsegood, M. R. J.; Aoyagi, M.; Nakanishi, T.; Ariga, K. *Inorg. Chem.* **2006**, *45*, 8288; (j) Bonizzoni, M.; Fabbrizzi, L.; Taglietti, A.; Tiengo, F. *Eur. J. Org. Chem.* **2006**, 3567; (k) Yu, M.; Lin, H.; Zhao, G. H.; Lin, H. K. *J. Mol. Recognit.* **2007**, *20*, 69.
8. Boiocchi, M.; Del Boca, L.; Gómez, D. E.; Fabbrizzi, L.; Licchelli, M.; Monzani, E. *J. Am. Chem. Soc.* **2004**, *126*, 16507.
9. (a) Amendola, V.; Boiocchi, M.; Fabbrizzi, L.; Palchetti, A. *Chem.—Eur. J.* **2005**, *11*, 120; (b) Boiocchi, M.; Del Boca, L.; Esteban-Gómez, D.; Fabbrizzi, L.; Licchelli, M.; Monzani, E. *Chem.—Eur. J.* **2005**, *11*, 3097.
10. Reis, D. C.; Machado, C.; Machado, V. G. *Tetrahedron Lett.* **2006**, *47*, 9339.
11. (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.
12. See for instance: (a) Keusgen, M.; Kloock, J. P.; Knobbe, D.-T.; Jünger, M.; Krest, I.; Goldbach, M.; Klein, W.; Schöning, M. J. *Sens. Actuators, B* **2004**, *103*, 380; (b) Lv, J.; Zhang, Z. J.; Li, J. D.; Luo, L. R. *Forensic Sci. Int.* **2005**, *148*, 15; (c) Mak, K. K. W.; Yanase, H.; Renneberg, R. *Biosens. Bioelectron.* **2005**, *20*, 2581; (d) Nelson, L. *J. Emerg. Nurs.* **2006**, *32*, S8.
13. Royo, S.; Martínez-Máñez, R.; Sancenón, F.; Costero, A. M.; Parra, M.; Gil, S. *Chem. Commun.* **2007**, 4839.
14. He, S.; Iacono, S. T.; Budy, S. M.; Dennis, A. E.; Smith, D. W., Jr.; Smith, R. C. *J. Mater. Chem.* **2008**, *18*, 1970.
15. Mishra, A.; Behera, R. K.; Behera, P. K.; Mishra, B. K.; Behera, G. B. *Chem. Rev.* **2000**, *100*, 1973.
16. (a) Brooker, L. G. S.; Keyes, G. H.; Sprague, R. H.; Van Dyke, R. H.; Van Lare, E.; Van Zandt, G.; White, F. L.; Cressman, H. W. J.; Dent, S. G. *J. Am. Chem. Soc.* **1951**, *73*, 5332; (b) Brooker, L. G. S.; Keyes, G. H.; Heseltine, D. W. *J. Am. Chem. Soc.* **1951**, *73*, 5350; (c) Benson, H. G.; Murrell, J. N. *J. Chem. Soc., Faraday Trans. 2* **1972**, *68*, 137; (d) Botrel, A.; Le Beuze, A.; Jacques, P.; Strub, H. *J. Chem. Soc., Faraday Trans. 2* **1984**, *80*, 1235; (e) Jacques, P. *J. Phys. Chem.* **1986**, *90*, 5535; (f) Catalan, J.; Mena, E.; Meutermans, W.; Elguero, J. *J. Phys. Chem.* **1992**, *96*, 3615.
17. Reichardt, C. *Solvents and Solvent Effects in Organic Chemistry*, 3rd ed.; Wiley-VCH: Weinheim, 2003; Chapters 6 and 7, pp 329–469.
18. Suppan, P.; Ghoneim, N. *Solvatochromism*, 1st ed.; Royal Society of Chemistry: Cambridge, 1997; Chapter 3, pp 68–95.
19. (a) Reichardt, C. *Chem. Rev.* **1994**, *94*, 2319; (b) Reichardt, C. *Pure Appl. Chem.* **2004**, *76*, 1903; (c) Reichardt, C. *Org. Process Res. Dev.* **2007**, *11*, 105; (d) Reichardt, C. *Pure Appl. Chem.* **2008**, *80*, 1415.
20. (a) Humeres, E.; Nunes, R. J.; Machado, V. G.; Gasques, M. D. G.; Machado, C. *J. Org. Chem.* **2001**, *66*, 1163; (b) Venturini, C. de G.; Andreaus, J.; Machado, V. G.; Machado, C. *Org. Biomol. Chem.* **2005**, *3*, 1751; (c) Testoni, F. M.; Ribeiro, E. A.; Giusti, L. A.; Machado, V. G. *Spectrochim. Acta, Part A* **2009**, *71*, 1704.
