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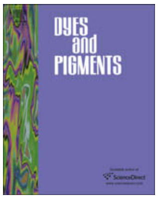

Chromogenic anionic chemosensors based on protonated merocyanine solvatochromic dyes: Influence of the medium on the quantitative and naked-eye selective detection of anionic species

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ABSTRACT

Three merocyanine solvatochromic dyes, Brooker's merocyanine, Reichardt's dye and 4-(2,4,6-triphenylpyridinium-1-yl)phenolate were solubilized in acetonitrile. Although solutions of each of the compounds are colored, the color disappears after protonation. Addition of various anions to solutions of the protonated dyes revealed that only CN^-, F^-, and $H_2PO_4^-$ led to the reappearance of the original color of protonated Reichardt's dye, and only CN^- and F^- ions caused the original color of the solutions containing the other two protonated dyes to reappear. Addition of small amounts of water to solutions containing the protonated compounds made them useful only for detecting CN^- among all the other anions. A model was used to explain the experimental results, which considers proton transfer from the protonated dye to the anion through three dye:anion stoichiometries, namely 1:1, 1:2, and 1:3.

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 [1–7]. 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 [8,9]. Another interesting target in terms of detection is CN^- [10,11], 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 [12]. Also, it is formed through hydrolysis in many foods, such as some fruit seeds and roots [12].

Of the strategies utilized 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 differential capabilities to interact with the receptor site in the chemosensor, for instance, through intermolecular hydrogen bonds (HB). In relation to this feature, the observation made by Steiner [13] should be considered, i.e., that all HBs can be characterized as incipient proton-transfer processes and that for strong HBs these reactions can be found in a very advanced state.

Merocyanine dyes are heterocyclic compounds that have many applications [14,15]. Among these compounds, two examples are well-known: 4-[(1-methyl-4(1*H*)-pyridinylidene)-ethylidene]-2,5-cyclohexadien-1-one, known as Brooker's merocyanine (**1a**) [16,17], and Reichardt's betaine dye, 2,6-diphenyl-4-(2,4,6-triphenylpyridinium-1-yl)phenolate (**2a**) [18–20]. 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 [18]. 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 [18–20]. These dyes have been used in recent years in the study of pure [16–20] and mixed solvents [18,19,21–24], microheterogeneity in solution [25,26], ionic liquids [27], fuels [28], and solutions containing cyclodextrins [29]. These dyes have also been used in the investigation of salt solutions [30–32], this phenomenon being recognized as halochromism [30–32]. More recently, these dyes

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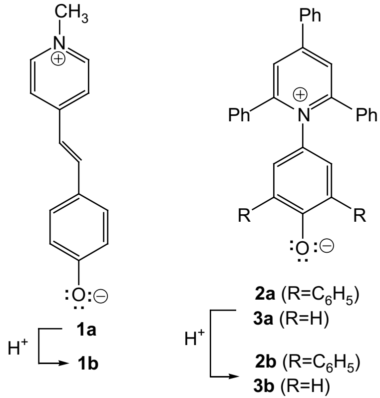
have been used as signalizing units in different strategies for anion chromogenic sensing [33–36]. For instance, pyridinium-*N*-phenolate **2a**, after protonation at the phenolate oxygen atom in trichloromethane, generates **2b**, which acts as an anionic chemosensor for F^- and $H_2PO_4^-$ [33]. These anions are able to abstract a proton from the phenol group in the protonated dye, which makes its solutions colored. Compound **2b** is also able to signal the presence of I^-, due to the interaction of the anion with the pyridinium group forming a charge-transfer complex [33]. In this paper, compounds **1b**–**3b** are compared as anionic chemosensors using acetonitrile as solvent. Moreover, small amounts of water were added to the acetonitrile solutions of protonated dyes to study a possible selective detection of anionic species in solution. All data were interpreted in terms of incipient and complete proton transfer involving anionic species and the protonated chromogenic chemosensors.



1a

1b

2a ($R=C_6H_5$)
3a ($R=H$)

2b ($R=C_6H_5$)
3b ($R=H$)

2. Results and discussion

Solutions of dyes **1a**–**3a** in acetonitrile are colored. Upon protonation the solutions become pale-yellow colored in the case of dye **1a** and colorless in the case of dyes **2a** and **3a**. A study was carried out by adding, individually, different anions (CN^-, F^-, Cl^-, Br^-, I^-, $H_2PO_4^-$, HSO_4^-, and NO_3^-) as tetra-*n*-butylammonium salts to solutions of the protonated dyes. It was found that the addition of CN^-, F^-, and to a lesser extent $H_2PO_4^-$ caused the reappearance of the original solvatochromic band of dye **2a** at $\lambda_{max} = 622$ nm (Fig. 1), which can be observed by the reappearance of the blue-green color (for interpretation of the references to colour in the text, the reader is referred to the web version of this article). For solutions of compounds **1b** and **3b**, only CN^- and F^- produce a reappearance of the original color. Following the addition of small amounts of water to solutions containing the protonated dyes **1b**–**3b**, only CN^- caused a return of the solution color.

