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# Use of the interaction of a boronic acid with a merocyanine to develop an anionic colorimetric assay

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**Abstract**—A displacement assay based on the interaction of Brooker's merocyanine (**BM**), a merocyanine dye, with an excess of phenylboronic acid (**BA**) was studied in acetonitrile. **BM** is colored in solution, but its reaction with **BA** yields a colorless covalently linked **BA**–**BM** species. This strategy was studied in the presence of different anions ($F^-$, $Cl^-$, $Br^-$, $I^-$, $H_2PO_4^-$, $HSO_4^-$, $CH_3COO^-$, and $NO_3^-$), but only fluoride, a strongly nucleophilic anion, and to a much lesser extent acetate, reacted with **BA**–**BM**, displacing **BM** through a bimolecular nucleophilic substitution mechanism, and coloring the solution. Experimental data were collected and are shown here in order to gain a better understanding of this chromogenic sensor.


The fact that anions play a fundamental role in many chemical and biological processes has led to many studies in the field of the recognition and the detection of anionic species.[1] Many sensors have been developed in the last few years which not only allow visual selective anion detection, but also the quantification of such species.[2–5] However, the development of colorimetric assays for anionic species,[3–5] which are simple in comparison with the most common fluorescence anion sensors,[2,4] is still limited. 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.[6] These displacement assays work well as anionic sensors if spectral differences can be detected for the free and the complexed indicator and also if the receptor site is able to recognize mainly one of many substrates.

Boronic acids are very attractive molecules due to the fact that they are able to bind selectively anions and monosaccharides. Although they have been used in the design of chromo- and fluorogenic chemosensors as receptor sites for monosaccharides[7] and also for anions,[8] no studies can be found in the literature involving colorimetric sensors based on displacement assays using an organic boronic acid, an indicator, and anionic species.

Merocyanine dyes are heterocyclic compounds with many applications.[9] A classical example of these dyes is 4-[(1-methyl-4(1*H*)-pyridinylidene)-ethylidene]-2,5-cyclohexadien-1-one, known as Brooker's merocyanine, (**BM**), which has been extensively used in many studies,[9] especially as a solvatochromic probe in the investigation of pure and mixed solvents.[10]

Here, we show that a displacement assay based on the interaction of phenylboronic acid (**BA**) with **BM** can be carried out in acetonitrile. Subsequently, a strongly nucleophilic anion such as fluoride can react with **BA**–**BM** species, displacing **BM**. Since **BM** is colored in acetonitrile, and the covalently linked **BA**–**BM** is colorless, the color in the solution can, in principle, be returned by adding fluoride anions, as shown in Scheme 1. The formation of species such as **BA**–**F** under conditions where the anion is present in low concentrations is extensively discussed in the literature.[8]

**BM** was initially solubilized in acetonitrile and the violet resultant solution was titrated with increasing amounts of **BA** in acetonitrile solution.[11] It was observed that the addition of **BA** led to the disappearance of the solvatochromic band of **BM** at 571.0 nm followed by the

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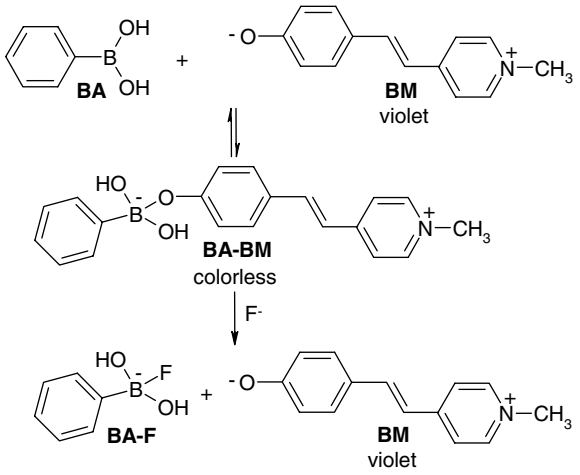


