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An anionic chromogenic sensor based on the competition between the anion and a merocyanine solvatochromic dye for calix[4]pyrrole as a receptor site

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Abstract—The interaction of Brooker's merocyanine (**BM**), a merocyanine dye, with calix[4]pyrrole (**CP**) was studied in acetonitrile. **BM** is violet in solution, but its interaction with **CP** changes the color of the solution due to the formation of **CP**–**BM** species associated through hydrogen bonding. A displacement assay was then carried out in the presence of different anions (F^-, Cl^-, Br^-, I^-, $H_2PO_4^-$, HSO_4^-, and NO_3^-). It was verified that F^-, and to a lesser extent Cl^- and $H_2PO_4^-$, displace **BM** through the formation of a complex with **CP**, coloring the solution. Addition of HSO_4^- makes the solution almost colorless because it is sufficiently acidic to transfer a proton to **BM**, removing it from the receptor site in **CP** and protonating the dye, thereby allowing the visual detection of the anion in relation to the other anions.

Anionic species play a very important role in many chemical and biological processes, which has led to many recent studies in the field of their recognition and detection.[1–3] Thus, many sensors have been developed in recent years for the visual selection and quantification of anions.[2,3] Of the studies concerning the development of novel sensors, an interesting strategy involves the use of equilibrium competition between an indicator and the anionic substrate for a receptor site.[3,4] These displacement assays work well as anionic sensors if the receptor unit is able to recognize predominantly one of several anions, and also if spectral differences can be verified for the free and complexed indicator.

Many receptor molecules exhibiting the capability to recognize anions have been synthesized in recent years and their design comprises the use of hydrogen bonds alone, or in conjugation with electrostatic forces, to generate binding sites for anionic species. Among the most popularized anion receptors, an example of great interest, is *meso*-octamethylporphyrinogen, commonly known as *meso*-octamethylcalix[4]pyrrole (**CP**). This receptor was originally discovered by Baeyer in 1886[5] but only recently were its anion binding properties recognized.[6] The main advantages of its use in anion recognition and sensing are its easy and quick synthesis,[7] associated with the fact that functionalized anion sensors can be planned by means of the integration of **CP** with different signaling subunits.[8,9] An interesting example of a colorimetric sensor was discovered by Gale and co-workers and involved an efficient displacement assay for anions with the use of **CP** as a receptor and *p*-nitrophenolate as the signalizing unit.[9]

Merocyanine dyes are heterocyclic compounds that exhibit many applications.[10] Among these dyes, the compound 4-[(1-methyl-4(1*H*)-pyridinylidene)-ethylidene]-2,5-cyclohexadien-1-one, known as Brooker's merocyanine (**BM**), has been extensively utilized in the studies in solution,[10,11] because of its capability to act as a solvatochromic probe in the investigation of pure and mixeds solvents.[11]

Here, we show that a displacement assay based on the interaction of **CP** with **BM** can be carried out in acetonitrile (Scheme 1). A solution of **BM** is colored and it is expected that the addition of **CP** would change the solution color due to the formation of hydrogen-bonded **CP**–**BM** species. Later, the original color of the solution would return with the addition of an appropriate anion

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Keywords: Anionic sensor; Fluoride; Hydrogen sulfate; Displacement assay; Brooker's merocyanine; Calix[4]pyrrole.