Fig. 2 shows the influence of the anionic species on the Vis spectra of **1b** and **2b** in acetonitrile and in acetonitrile with small amounts of water. Firstly, it can be verified that **1a** has a charge-transfer solvatochromic band in acetonitrile with $\lambda_{max} = 571$ nm and that this band disappears after protonation (Fig. 2A). Addition of CN^- led to an almost complete reappearance of the Vis-absorption band of **1a**. F^- also caused a return of the original coloration of solutions containing **1b**. All other anions used were unable to deprotonate the compound **1b** in solution, or to cause the appearance of an intermediate band typical of hydrogen-bonded species. It is important to notice the hypsochromic shift of the solvatochromic band of **1a** due to its preferential solvation caused by the water added [23,24], and that with the addition of 3% (v/v) water only CN^- was able to act as a base and to deprotonate the dye. Dye **2a** has a solvatochromic band at $\lambda_{max} = 622$ nm in pure acetonitrile. This band undergoes a hypsochromic shift after the addition of small amounts of water (Fig. 2B) [21,22], similar to the behavior obtained for dye **1a**. The general trends were very similar to that observed for **1b**, except for the fact that $H_2PO_4^-$ also caused a return of the original coloration of solutions containing **2b**, with the results showing the following decreasing order of the spectral effect: $CN^- > F^- > H_2PO_4^-$. Thus, our experimental results indicate that a complete proton transfer occurs from the protonated dye (HBD) to the anion (HBA), since a HBD/HBA interaction involving the protonated dye and the anion would lead to a visible band corresponding to a hypsochromic shift in relation to the same band of the deprotonated dye in acetonitrile.

Fig. 3 shows the relative absorbance values for solutions of **1b**–**3b** in the presence of F^- and CN^-, dissolved in acetonitrile and acetonitrile-water mixtures. The highest absorbance values found in acetonitrile for **1b** and **2b** were in the presence of CN^- and for **3b** with F^- added. In addition, the experimental data demonstrated that the chromogenic chemosensors have a low selectivity with respect to these two anions in acetonitrile. With the addition of very small amounts of water a different behavior for compounds **1b**–**3b** emerged: a very high selectivity for CN^- over F^- could be verified.

3. Titration of protonated dyes with the anionic species

Compounds **1b**–**3b** were titrated in acetonitrile and acetonitrile-water mixtures with those anions which led to a change in the color of their solutions. Plots of the absorbance values for solvatochromic bands of the deprotonated dyes as a function of the concentration of the anion added were constructed in order to determine the binding constants, which were obtained by fitting the experimental data with equations (1)–(5) [37,38], which relate to the following situations according to different dye:anion stoichiometries:

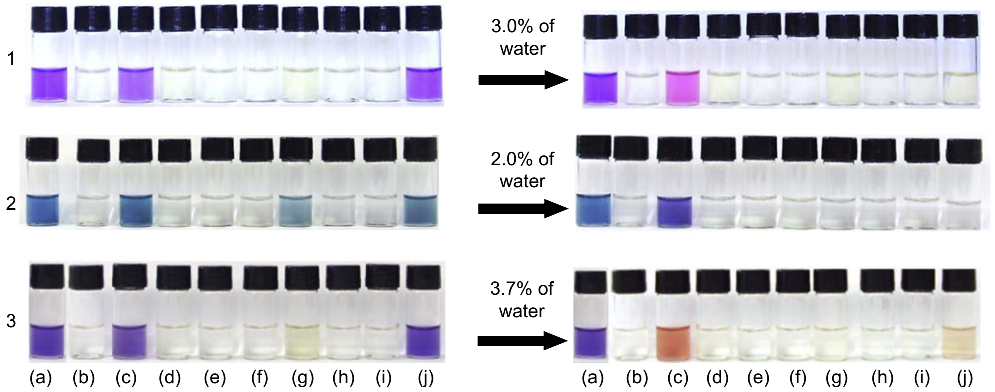


Fig. 1. Solutions in acetonitrile and acetonitrile with water of (a) dyes **1a**–**3a**, (b) **1b**–**3b**, and protonated dyes in the presence of (c) CN^-, (d) Cl^-, (e) Br^-, (f) I^-, (g) $H_2PO_4^-$, (h) HSO_4^-, (i) NO_3^-, and (j) F^-, as tetra-*n*-butylammonium salts. For concentrations of dyes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

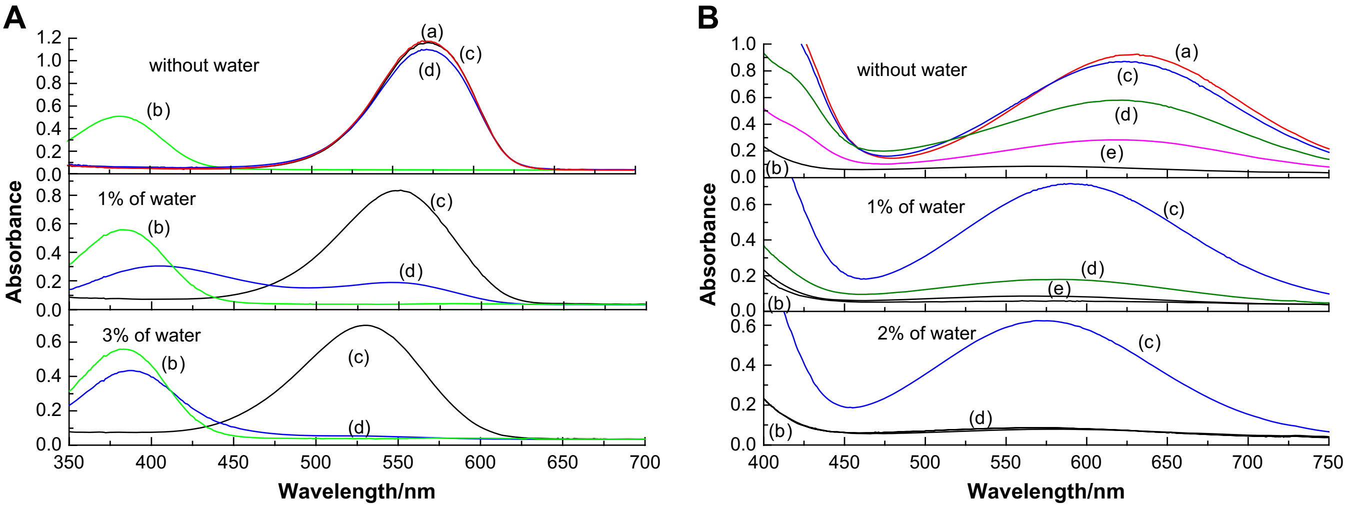


Fig. 2. Vis spectra of solutions in acetonitrile and acetonitrile with water. (A) Dye **1a** (a), **1b** (b), and **1b** in the presence of CN^- (c) and F^- (d). (B) Dye **2a** (a), **2b** (b), and **2b** in the presence of CN^- (c), F^- (d), and $H_2PO_4^-$ (e). UV–vis spectra of **1a** or **2a** in acetonitrile with water coincides with the UV–vis spectra of protonated dye with CN^- in the same conditions. For concentrations of dyes and anions, see the text.