**Scheme 1.**

appearance of a new band at 385.9 nm (Fig. 1). In addition, an isosbestic point could be detected at 470.1 nm. These effects cannot be explained by the formation of hydrogen-bonded complexes of **BA** and **BM** in acetonitrile. It is well known that the addition of hydrogen-bond donor (HBD) solvents to **BM** acetonitrile solutions causes hypsochromic shifts in the solvatochromic band of **BM**,[10] due to the specific interaction through hydrogen bonding between the OH group of the HBD solvent with the phenolate group of **BM**. However, these effects are not as dramatic as those observed here: very large volumes of the HBD solvent are needed to cause a strong hypsochromic shift but without suppression of the solvatochromic band in the visible region and with the absence of an isosbestic point.

Increasing amounts of different anions[12] ($F^-$, $Cl^-$, $Br^-$, $I^-$, $H_2PO_4^-$, $HSO_4^-$, $CH_3COO^-$, and $NO_3^-$) were added to the colorless solution of **BA**–**BM**. It was found that only the addition of $F^-$, and to a much lesser extent

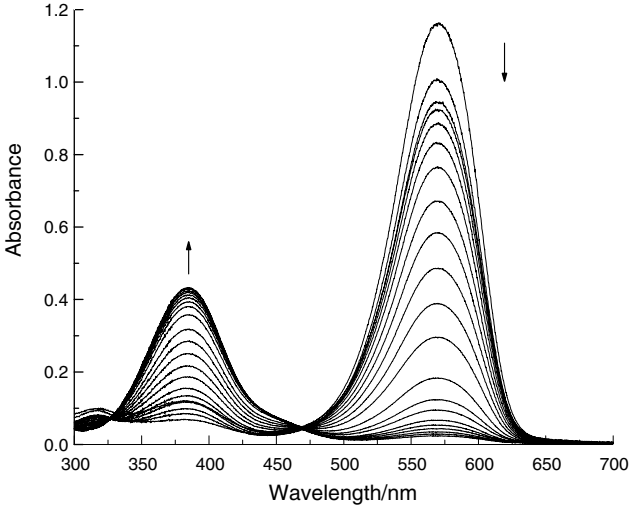


**Figure 1.** UV–vis spectra of **BM** ($2.0 \times 10^{-5}$ mol dm$^{-3}$) in acetonitrile at 25 °C after addition of increasing amounts of **BA**. The final concentration of **BA** was $2.4 \times 10^{-4}$ mol dm$^{-3}$.

acetate, caused the reappearance of the original solvatochromic band at 571.0 nm (Figs. 2 and 3). The system studied here therefore represents an alternative efficient strategy, in comparison with others described in the literature,[13] for visually and quantitatively detecting $F^-$ in a selective way. It was also verified that the presence of a mixture of all anions used in the studies led to a result qualitatively similar to that observed when only fluoride was present. However, the reappearance of the solvatochromic band at 571.0 nm occurred at a lower absorbance value for the mixture of anions, suggesting that the increase in the ionic strength caused by the high concentration of electrolytes lowers the value of the equilibrium constant for the process. This is to be expected since the negative charge on the phenolate group in BM is dispersed when compared with the concentrated negative charge on fluoride.

Titration experiments were performed to quantify the influence of an increase in the fluoride concentrations on the **BA**–**BM** solutions, and the sequence of UV–vis spectra is shown in Figure 4.[14] It can be observed that the addition of the anion led to the disappearance of the **BA**–**BM** band at 385.9 nm with the simultaneous appearance of the solvatochromic band of **BM** at