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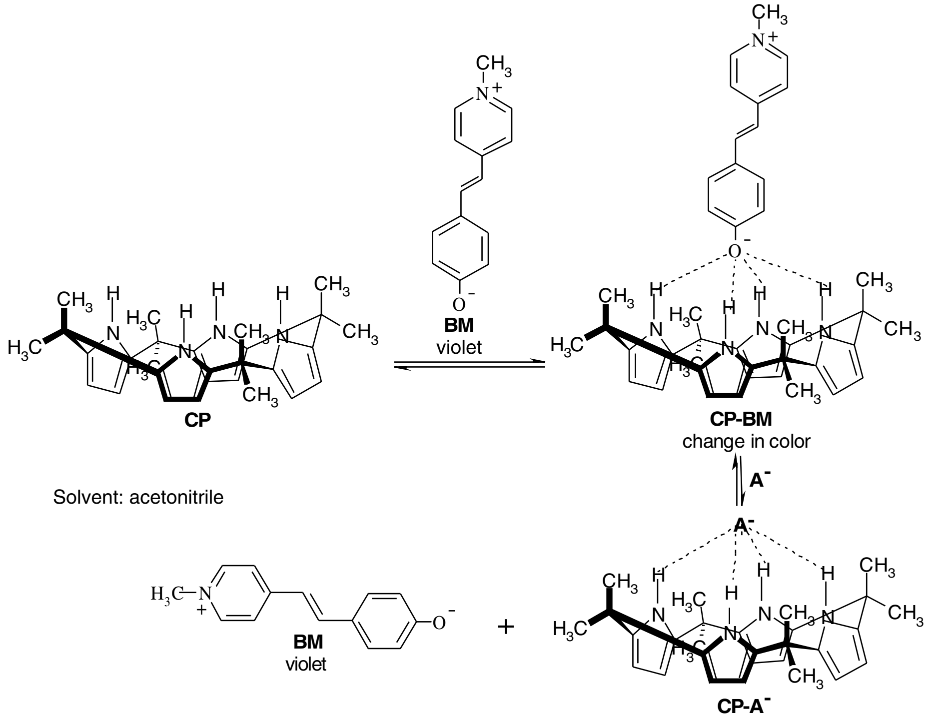

Scheme 1.

to bind selectively to **CP** and, consequently, perform the displacement of the free solvatochromic dye. The formation of **CP**-**A**$^-$ species with anions such as F$^-$, Cl$^-$, and $H_2PO_4^-$ is extensively discussed in the literature.[6,8,9,12]

Firstly, a violet solution of **BM** was prepared in acetonitrile, which was then titrated with increasing amounts of **CP** in acetonitrile solution.[13] It was observed that the addition of **CP** led to the disappearance of the solvatochromic band of **BM** at 571.0 nm, with the simultaneous appearance of a new band at 481.0 nm (Fig. 1). In addition, an isosbestic point could be detected at 518.0 nm, suggesting the presence of two equilibrium species. No alteration in the UV–vis spectrum with the addition of **CP** was observed at concentrations higher than 5.0×10^{-4} mol dm^{-3}. A Job plot[14] was also obtained and the experiment confirmed that **CP**–**BM** was formed in acetonitrile with a 1:1 stoichiometry. This experimental evidence was used in order to obtain the binding constant for the formation of **CP**–**BM**. A plot of the absorbance values at 481.0 nm as a function of the concentration of **CP** added was obtained and a fitting of the experimental data[15,16] considering **CP** and **BM** in a 1:1 stoichiometry gave a binding constant of $(5.66 \pm 0.34) \times 10^3$ dm^3 mol^{-1}. The addition of hydrogen-bond donor (HBD) solvents to **BM** in its acetonitrile solutions causes hypsochromic shifts in the solvatochromic band of **BM**,[11] due to the specific interaction through hydrogen bonding between the OH group of the HBD solvent with the phenolate group of **BM**. Data obtained in this study thus suggest that **CP**–**BM** is formed by means of a strong association of the phenolate donor in **BM** with the **NH** pyrrole groups in **CP**.

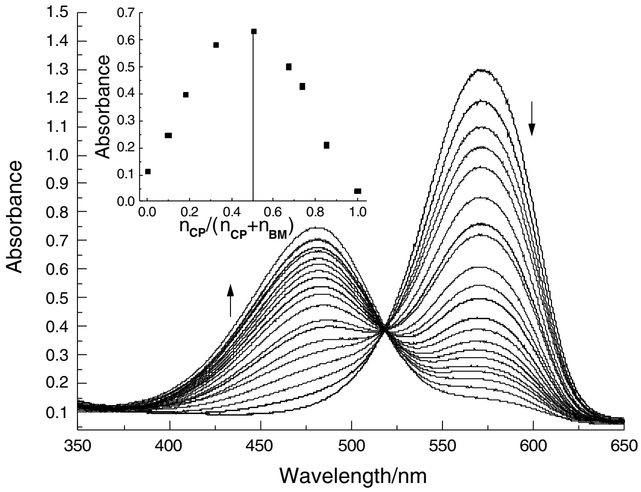

Figure 1. UV–vis spectra of **BM** (1.8×10^{-5} mol dm^{-3}) in acetonitrile at 25 °C after addition of increasing amounts of **CP**. The final concentration of **CP** was 2.2×10^{-4} mol dm^{-3}. Inset: Job plot for **CP** and **BM**.