Case 1 (1:1 and 1:2 stoichiometries)

$$\text{Abs} = \left[\text{Abs}_\text{o} + \text{Abs}_{11}K_{11}C_{\text{A}^-} + \text{Abs}_{12}K_{11}K_{12}C_{\text{A}^-}^2\right] / \left[1 + K_{11}C_{\text{A}^-} + K_{11}K_{12}C_{\text{A}^-}^2\right] \tag{1}$$

Case 2: 1:1 and 1:3 stoichiometries

$$\text{Abs} = \left[\text{Abs}_\text{o} + \text{Abs}_{11}K_{11}C_{\text{A}^-} + \text{Abs}_{13}K_{11}K_{13}C_{\text{A}^-}^3\right] / \left[1 + K_{11}C_{\text{A}^-} + K_{11}K_{13}C_{\text{A}^-}^3\right] \tag{2}$$

Case 3: 1:2 and 1:3 stoichiometries

$$\text{Abs} = \left[\text{Abs}_\text{o} + \text{Abs}_{12}K_{12}C_{\text{A}^-}^2 + \text{Abs}_{13}K_{12}K_{13}C_{\text{A}^-}^3\right] / \left[1 + K_{12}C_{\text{A}^-}^2 + K_{12}K_{13}C_{\text{A}^-}^3\right] \tag{3}$$

Case 4: 1:2 stoichiometry

$$\text{Abs} = \left[\text{Abs}_\text{o} + \text{Abs}_{12}K_{12}C_{\text{A}^-}^2\right] / \left[1 + K_{12}C_{\text{A}^-}^2\right] \tag{4}$$

Case 5: 1:3 stoichiometry

$$\text{Abs} = \left[\text{Abs}_\text{o} + \text{Abs}_{13}K_{13}C_{\text{A}^-}^3\right] / \left[1 + K_{13}C_{\text{A}^-}^3\right] \tag{5}$$

In these equations, Abs is the absorbance value after each addition of the anion, Abs_o is the initial absorbance without anion, 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, respectively. C_{A^-} is the concentration of the anion in each addition and K_{11}, K_{12}, and K_{13} are the binding constants. The results are given in Table 1 and show very good fits for all systems studied (S.D. $< 6.0 \times 10^{-4}$).

The Vis spectra in Fig. 4 show the behavior of **2b** in acetonitrile with the addition of tetra-*n*-butylammonium fluoride. It can be seen that with addition of the anion the solvatochromic band of **2a** appears at $\lambda_{\max} = 622$ nm. A plot of the absorbance values at 622 nm as a function of F^- concentration (not shown) showed a behavior typical of a 1:3 **2b**:anion stoichiometry and a fitting of the experimental data gave a binding constant of $(2.21 \pm 0.15) \times 10^9\ \text{dm}^9\,\text{mol}^{-3}$. A Job plot (as shown in the inset of Fig. 4) confirmed a 1:3 dye:anion stoichiometry. Addition of small amounts of water led to a decrease in the K_{13} value due to the strong interaction of this solvent with the anion, which inhibits its action as a base. A study of the addition of CN^- to a solution of **2b** in acetonitrile revealed that this anion abstracts the proton in a 1:1 and 1:3 dye:anion stoichiometry, and that the addition of 1% (v/v) water is responsible for the decrease in the K_{13} value, simultaneously with an increase in the K_{11} value. A similar behavior was observed using $H_2PO_4^-$ as anion. In this case the addition of 1% (v/v) water showed a significant effect by decreasing the binding

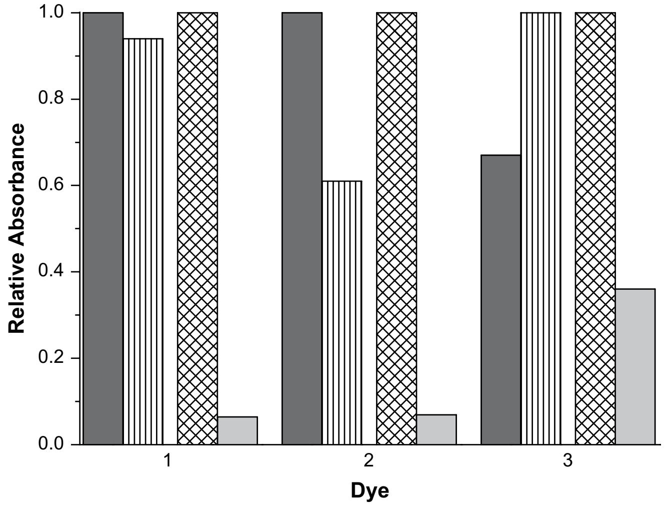


Fig. 3. Relative absorbances for F^- and CN^- added to solutions of the-**1b–3b** in acetonitrile (■ CN^- and ▥ F^-) and acetonitrile–water mixtures (▩ CN^- and ▭ F^-). For percentages of water in each solvent mixture, see Figs. 1 and 2.

