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Short Report

Fluorescence Quenching of Two *meso*-Substituted Tetramethyl BODIPY Dyes by Fe(III) Cation

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Ferric ion (Fe(III)) is a biologically and environmentally relevant cation so that its analysis in environmental and biological samples is often required. Borodiazaindacenes (BODIPYs) are known for their good photophysical properties; however, there are few BODIPY-based Fe(III) sensors reported. Herein, we show the characterisation of two BODIPY dyes whose fluorescence emission is diminished by such cation. Both "turn-off" probes, a catecholyl-substituted BODIPY and a pyridyl-substituted BODIPY, were synthetically obtained and an initial screening showed a relatively good specificity for Fe(III) when compared to other cations. Catecholyl-substituted BODIPY was more sensitive to Fe(III), however, with a pH-dependent analytical performance and low brightness. On the other hand, pyridyl-substituted BODIPY was very bright and its analytical performance was apparently pH-independent, however, it was less sensitive to the analyte. In conclusion, we show herein the obtainment and characterisation of two probes with promising analytical value in the analysis of Fe(III).

Keywords: BODIPY, iron probe, ferric ion, pH sensor, fluorescence quenching, turn-off sensor

Introduction

Ferric ion (Fe(III)) is a trivalent metallic cation indispensable for eukaryotic cells. Iron homeostasis is a crucial process, and its deficiency or excess can lead to several disorders in humans.[1] In plants, Fe(III) is essential for photosynthesis, which accounts for its intentional introduction in the environment as a fertilizer, despite some environmental concerns.[2] Iron sensing systems possess potential applications as analytical tools for the analysis of biological or environmental samples, and iron-sensitive fluorescent molecules can be applied for the fluorimetric analysis of such analyte.

Fluorescence turn-off Fe(III) probes, in which Fe(III) acts as a fluorescence quencher, are the most common system, and result from the opening of novel deexcitation pathways of excited state due to fluorophore-ion complexation. Molecular and supramolecular fluorescent sensors have been widely investigated in order to develop sensitive and selective methods for Fe(III) monitoring,[3] which is still a very active field of research.[4]

Borodiazaindacene (BODIPY) dyes encompass simple fluorescent compounds, initially described by Treibs and Kreuzer,[4] which have arisen in the last two decades as important analytical tools.[5] Prepared from the complexation between a dipyrrin dye and difluoroboryl unity, BODIPYs are usually associated with sharp emission and absorption peaks, good photo and chemo stability, and relatively easy chemical modification and fluorescence tuning.[6] BODIPY scaffold has been explored for the obtainment of some ion probes,[7] and some BODIPY-based Fe(III) probes have been reported.[8] Herein we show a relatively specific Fe(III)-induced fluorescence quenching of two structurally simple BODIPY. These are easily synthesized compounds and could be applied for analytical purposes in the life and environmental sciences.

Experimental

Reagents and syntheses

Reagents were obtained from Sigma-Aldrich Brasil Ltda. (São Paulo, SP, Brazil) and from Merck Millipore Brazil (São Paulo, SP, Brazil) while solvents were obtained from local suppliers and treated according to established protocols of purification. Chemical structures were determined by carbon-13 and hydrogen nuclear magnetic resonance (^{13}C and ^{1}H NMR) in a DRX NMR

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system from Bruker Daltonics® (Billerica, MA, USA), infrared spectroscopy (IR) in an IR-Prestige 21 system from Shimadzu (Kyoto, Japan), and high-resolution mass spectrometry with electrospray ionisation (HRMS-ESI) using the ultrOTOFQ ESI-time-of-flight (TOF) system from Bruker Daltonics®.

Fluorescence and absorption spectroscopy

Absorption spectra were obtained on a UV-380G UV-Vis spectrophotometer from GEHAKA (São Paulo, Brazil) and steady state fluorescence spectra were obtained on a RF5301PC spectrofluorimeter from Shimadzu using an excitation wavelength (λ_{exc}) of 460 nm. Molar extinction coefficients (ε) were calculated from the slope of the regression linear curve of absorbance and concentration, with ten diluted solutions (absorbance under 0.1) of each compound being analysed.

The EasyLife™ V (Optical Building Blocks, Birmingham, NJ, USA) fluorescence lifetime system was used to obtain time-resolved fluorescence spectroscopy. Instrument response function was obtained using colloidal silica, and an exponential decay curve was fitted to the experimental data in order to calculate fluorescence lifetime. Chi-square (χ^2), Durbin-Watson (DW) and Z (Z) statistics were calculated for each fitted curve, and reasonable statistical parameters were obtained: $0.9 < \chi^2 < 1.2$; DW > 1.7; Z > –1.96.

Quantum yields were calculated using a comparative method[9] with a fluorescein standard (fluorescein in 0.1 mol L^{-1} NaOH(aq); ϕ = 0.91, λ_{exc} = 470 nm).[10] The emission spectra from five samples of each fluorophore (absorbance between 0.1 and 0.02 at the excitation wavelength of 470 nm) were obtained. The results were plotted with the integrated fluorescence intensity *vs.* absorbance to obtain the slope of the curve for each tested compound and the standard. The quantum yield of the tested compound (ϕ_x) was calculated using equation 1, where ϕ_{st} is the quantum yield of the standard, m_x and m_{st} are the slopes for the test compound and standard compound, and n_x and n_{st} are the refractive indexes of the solvents.

$$\phi_x = \phi_{st}\left[\frac{m_x}{m_{st}}\right]\left[\frac{n_x}{n_{st}}\right]^2 \quad (1)$$

Synthetic procedures and spectroscopical characterisation are available as Supplementary Information.