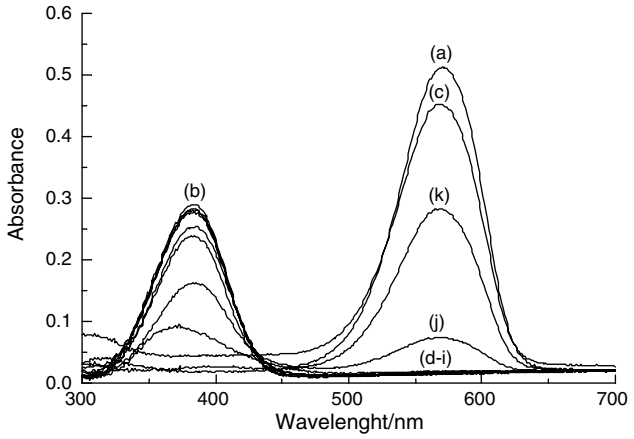


**Figure 2.** UV–vis spectra of acetonitrile solutions of (a) **BM**, (b) **BA**–**BM**, and **BA**–**BM** in the presence of (c) $F^-$, (d) $Cl^-$, (e) $Br^-$, (f) $I^-$, (g) $H_2PO_4^-$, (h) $HSO_4^-$, (i) $NO_3^-$, (j) $CH_3COO^-$, and (k) a mixture containing $F^-$, $Cl^-$, $Br^-$, $I^-$, $H_2PO_4^-$, $HSO_4^-$, $NO_3^-$, and $CH_3COO^-$, at a concentration of $1.0 \times 10^{-4}$ mol dm$^{-3}$ for each anion. The concentrations of **BM** and **BA** were $1.0 \times 10^{-5}$ and $2.78 \times 10^{-4}$ mol dm$^{-3}$, respectively.

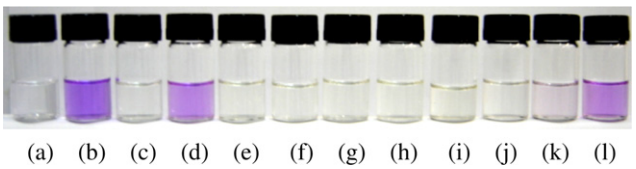


**Figure 3.** Acetonitrile solutions of (a) **BA**, (b) **BM**, (c) **BA**–**BM**, and **BA**–**BM** in the presence of (d) $F^-$, (e) $Cl^-$, (f) $Br^-$, (g) $I^-$, (h) $H_2PO_4^-$, (i) $HSO_4^-$, (j) $NO_3^-$, (k) $CH_3COO^-$, and (l) a mixture containing $F^-$, $Cl^-$, $Br^-$, $I^-$, $H_2PO_4^-$, $HSO_4^-$, $NO_3^-$, and $CH_3COO^-$, at a concentration of $1.0 \times 10^{-4}$ mol dm$^{-3}$ for each anion. The concentrations of **BM** and **BA** were $1.0 \times 10^{-5}$ and $2.78 \times 10^{-4}$ mol dm$^{-3}$, respectively.

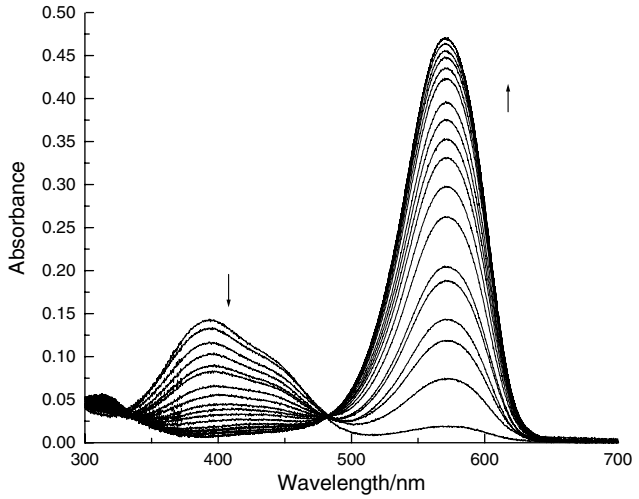


**Figure 4.** UV–vis spectra of **BA**–**BM** in acetonitrile at 25 °C after addition of increasing amounts of tetrabutylammonium fluoride. The concentrations of **BA** and **BM** were $2.78 \times 10^{-4}$ and $1.0 \times 10^{-5}$ mol dm$^{-3}$, respectively and the final concentration of fluoride was $8.2 \times 10^{-5}$ mol dm$^{-3}$.