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

Dye	Experimental conditions	Anion	K_{11}/dm^3 mol^{-1}	K_{12}/dm^6 mol^{-2}	K_{13}/dm^9 mol^{-3}	S.D.
1	acetonitrile	CN^-	–	$(2.26 \pm 0.23) \times 10^9$	$(2.70 \pm 0.94) \times 10^4$	6×10^{-4}
	with 3% water	CN^-	$(2.53 \pm 0.32) \times 10^4$	$(1.96 \pm 0.25) \times 10^5$	–	3×10^{-5}
	acetonitrile	F^-	–	$(2.07 \pm 0.42) \times 10^8$	$(1.43 \pm 0.85) \times 10^4$	2×10^{-4}
	with 3% water	F^-	$(1.58 \pm 0.35) \times 10^3$	$(2.96 \pm 0.26) \times 10^3$	–	3×10^{-6}
2	acetonitrile	CN^-	$(3.79 \pm 0.19) \times 10^2$	–	$(1.94 \pm 0.14) \times 10^6$	4×10^{-5}
	with 1% water	CN^-	$(1.53 \pm 0.26) \times 10^3$	–	$(6.62 \pm 1.33) \times 10^5$	2×10^{-4}
	acetonitrile	F^-	–	–	$(2.21 \pm 0.15) \times 10^9$	3×10^{-4}
	with 1% water	F^-	–	–	$(3.39 \pm 0.15) \times 10^8$	3×10^{-4}
	with 2% water	F^-	–	–	$(9.50 \pm 0.27) \times 10^7$	6×10^{-5}
	acetonitrile	$H_2PO_4^-$	$(2.45 \pm 0.37) \times 10^2$	–	$(1.63 \pm 0.15) \times 10^6$	5×10^{-6}
	with 1% water	$H_2PO_4^-$	$(4.48 \pm 0.37) \times 10^1$	–	$(1.60 \pm 1.47) \times 10^2$	2×10^{-6}
3	acetonitrile	CN^-	–	$(3.71 \pm 0.09) \times 10^7$	–	6×10^{-5}
	with 3.7% water	CN^-	$(1.44 \pm 0.14) \times 10^3$	$(1.00 \pm 0.09) \times 10^4$	–	8×10^{-6}
	acetonitrile	F^-	$(5.75 \pm 3.04) \times 10^3$	–	$(3.01 \pm 0.36) \times 10^6$	2×10^{-4}
	with 3.7% water	F^-	$(3.07 \pm 0.12) \times 10^2$	–	$(1.90 \pm 1.60) \times 10^2$	3×10^{-6}

constant values, also due to the specific solvation of the anion by water through HB.

The Vis spectra in Fig. 5 show the effect of the addition of CN^- on a solution of **1b** in acetonitrile. It can be observed that the band at $\lambda_{max} = 382$ nm relating to the protonated merocyanine diminishes with the addition of the anion, simultaneously with the appearance of the solvatochromic band of **1a** with a maximum at 571 nm. A better observation of the Vis spectra reveals that the experiment can be separated into two steps, as shown in Fig. 5B and C. In Fig. 5B, the Vis spectra show that with the addition of the anion, the band at 382 nm diminishes, with an isosbestic point at 427 nm. In this first part of the titration, there is not a significant increase in the absorbance value at 571 nm. Later, it is observed that the band at 382 nm rapidly diminishes with the addition of the anion until its disappearance, followed by a considerable increase in the absorbance at 571 nm, with an isosbestic point at 473 nm (Fig. 5C). A similar behavior was observed for the titration of the same protonated dye with F^-, and with the two anions the fitting of experimental data with equation (3) gave the K_{12} and K_{13} values. Fig. 6 shows the results for the titration of **1b** with CN^- in acetonitrile with 3% (v/v) of water. With addition of the anion, the band corresponding to **1b** at 382 nm decreases with the simultaneous appearance of the solvatochromic band relating to the deprotonated dye at 530 nm. In contrast with the titration in pure acetonitrile, only one well-defined isosbestic point, at 433 nm, was observed. Experimental data for the titrations in an acetonitrile–water mixture using CN^- or F^- were fitted with equation (1), exactly as shown for CN^- in the inset of Fig. 6, indicating a change in the dye:anion stoichiometry of the process from 1:2 and 1:3 toward 1:1 and 1:2.

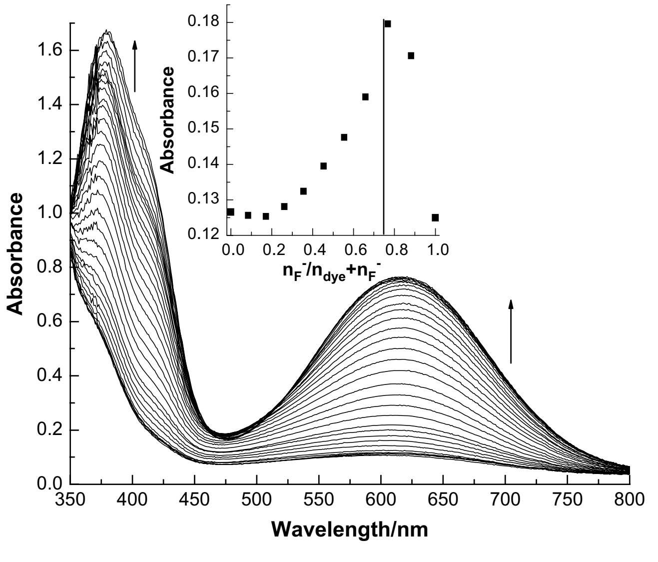


Fig. 4. Vis spectra of **2b** (2.0×10^{-4} mol dm^{-3}) in acetonitrile at 25 °C after addition of increasing amounts of F^-. The final concentration of F^- was 2.5×10^{-3} mol dm^{-3}. Inset: Job plot for F^- and **2b**.