Upon addition of **CP** to the violet solution of **BM** in acetonitrile, the color changes to orange. A study was carried out adding, individually, different anions[17] (F$^-$, Cl$^-$, Br$^-$, I$^-$, $H_2PO_4^-$, HSO_4^-, and NO_3^-) as tetrabutylammonium salts to the **CP**–**BM** solution. It was found that the addition of F$^-$ caused the reappearance of the original solvatochromic band at 571.0 nm (Fig. 2), which can be visualized by the reappearance of the violet color. Addition of Cl$^-$ or $H_2PO_4^-$, to a much lesser extent, caused changes in color which were intermediate in

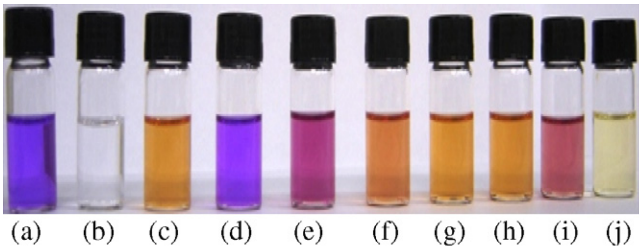

Figure 2. Acetonitrile solutions of (a) **BM**, (b) **CP**, (c) **CP**–**BM**, and **CP**–**BM** in the presence of (d) F^-, (e) Cl^-, (f) Br^-, (g) I^-, (h) NO_3^-, (i) $H_2PO_4^-$, and (j) HSO_4^-, at a concentration of 1.5×10^{-3} mol dm^{-3} for each anion. The concentrations of **CP** and **BM** were 1.6×10^{-4} and 1.8×10^{-5} mol dm^{-3}, respectively.[17]

relation to the violet solution of **BM** and orange color of the **CP**–**BM** solution. Interestingly, the addition of HSO_4^- makes the solution of **CP**–**BM** almost colorless, allowing the visual detection of the anion vis-à-vis to the other species studied. UV–vis spectra of the **CP**–**BM** solutions in the presence of each halide are depicted in Figure 3. The spectra show that the largest spectral change was observed for F^-, followed by Cl^-. A strong reduction in the absorbance at 481.0 nm occurred simultaneously with the reappearance of the solvatochromic band of **BM** at 571.0 nm. Thus, the data confirm that the anions that associate more strongly to **CP** are able to dislodge **BM** from the receptor site, making it free to absorb light at 571.0 nm and therefore to signalize the presence of the analytes. It is also verified that the absorbance obtained in solution at 571.0 nm when fluoride is added is close to the initial absorbance of free **BM**, while a very small effect is verified for Br^- and I^-.

Figure 4 shows UV–vis spectra for the **CP**–**BM** solutions in acetonitrile in the presence of F^-, $H_2PO_4^-$, HSO_4^-, and NO_3^-. It can be observed that while the addition of nitrate does not have any effect on the spectrum of **CP**–**BM**, appreciable spectral shifts occur when the other anions are added. Addition of $H_2PO_4^-$ leads to the diminution of the band at 481.0 nm followed by an increase in the solvatochromic band at 571.0 nm. The effect is, however, less than that caused by the addition of F^- and Cl^-. Thus, the data show that at the concentration employed there remains in solution an appreciable amount of **BM** bound to **CP**, a much larger anion concentration being required to perform a quantitative substitution of **BM**. The addition of HSO_4^- was responsible for the strongest spectral shift observed. It can be seen that the band referent to **CP**–**BM** and the residual band of **BM** in the initial spectrum have disappeared completely while, simultaneously, a new band has appeared with a maximum at the wavelength of 384.0 nm. This hypsochromic shift of 97.0 nm makes the solution almost colorless. It is well known that **BM** in solution can be made colorless under protonation. Therefore, the data suggest that HSO_4^- is sufficiently acidic to transfer a proton to **BM**, removing it from the **CP** receptor site by protonating the dye and leading it to absorb only in the UV region of the spectrum.