21. (a) Zanotto, S. P.; Scremin, M.; Machado, C.; Rezende, M. C. *J. Phys. Org. Chem.* **1993**, *6*, 637; (b) Morley, J. O.; Morley, R. M.; Docherty, R.; Charlton, M. H. *J. Am. Chem. Soc.* **1997**, *119*, 10192; (c) Morley, J. O.; Morley, R. M.; Fitton, A. L. *J. Am. Chem. Soc.* **1998**, *120*, 11479; (d) da Silva, D. C.; Ricken, I.; Silva, M. A. R.; Machado, V. G. *J. Phys. Org. Chem.* **2002**, *15*, 420; (e) Bevilaqua, T.; da Silva, D. C.; Machado, V. G. *Spectrochim. Acta, Part A* **2004**, *60*, 951; (f) Cavalli, V.; da Silva, D. C.; Machado, C.; Machado, V. G.; Soldi, V. *J. Fluoresc.* **2006**, *16*, 77; (g) Koopmans, C.; Ritter, H. *J. Am. Chem. Soc.* **2007**, *129*, 3502.
22. (a) Gageiro, V.; Aillón, M.; Rezende, M. C. *J. Chem. Soc., Faraday Trans.* **1992**, *88*, 201; (b) Reichardt, C.; Asharin-Fard, S.; Schäfer, G. *Chem. Ber.* **1993**, *126*, 143; (c) Bosch, E.; Rosés, M.; Herodes, K.; Koppel, I.; Leito, I.; Koppel, I.; Taal, V. *J. Phys. Org. Chem.* **1996**, *9*, 403; (d) Herodes, K.; Leito, I.; Koppel, I.; Rosés, M. *J. Phys. Org. Chem.* **1999**, *12*, 109; (e) Mancini, P. M. E.; Terenzani, A.; Adam, C.; Vottero, L. R. *J. Phys. Org. Chem.* **1999**, *12*, 430; (f) Tada, E. B.; Novaki, L. P.; El Seoud, O. A. *Langmuir* **2001**, *17*, 652; (g) Mchedlov-Petrossyan, N. O.; Vodolazkaya, N. A.; Reichardt, C. *Colloids Surf. A* **2002**, *205*, 215; (h) Herodes, K.; Koppel, J.; Reichardt, C.; Koppel, I. A. *J. Phys. Org. Chem.* **2003**, *16*, 626; (i) Reichardt, C. *Green Chem.* **2005**, *7*, 339; (j) Budag, R.; Giusti, L. A.; Machado, V. G.; Machado, C. *Fuel* **2006**, *85*, 1494.
23. (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.
24. (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.
25. Lee, K. H.; Lee, H.-Y.; Lee, D. H.; Hong, J.-I. *Tetrahedron Lett.* **2001**, *42*, 5447.
26. See for instance: (a) Gruda, I.; Bolduc, F. *J. Org. Chem.* **1989**, *49*, 3300; (b) Tsukada, M.; Mineo, Y.; Iteoh, K. *J. Phys. Chem.* **1989**, *93*, 7989.

27. (a) Dawber, J. G.; Williams, R. A. *J. Chem. Soc., Faraday Trans. 1* **1986**, *82*, 3097; (b) Dawber, J. G.; Ward, J.; Williams, R. A. *J. Chem. Soc., Faraday Trans. 1* **1988**, *84*, 713; (c) Ortega, J.; Ràfols, C.; Bosch, E.; Rosés, M. *J. Chem. Soc., Perkin Trans. 2* **1996**, 1497.
28. Valeur, B.; Pouget, J.; Bourson, J.; Kaschke, M.; Ernsting, N. P. *J. Phys. Chem.* **1992**, *96*, 6545.
29. Connors, K. A. *Binding Constants*; Wiley Interscience: New York, NY, 1987; Chapter 2.
30. (a) Shen, X.-H.; Belletête, M.; Durocher, G. *Chem. Phys. Lett.* **1998**, *298*, 201; (b) Chen, Y.-L.; Xu, T.-K.; Shen, X.-H.; Gao, H.-C. *J. Photochem. Photobiol., A* **2005**, *173*, 42.
31. A discussion on the deduction and the use of Eqs. 1–4 can be found in the literature (see Refs. 28–30).
32. The reaction occurs only with the deprotonated form of the dye, and of the anions studied only CN^- reacted with the dye. Data suggest that the anion acts as a nucleophile reacting with the quinonoid form of the dye in the solvent. This study is still in progress.
33. The limit of water in trichloromethane is 0.056% (0.031 mol dm^{-3}) at 20 °C (http://macro.lsu.edu/howto/solvents/chloroform.htm, accessed on 01.10.08).
34. Dickins, R. S.; Parker, D. In *Macrocyclic Chemistry: Current Trends and Future Perspectives*; Gloe, K., Ed.; Springer: Dordrecht, 2005; Chapter 8, pp 121–136.