Fig. 7 gives the Vis spectra showing the effect of addition of CN^- to a solution of **3b** in acetonitrile. An increase in intensity of the solvatochromic band for the deprotonated dye at $\lambda_{max} = 550$ nm can be observed and again the titration can be separated into two steps: the first with an increase in the absorbance at 450 nm with an isosbestic point at 374 nm and a very slight increase at 550 nm, and the second with a very large increase in the absorbance values at 550 nm and an isosbestic point at 462 nm. These spectral changes are accompanied by color changes: whereas in the first step of the titration an orange color predominates, suggesting HB between **3b** and the anion, at the end of the titration the color changes to violet, typical of the deprotonated dye in solution (see inset in Fig. 7, for interpretation of the references to colour in the text, the reader is referred to the web version of this article). Addition of 3.7% (v/v) of water caused a considerable change in the profile of the titration (Fig. 8), since the addition of the anion led to the appearance of a band at 470 nm, with the occurrence of one isosbestic point at 386 nm. Fig. 9 shows the titration curves in the form of plots of the absorbance values for the solvatochromic band of **3a** as a function of the CN^- added. It is possible to observe a difference in the shapes of the curves measured in acetonitrile and in acetonitrile with 3.7% of water. For acetonitrile, a fitting of titration data using equation (4) gave a binding constant of $K_{12} = (3.71 \pm 0.09) \times 10^7$ dm^6 mol^{-2}. For the binary solvent mixture, the experimental data were fitted using equation (1) and gave K_{11} and K_{12} values of $(1.44 \pm 0.14) \times 10^3$ dm^3 mol^{-1} and $(1.00 \pm 0.09) \times 10^4$ dm^6 mol^{-2}, respectively. For the titrations with F^-, in acetonitrile the dye:anion stoichiometries were 1:1 and 1:3, while after addition of 3.7% of water there was a decrease in the K_{11} and K_{13} values, reflecting the influence of water in solvating the species in solution.

4. A model to explain the behavior of protonated dyes in the presence of anionic species

The results show that various stoichiometries can be obtained for the studied systems depending on the protonated dye, the

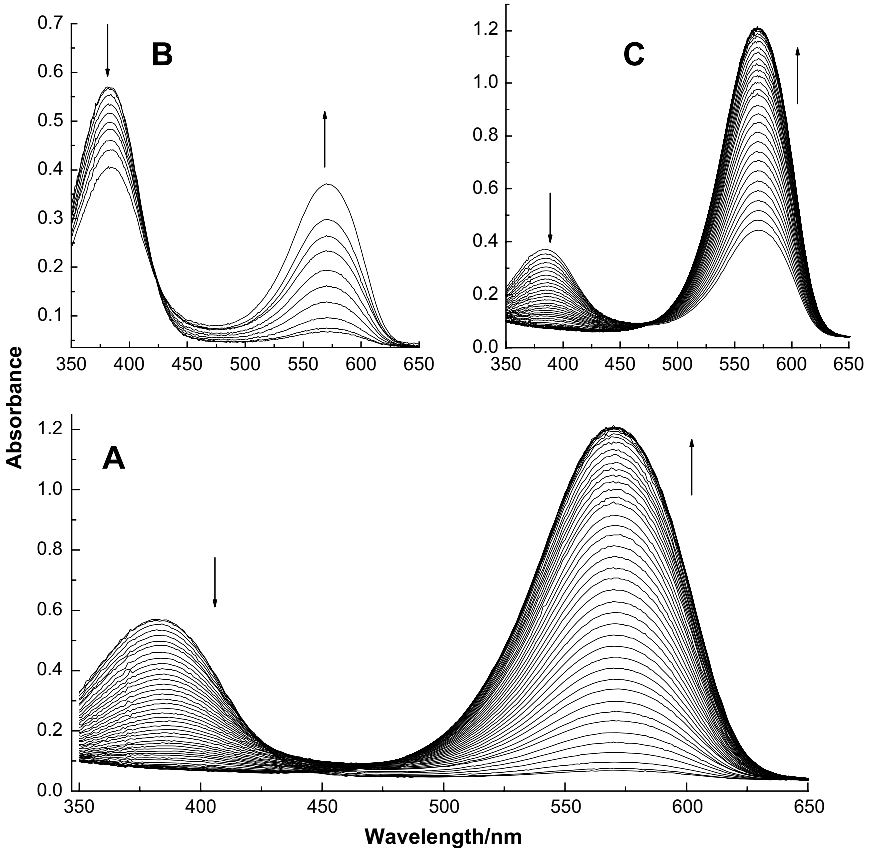


Fig. 5. Vis spectra sets of **1b** (2.0×10^{-5} mol dm^{-3}) in acetonitrile at 25 °C after addition of increasing amounts of CN^-. (A) Vis spectra of the entire titration, with the final concentration of CN^- of 4.9×10^{-5} mol dm^{-3}. (B) Vis spectra of the first part of the titration, with the concentration of CN^- varying between zero and 1.5×10^{-5} mol dm^{-3}. (C) Vis spectra of the latter part of the titration, with the concentration of CN^- varying between 1.6×10^{-5} mol dm^{-3} and 4.9×10^{-5} mol dm^{-3}.

medium, and the anion utilized. Scheme 1 illustrates the different behaviors for **1b** and this can be generalized for 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 which 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 [39–43], 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 the abstraction of the proton, with the formation of a $[HA_2]^-$ complex, such as $[HF_2]^-$ species whose formation and stability have been verified by theoretical studies [44].