571.0 nm. The presence of an isosbestic point at 385.9 nm can also be observed. A plot of the absorbance values at 571.0 nm as a function of the concentration of fluoride displayed an almost linear behavior until the dye and fluoride concentrations were the same (Fig. 5). This provides important evidence for the fact that fluoride acts as a nucleophile, displacing **BM** and forming **BA**–**F** species, in a mechanism of bimolecular nucleophilic substitution. A fitting of the experimental data[15,16] considering **BA** and the anion in a 1:1 stoichiometry gave a binding constant of $1.93 \pm 0.53 \times 10^5$ dm$^3$ mol$^{-1}$.

An important aspect to be considered is that our experiments were performed with a large excess of **BA**, more than 26 times, in relation to **BM**. Thus, in principle, when fluoride is added, it can react with **BA** or with **BA**–**BM** and, if the excess in **BA** is considered, the preference initially would be for **BA**. This would lead to the plot shown in Figure 5 having a sigmoid shape, which was not the case. This indicates that fluoride preferentially reacts with **BA**–**BM** species in comparison with **BA**. Thus, the data suggest that, in the **BA**–**BM** species, **BM** plays a double role, making the boron center more electrophilic than that in **BA** and also acting as an excellent leaving group. In addition, it is possible that acetonitrile plays an important role, solvating the boron center in **BA** and inhibiting the fluoride access.

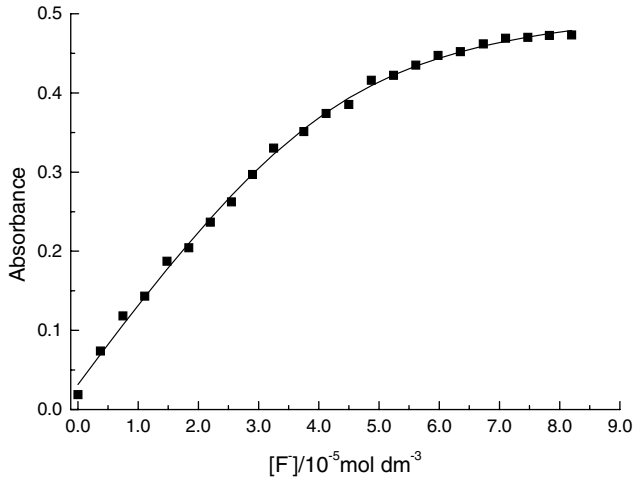


**Figure 5.** Variation in the absorbance at 571.0 nm due to the appearance of **BM** in acetonitrile from the **BA**–**BM** solution with the addition of increasing amounts of tetrabutylammonium fluoride. The experiment was performed at 25 °C and the concentrations of **BM** and **BA** were $1.0 \times 10^{-5}$ and $2.78 \times 10^{-4}$ mol dm$^{-3}$.[15]

It is important to observe that the very important nucleophilic effect of fluoride in comparison with the other anions is related to its smaller size, higher charge density, and higher electron affinity, properties which allow it to interact strongly with the boron center in **BA**–**BM**. These aspects have been highlighted in other publications in relation to the high selectivity for fluoride in comparison with other anions, of phenol-based chromo- and fluorogenic sensors.[13]

An additional question related to the study here presented concerns the possibility that the effect observed when **BA** is added to **BM** solution may be due to a simple proton transfer from **BA** to **BM**. In this case, fluoride would act as a base removing the proton of the protonated dye and regenerating the color in solution. Thus, a simple experiment was carried out: a **BM** solution in acetonitrile containing traces of water was protonated by $H_2CO_3$ formed in solution by bubbling with $CO_2$. The protonation made the dye solution colorless. The addition of fluoride to this solution did not cause any change in the color of the solution, even with the addition of fluoride in a concentration three times higher ($3.0 \times 10^{-4}$ mol dm$^{-3}$) than that used in the other experiments. Thus, the possibility for this assay to involve simple proton transfer was discarded, and the model in Scheme 1 was proposed.