35. Titration of **3b** with $H_2PO_4^-$ showed, on starting the addition of anion, an increase in absorbance between 400 and 441 nm and, less significantly, at 609 nm. The system then became cloudy due to the precipitation of the anion added. By adding more anion, the system became transparent again, but the absorbance value at 609 nm did not surpass 0.120.
36. Davidson, S. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1969**, *91*, 225.
37. Hamasaki, K.; Nakamura, A.; Ueno, A.; Toda, M. R. *J. Inclusion Phenom. Mol. Recognit. Chem.* **1992**, *13*, 349.
38. Wolfbeis, O. S.; Kessler, M. A. *Chem. Phys. Lipids* **1989**, *50*, 51.
39. Hajdu, J.; Smith, G. M. *J. Am. Chem. Soc.* **1981**, *103*, 6192.
40. Hajdu and Smith[39] have observed that, for instance, acetic acid is more acidic than *p*-nitrophenol in water by a ΔpK_a=2.45. However, in DMSO, it is observed that *p*-nitrophenol (pK_a=10.4) is more acidic than carboxylic acid (pK_a=11.9). In another example, pK_a values for *p*-nitrophenol and phenol in water are 7.2 and 9.9 (ΔpK_a=2.7), respectively, against a ΔpK_a=3.21 in DMF (pK_a=12.19 for *p*-nitrophenol and pK_a=15.4 for phenol).
41. Jover, J.; Bosque, R.; Sales, J. *QSAR Comb. Sci.* **2007**, *26*, 385.
42. Coleman, C. A.; Murray, C. J. *J. Org. Chem.* **1992**, *57*, 3578.
43. Wu, Y.; Peng, X.; Fan, J.; Gao, S.; Tian, M.; Zhao, J.; Sun, S. *J. Org. Chem.* **2007**, *72*, 62.
44. (a) Gunnlaugsson, T.; Kruger, P. E.; Lee, T. C.; Parkesh, R.; Pfeffer, F. M.; Hussey, G. M. *Tetrahedron Lett.* **2003**, *44*, 6575; (b) Costero, A. M.; Bagñuls, M. J.; Aurell, M. J.; Ward, M. D.; Argent, S. *Tetrahedron* **2004**, *60*, 9471; (c) Gómez, D. E.; Fabbrizzi, L.; Licchelli, M.; Monzani, E. *Org. Biomol. Chem.* **2005**, *3*, 1495; (d) Babu, J. N.; Bhalla, V.; Kumar, M.; Singh, H. *Lett. Org. Chem.* **2006**, *3*, 787.
45. Gronert, S. *J. Am. Chem. Soc.* **1993**, *115*, 10258.
46. Zimmermann-Dimer, L. M.; Machado, V. G. *Dyes Pigments* **2009**, *82*, 187.
47. (a) Kosower, E. M. *J. Am. Chem. Soc.* **1958**, *80*, 3253; (b) Mackay, R. A.; Poziomek, E. J. *J. Am. Chem. Soc.* **1970**, *92*, 2432; (c) Medda, K.; Pal, M.; Bagchi, S. *J. Chem. Soc., Faraday Trans. 1* **1988**, *84*, 1501.
48. The large hypsochromic shift observed for the solvatochromic band of **3a** in trichloromethane with addition of a small amount of methanol from λ_{max}=609 nm to 547 nm is due to the preferential solvation of the dye's phenolate moiety by the alcohol in the mixture.[20c]
49. Marcus, Y. *J. Chem. Soc., Faraday Trans.* **1991**, *87*, 2995.
50. (a) Ros-Lis, J. V.; Martínez-Máñez, R.; Soto, J. *Chem. Commun.* **2002**, 2248; (b) Ros-Lis, J. V.; Martínez-Máñez, R.; Soto, J. *Chem. Commun.* **2005**, 5260.
51. (a) Tomasulo, M.; Raymo, F. M. *Org. Lett.* **2005**, *7*, 4633; (b) Tomasulo, M.; Sortino, S.; White, A. J. P.; Raymo, F. M. *J. Org. Chem.* **2006**, *71*, 744.
52. Furniss, B. S.; Hannaford, A. J.; Smith, P. W. G.; Tatchell, A. R. *Vogel's Textbook of Practical Organic Chemistry*, 5th ed.; Longman: London, 1989.
53. Minch, M. J.; Shah, S. S. *J. Chem. Educ.* **1977**, *54*, 709.
54. Rezende, M. C.; Radetski, C. M. *Quim. Nova* **1988**, *11*, 353; *Chem. Abstr.* **1989**, *111*, 8876w.
55. Chadwick, T. C. *Anal. Chem.* **1974**, *46*, 1326.
56. Dimroth, K.; Reichardt, C.; Siepmann, T.; Bohlmann, F. *Justus Liebigs Ann. Chem.* **1963**, *661*, 1.