As a new feature in relation to other chromogenic systems based on proton transfer found in the literature, in our study we obtained, in many cases, a 1:3 dye:anion stoichiometry for the experimental conditions less favorable to proton transfer. It is well known that *N*-alkyl pyridinium cations can form by ion pairing charge-transfer complexes with I^- in solution [33,45]. 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 the formation of a $[HA_2]^-$ complex. In order to confirm the role of the first equivalent of the anion in the abstraction of the proton, we titrated **1b** with F^- in acetonitrile using increasing amounts of I^-. The results are shown in Table 2 and it can be observed that in the absence of I^- the mathematical model that best fits the experimental data was equation (3), which considers 1:2 and 1:3

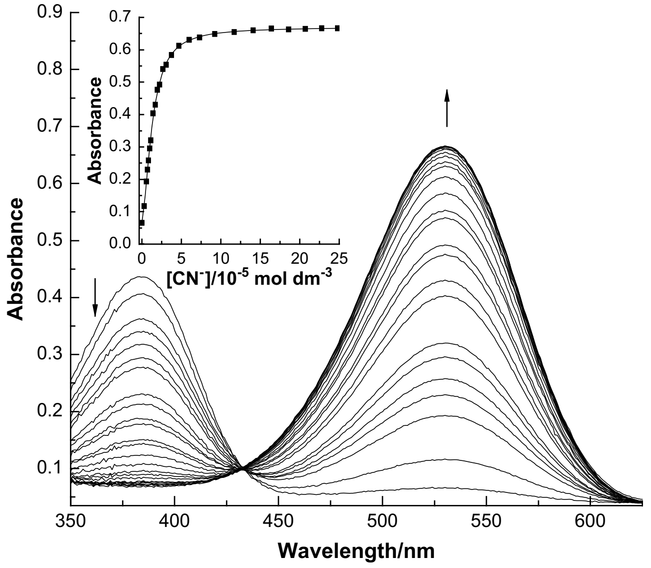


Fig. 6. Influence of the addition of increasing amounts of CN^- on the Vis spectra of **1b** (2.0×10^{-5} mol dm^{-3}) in acetonitrile with 3.0% (v/v) of water at 25 °C. The final concentration of CN^- was 2.5×10^{-4} mol dm^{-3}. The inset displays the variation in the absorbance at 530 nm of **1b** with the addition of increasing amounts of CN^-.

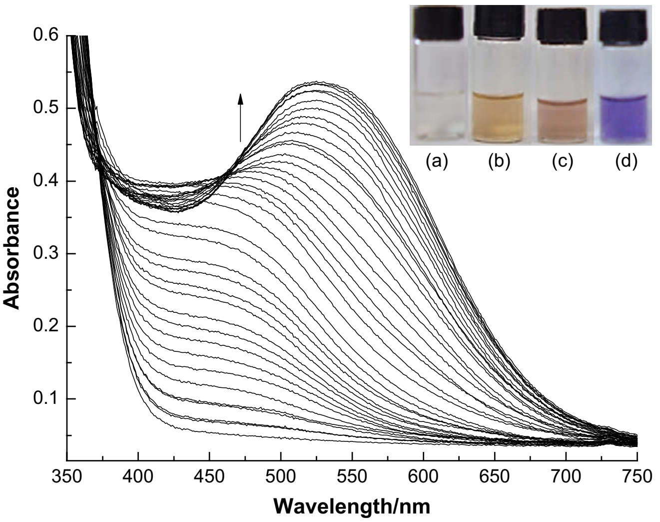


Fig. 7. Vis spectra of **3b** (2.3×10^{-4} mol dm^{-3}) in acetonitrile at 25 °C after addition of increasing amounts of CN^-, with the final concentration of the anion of 2.2×10^{-3} mol dm^{-3}. The inset shows solutions of the protonated dye in absence (a) and in the presence of CN^- in a concentration of 1.7×10^{-4} mol dm^{-3} (b), 2.5×10^{-4} mol dm^{-3} (c), and 1.5×10^{-3} mol dm^{-3} (d).

dye:anion stoichiometries. In this situation, the cationic pyridinium group is free to be complexed by F^-, leading to the observed results. The complexation of I^- with the pyridinium group makes its interaction with F^- more difficult, reducing the magnitude of the K_{13} value and increasing the K_{12} value. This was confirmed by addition of six equivalents of I^- to the system which led to an experimental condition that favored only a 1:2 stoichiometry. In addition, it can be seen in Table 1 that, on adding water to the system containing F^-, a decrease in the K_{13} values for **2b** and **3b** occurs. This is due to the action of water impairing, through solvation of the anion, the interaction of the latter with the pyridinium center. A similar behavior was observed for **2b** with CN^- and $H_2PO_4^-$ as anions. Moreover, addition of water to the system containing **1b** and CN^- or F^- led to a change from 1:2 and 1:3 to 1:1 and 1:2 stoichiometries, respectively. This change in the profile of the titration is due to the hydrophilicity of this compound in comparison with the other dyes studied, leading to the microenvironment of the dye being solvated also by water and inhibiting the pyridinium:anion ion pair.

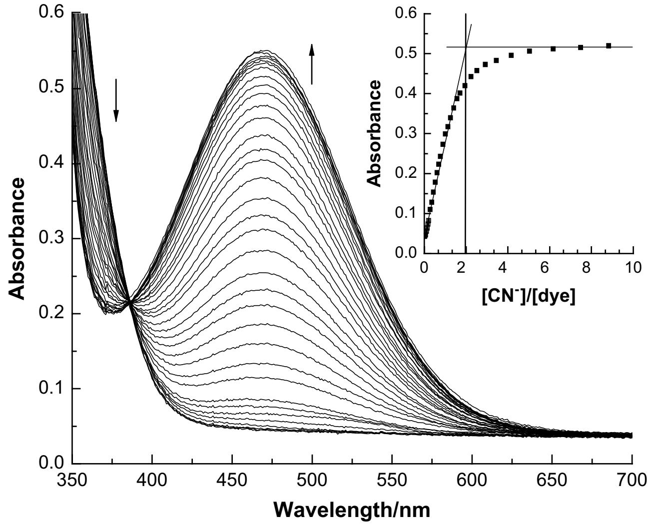


Fig. 8. Influence of the addition of increasing amounts of CN^- on the Vis spectra of **3b** (2.3×10^{-4} mol dm^{-3}) in acetonitrile with 3.7% (v/v) of water at 25 °C. Absorbance values were taken at 485 nm and the final concentration of CN^- was 3.2×10^{-3} mol dm^{-3}. The inset displays a mole-ratio plot for the interaction of **3b** with CN^-, which clearly indicates a 1:2 dye:anion stoichiometry.