In conclusion, it is shown here that the reaction of **BM** with **BA** in excess leads to the formation of a **BA**–**BM** colorless species, which is able to act as a fluoride chromogenic sensor. The strategy studied here combines the conception of the chemosensors based on displacement assays[6] with the design of sensors based on the formation/breaking of covalent bonds.[17] Since boronic acids are very important receptors which bind selectively cyanide[8] and monosaccharides,[7] especially glucose, the assay presented here can be easily modified to provide chromogenic sensors for these analytes. Finally, **BM** is fluorescent,[18] which may allow the use of this strategy for the development of fluorogenic sensors based on displacement assays.

## Acknowledgments

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11. A solution of **BM** ($2.0 \times 10^{-5}$ mol dm$^{-3}$) in acetonitrile was prepared. This violet solution was then used to prepare the stock solution of **BA** ($5.0 \times 10^{-3}$ mol dm$^{-3}$). Titration experiments were performed by adding small amounts of this solution with a microsyringe to closed quartz cuvettes containing the solution of **BM**. These solution transfer experiments were carried out using flasks and the cuvettes hermetically closed with rubber stoppers in order to minimize problems with the evaporation of the solvent and all experiments were carried out at 25 °C.
12. All anions were used as tetrabutylammonium salts, with the exception of acetate. In this case, a solution in acetonitrile of Kryptofix® ($7.44 \times 10^{-3}$ mol dm$^{-3}$) was prepared and after potassium acetate was added up to a stock solution of $6.66 \times 10^{-3}$ mol dm$^{-3}$. One part of this solution was diluted with acetonitrile to give a solution with a concentration of $1.0 \times 10^{-4}$ mol dm$^{-3}$.
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14. A solution of **BM** ($1.0 \times 10^{-5}$ mol dm$^{-3}$) in acetonitrile was prepared and used to prepare other solutions containing **BA** ($2.78 \times 10^{-4}$ mol dm$^{-3}$). This excess of **BA** was used in order to favor the presence of only **BA**–**BM** species, because in low **BA** concentrations we collected evidences for the presence of **BA**–(**BM**)$_2$ and **BA**–(**BM**)$_3$ species, in agreement with that studied in the literature concerning the reaction of organic boronic acids with anionic species, such as hydroxide, fluoride, and cyanide.[8] This resultant colorless solution containing **BA**–**BM** was used to prepare a stock solution of fluoride ($7.90 \times 10^{-3}$ mol dm$^{-3}$). Titration experiments were performed by adding small amounts of this fluoride solution with a microsyringe to closed quartz cuvettes containing the solution of **BA**–**BM**. These solution transfer experiments were carried out at 25 °C under experimental conditions similar to those used in previous experiments[11] to minimize problems with the evaporation of the acetonitrile.
15. Data related to the titration of **BA**–**BM** with fluoride were fitted with the use of the following equation:[16] $\mathrm{Abs} = \mathrm{Abs}_0 + (\mathrm{Abs}_{max} - \mathrm{Abs}_0)/2C_{BM}\{C_{BM} + C_{F^-} + 1/K - [(C_{BM} + C_{F^-} + 1/K)^2 - 4C_{BM}C_{F^-}]^{1/2}\}$. In this equation, Abs is the absorbance value after each addition of the anion, $\mathrm{Abs}_0$ is the initial absorbance without anion added, $\mathrm{Abs}_{max}$ is the maximal absorbance value obtained by addition of the anion, $C_{BM}$ is the concentration of **BM**, $C_{F^-}$ is the concentration of fluoride in each addition and $K$ is the binding constant. The value of $\chi^2$ for the plot was $4 \times 10^{-5}$.
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