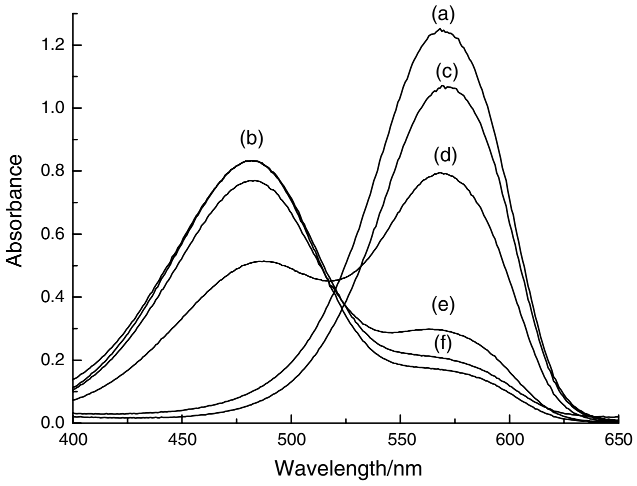

Figure 3. UV–vis spectra of acetonitrile solutions of (a) **BM**, (b) **CP**–**BM**, and **CP**–**BM** in the presence of (c) F^-, (d) Cl^-, (e) Br^-, and (f) I^-, at a concentration of 1.5×10^{-3} mol dm^{-3} for each anion. The concentrations of **CP** and **BM** were 1.6×10^{-4} and 1.8×10^{-5} mol dm^{-3}, respectively.[17]

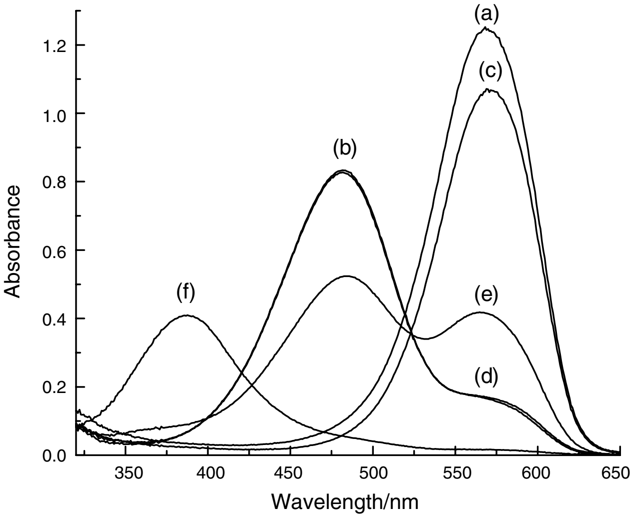

Figure 4. UV–vis spectra of acetonitrile solutions of (a) **BM**, (b) **CP**–**BM**, and **CP**–**BM** in the presence of (c) F^-, (d) NO_3^-, (e) $H_2PO_4^-$, and (f) HSO_4^-, at a concentration of 1.5×10^{-3} mol dm^{-3} for each anion. The concentrations of **CP** and **BM** were 1.6×10^{-4} and 1.8×10^{-5} mol dm^{-3}, respectively.[17]

Titration experiments were performed to quantify the influence of an increase in the F^- concentrations on the **CP**–**BM** solutions.[18] It was observed that the addition of F^- led to the disappearance of the **CP**–**BM** band at 481.0 nm with the simultaneous appearance of the solvatochromic band of **BM** at 571.0 nm. The presence of an isosbestic point at 518.0 nm was also observed. Plots of the absorbance values at 571.0 nm as a function of the concentration of fluoride using different concentrations of **CP** had a sigmoidal profile (Fig. 5). It was observed that for a **CP** concentration in the system of 4.2×10^{-4} mol dm^{-3}, the addition of fluoride up to a concentration of 4.0×10^{-4} mol dm^{-3} caused a very small spectral shift. Apart from at this concentration, the effect of the anion added is very strong, being very similar to the titrations made with lower **CP** concentra-