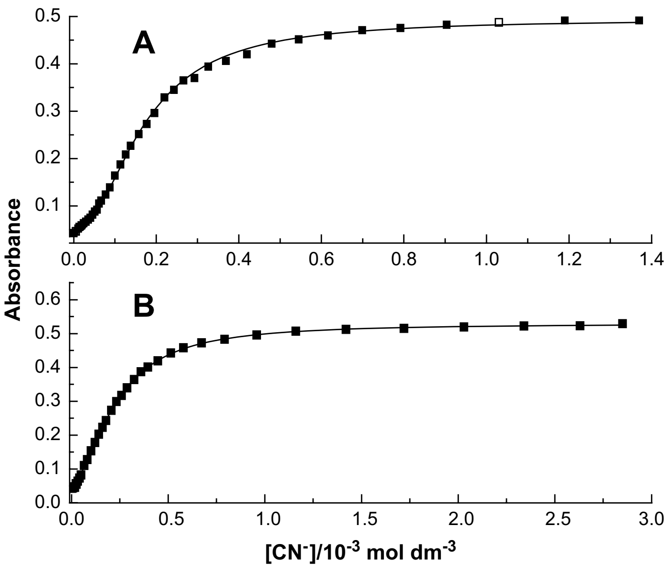


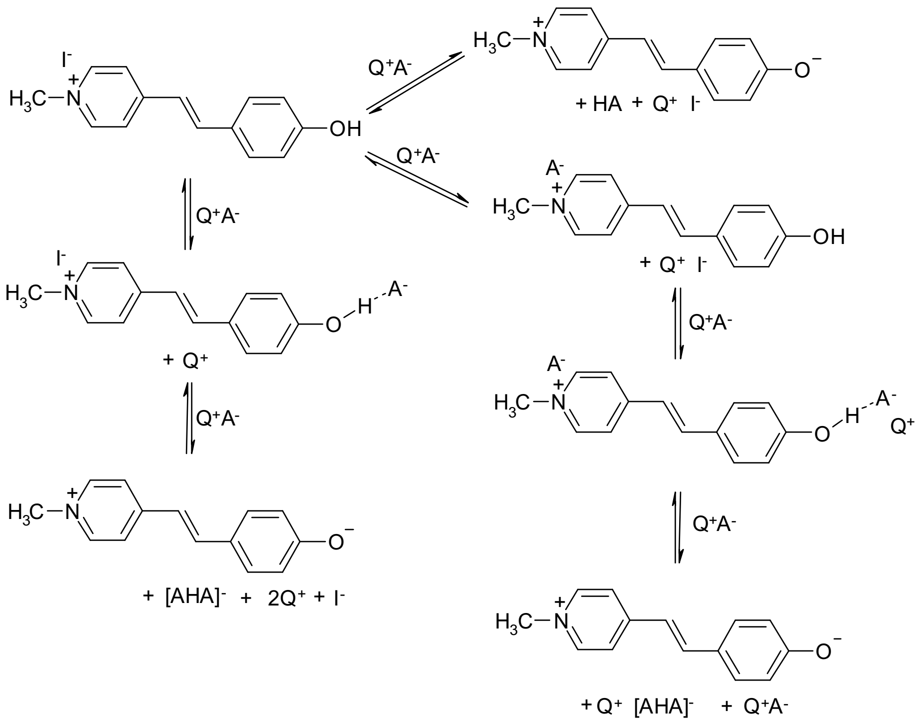
Fig. 9. Variation in the absorbance, with the addition of increasing amounts of CN^-, for the appearance of **3a** at (A) 550 nm in acetonitrile and (B) 485 nm in acetonitrile with 3.7% (v/v) of water. The experiments were performed at 25 °C and the concentration of **3b** was 2.3×10^{-4} mol dm^{-3}.

The experimental data demonstrate that, although the chromogenic chemosensors studied here are selective toward CN^- and F^- (and $H_2PO_4^-$ for **2b**) in relation to the other anions employed, after adding very small amounts of water the systems become 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}) [46,47]. Thus, addition of water to acetonitrile solutions of F^- and $H_2PO_4^-$ salts lead to a strong preferential solvation of the anion by water, which makes the hydrated species less able to act as a base. Since CN^- is less hydrated after the addition 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. The influence of water in diminishing the capability of F^- ions to act as a base has been previously reported [48–50]. The addition of water to the medium has another important role, which is to stabilize the deprotonated dye through HB, leading to the protonated compound becoming more acidic. This suggests that, for instance, in the mixtures of **3b** with CN^-, the addition of water causes a change in the dye:anion stoichiometry from 1:2 toward 1:1 and 1:2.

5. Experimental

5.1. Materials

Acetonitrile was purified according to a procedure described in the literature and then stored [51]. Karl–Fischer titrations were performed with this solvent and demonstrated the presence of water in a concentration of 7.11×10^{-3} mol dm^{-3} (0.0286% water). 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^-, NO_3^-, HSO_4^-, CN^-, and $H_2PO_4^-$) were used as



Scheme 1. Possible interactions of **1b** with an anionic species.

tetra-*n*-butylammonium salts with purity ~97–99%. The anions were purchased from Fluka (F^- (>97%), 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 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: CN^- (0.116% water), F^- (1.125% water), and $H_2PO_4^-$ (0.111% water). Compound **1b** was synthesized as an I^- salt, as described previously [52]. Dye **2a** was synthesized according to a method described in the literature [53], 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 acetonitrile were added with a microsyringe to the solutions of the dye until its protonation. Compound **3b** was prepared through reaction of 2,4,6-triphenylpyrylium perchlorate with 4-aminophenol [54,55].

5.2. UV–vis studies of the protonated dyes in the presence of 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 (λ_{max}) were calculated from the first derivative of the absorption spectrum.

The experiments were performed as follows: a stock solution of the dye (2.0×10^{-5} mol dm^{-3} for **1b**, 2.0×10^{-4} mol dm^{-3} for **2a**, and 2.3×10^{-4} mol dm^{-3} for **3b**) was prepared in acetonitrile. A few drops of acetic acid solution in acetonitrile were then added to the solution of **2a** with a microsyringe in order to protonate the dye. The protonated dye solution was then used to prepare the solution of each anion in a concentration of 1.5×10^{-4} mol dm^{-3} for **1b**, 2.0×10^{-3} mol dm^{-3} for **2b**, and 1.5×10^{-3} mol dm^{-3} for **3b**. These solutions were prepared and transferred using volumetric flasks and cuvettes hermetically closed with rubber stoppers in order to minimize the evaporation of the acetonitrile and to avoid the entrance of water to the system. All experiments were carried out at 25 °C.

The experiments involving acetonitrile–water mixtures were carried out for all dyes using the following procedure. Stock solutions of the protonated dye and the solutions of 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 of color in acetonitrile, and UV–vis spectra were taken in order to evaluate whether this amount was sufficient to allow a naked-eye selective detection. No more than 3.7% (v/v) of water was used for all experiments.

5.3. Titration experiments

Titration experiments in acetonitrile were performed with the preparation of the solution of the 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 571 nm for dye **1a**, 622 nm for dye **2a**, and 550 nm for dye **3a**. Titration experiments were also performed in acetonitrile–water systems, using the minimal water content which allowed selective detection of the anion, defined in

Table 2
Binding constants for the titration of **1b** with F^- in acetonitrile at 25 °C in the presence of increasing amounts of I^-.

Experimental conditions	K_{12}/dm^6 mol^{-2}	K_{13}/dm^9 mol^{-3}	S.D.
without I^- [a]	$(2.35 \pm 1.56) \times 10^7$	$(1.24 \pm 0.92) \times 10^5$	4×10^{-5}
with 1 equivalent of I^-	$(2.07 \pm 0.42) \times 10^8$	$(1.43 \pm 0.85) \times 10^4$	2×10^{-4}
with 6 equivalents of I^-	$(3.51 \pm 0.27) \times 10^9$	–	9×10^{-4}

[a] I^- as counteranion of **1b** was precipitated from the medium using $AgBF_4$.

the previous UV–vis studies. Titrations of **1b** in acetonitrile with F^- were also carried out by adding $AgBF_4$ to precipitate I^- from the medium and with an excess of tetra-*n*-butylammonium iodide.

5.4. Stoichiometry determinations

The experiments for the stoichiometry determinations were performed with the mole-ratio method and with the method of continuous variations (Job's method) [37].

The mole-ratio determinations were made as follows: a solution of **3b** (2.3×10^{-4} mol dm^{-3}) was prepared in acetonitrile with 3.7% (v/v) of water. A volume of 1.16 cm^3 was transferred from this solution to a quartz cuvette sealed with a rubber septum and the sample was placed in the UV–vis apparatus, with the cell-compartment thermostated at 25 °C. Small aliquots of CN^- stock solution prepared using the previous protonated dye solution were then transferred to the cuvette with a microsyringe with intervals of two minutes between each addition. The mixture was stirred after each addition and the maximum absorbance at 485 nm was read.

A Job plot was obtained considering the formation of **2a** in acetonitrile produced from **2b** and F^-. These studies were performed at 25 °C in acetonitrile and the stock solutions in the experiments had concentrations of 3.5×10^{-4} mol dm^{-3} for **2b** and 2.9×10^{-4} mol dm^{-3} for F^-. The absorbances were read at $\lambda_{max} = 622$ nm.

5.5. Calculations

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

6. Conclusions

We show here that merocyanine dyes in their protonated form have the potential to recognize the more basic anions. Also, we demonstrate the potential for structural changes in the framework of the chromogenic chemosensor which can be used to tune the selectivity toward F^- and CN^- over other anionic species in solution. Recent papers have demonstrated that the selectivity of a chemosensor toward the F^- can be tuned through the choice of substituents in the chemosensor with adequate electron push–pull features [56,57]. In addition, the important role of the medium in the performance of the chromogenic chemosensor was demonstrated, since it was able to determine the selection of CN^- over F^-, due to the strong preferential solvation of F^- by water through HB in its mixtures with acetonitrile.

In summary, the efficiency of chromogenic acidic chemosensors in solution is governed by the interaction between three factors, the microenvironment of the anion and the dye, the molecular structure of the dye, and the nature of the anionic species. Therefore, structural modifications in solvatochromic dyes such as **1a**–**3a**, and the utilization of other merocyanines [14–20], could be employed to promote a change in the acidity of these dyes in order to make chromogenic chemosensors more efficient not only in relation to anions but also to neutral analytes. The combination of these compounds with suitable aqueous–organic mixed solvents should make the resulting systems suitable for the recognition of specific anions. Finally, it is important to point out that some of these dyes, for instance compound **1a**, are fluorescent [58,59], widening the field of possible applications to include the development of fluorogenic chemosensors for anions and neutral species.

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