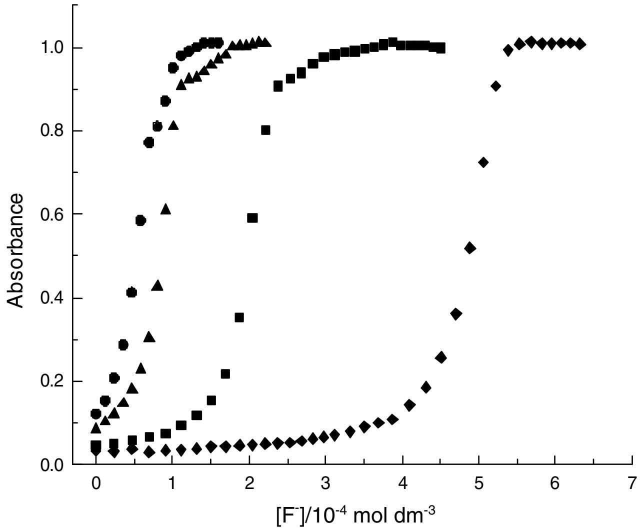

Figure 5. Addition of increasing amounts of F^- to **CP**–**BM** solutions in acetonitrile. Titrations were performed at 25 °C, and the concentration of **BM** was 1.8×10^{-5} mol dm^{-3}. The concentrations of **CP** were 7.4×10^{-5} (●), 1.1×10^{-4} (▲), 2.2×10^{-4} (■), and 4.2×10^{-4} (♦) mol dm^{-3}.[18]

tions. Thus, the data demonstrate the effective occurrence of a competition in the solution of the anion for free **CP** and **CP**–**BM** and that the lower the free **CP** concentration, the easier it is for the anion to displace **BM** from the **CP** receptor site and to form a complex with the receptor.

In conclusion, it is shown here that the mixture of **BM** with **CP** in excess, in acetonitrile, leads to the formation of a **CP**–**BM** species, which is able to act as an anionic chromogenic sensor. The system shown here thus represents an efficient strategy, as do others described in the literature,[19] for visually and quantitatively detecting different anionic species in a selective way. Finally, **BM** is fluorescent,[20] which may allow the use of this strategy for the development of a fluorogenic sensor based on displacement assays.

Acknowledgements

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13. A solution of **BM** (1.8×10^{-5} mol dm^{-3}) in acetonitrile was prepared. This violet solution was then utilized to make the stock solution of **CP** (4.6×10^{-3} mol dm^{-3}) in a 5 mL volumetric flask closed with a rubber stopper. In sequence, 1.5 cm^3 of the original solution of **BM** was placed in a quartz cuvette and this solution was titrated by adding small amounts of the solution containing **CP**. This solution transfer experiment was carried out at 25 °C using flasks and the cuvette hermetically closed with rubber stoppers in order to minimize problems with the evaporation of the solvent.
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15. Data related to the titration of **BM** with **CP** were fitted with the use of the following equation: $\mathrm{Abs} = (\mathrm{Abs}_0 + \mathrm{Abs}_{\max} K_{1:1} C_{\mathrm{CP}})/(1 + K_{1:1} C_{\mathrm{CP}})$.[16] In this equation, Abs is the absorbance value after each addition of **CP**, Abs_0 is the initial absorbance without **CP** added, $\mathrm{Abs}_{\max}$ is the maximal absorbance value obtained by addition of **CP**, C_{CP} is the concentration of **CP** in each addition and $K_{1:1}$ is the binding constant. The value of χ^2 for the plot was 1.1×10^{-4}.
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17. The solution containing **CP**–**BM** was prepared as described previously,[13] but with **CP** concentration of 1.6×10^{-4} mol dm^{-3}, and this stock solution was used to

make a 1.5×10^{-3} mol dm^{-3} solution of each anion. The UV–vis spectra for each solution were then obtained.

18. A solution of **BM** (1.8×10^{-5} mol dm^{-3}) in acetonitrile was prepared and subsequently used to prepare other solutions containing **CP**. These resultant solutions containing **CP**–**BM** were used to prepare stock solutions of F^{-} (1.5×10^{-3} mol dm^{-3}). Titration experiments were performed at 25 °C under experimental conditions similar to those used in previous experiments[13] by adding small amounts of these F^{-} solutions with a microsyringe to closed quartz cuvettes containing the solution of **CP**–**BM** and the absorbances were collected at 571.0 nm.
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