Fluorescence quenching and titrations

BODIPYs were dissolved at 5.0 × 10^{-5} mol L^{-1} in aqueous solution with 30% dimethyl sulfoxide (DMSO) as co-solvent. For an initial screening, fluorescence spectrum emission of each compound was recorded before and after the addition of several cations at a final concentration of 2.5 × 10^{-4} mol L^{-1} (5 eq). For that, aqueous stock solutions of the following 24 salts at 1.0 × 10^{-2} mol L^{-1} were prepared: $AgNO_3$, $Al_2(SO_4)_3$, $BaCl_2$, $Be(NO_3)_2$, $CaCl_2$, $CdCl_2$, $CoCl_2$, $Cr_4(SO4)_5OH_2$, Cs_2CO_3, CuCl, $CuCl_2$, $FeCl_3$, $FeSO_4$, $HgCl_2$, $InCl_3$, KCl, Li_2SO_4, $MgCl_2$, $MnCl_2$, NaCl, $Ni(NO_3)_2$, $Pb(NO_3)_2$, $SrCl_2$, and $Zn(OAc)_2$.

Titrations were realised by continuously adding small volumes of the cation stock solutions described earlier, and recording steady state and/or time-resolved fluorescence spectra. For temperature-controlled experiments, BODIPY solutions were cooled in a cooling bath at 0 °C or heated in a heating bath at 50 °C, and titrated in the same manner. For pH-controlled experiments, a digital pH meter was used to monitor the pH of the solution, which was corrected when necessary by adding concentrated aqueous solution of HCl or NaOH. For experiments at pH 2, no buffering agent was used, for pH 4 and 6 we used sodium acetate at 0.2 mol L^{-1} as buffering agent, and for experiments at pH 8, sodium bicarbonate at 0.2 mol L^{-1} was used.

Results and Discussion

Synthesis and spectroscopical characterisation

Four BODIPY dyes were synthesised and characterised in aqueous DMSO (30% v/v) regarding wavelength at maximum absorption (λ_{abs}), wavelength at maximum emission (λ_{em}), molar absorption coefficient (ε), fluorescence quantum yield (φ_{fl}) and fluorescence lifetime (τ) (Figure 1). Tetramethyl-BODIPY (**1**) was obtained as a red solid from the self-condensation of 3,5-dimethyl-2-carbaldehyde in 43.5% yield using equimolar $POCl_3$ in dichloromethane in a one pot process. *Meso*-substituted compounds **2** [a 8-(2-thienyl)-BODIPY] and **3** [a 8-(4-pyridyl)-BODIPY] were obtained respectively in 15.2 and 23.7% overall yield from the trifluoroacetic acid (TFA)-catalysed condensation of the respective aromatic aldehyde and 2,4-dimethyl pyrrole, followed by oxidation of the dipyrromethane with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and complexation of the dipyrrin with $BF_3.Et_2O$.[11] Catechol-substituted BODIPY (**4**) was obtained in a similar manner, however benzyloxy-protected derivative was initially produced in 56% yield, from which compound **4** was synthesised by palladium catalysed hydrogenolysis in 68% yield.[12]

The aromatic substituents barely affected λ_{abs}, ε and λ_{em} due to the absence of efficient resonance interaction between the *meso*-substituent and the BODIPY core. A

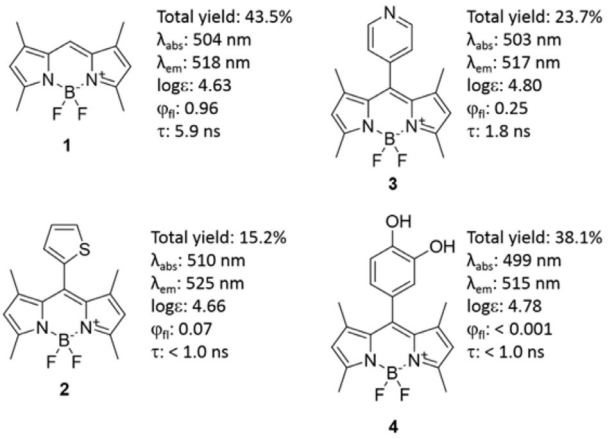

Figure 1. Structure and photophysical parameters of the BODIPYs synthesised, showing typical features of BODIPY dyes.

slight bathochromism (5-7 nm deviation) in both absorption and emission was observed in the thienyl substituted derivative **2**, due to the better co-planarity of such aromatic ring and the BODIPY core. As for fluorescence quantum yields and lifetime, expressive differences were observed among the synthesised compounds. Reduction of φ_{fl} and τ was observed for the *meso*-substituted compounds, which is explained by the addition of a new path of non-radiative energy dissipation. This reduction was especially intense for compound **4,** in which the fluorescence was almost absent, a feature previously found to be related to a photo-induced electron transfer from the catecholic hydroxyls to the BODIPY core.[12]

Fluorescence quenching

We screened the recognition behaviour of BODIPYs **1**-**4** towards several metal cations and no relevant bathochromism or hypsochromism was observed in the emission or absorption spectra. Bathochromism has been previously reported for compound **4** after addition of Pb ions,[13] which differs from our observations probably due to the use of different aqueous systems.

Interestingly, fluorescence emission of compounds **3** and **4** were significantly and specifically quenched by $FeCl_3$ (Figure 2), while no significant changes were observed in the absorption spectroscopy. Not only $FeCl_3$ but also $Fe_2(SO_4)_3$ has quenched the fluorescence emission of both compounds showing that the fluorescence quenching results from the interaction of fluorescent probes and Fe(III) cation.

Previously published data have showed fluorescence quenching of BODIPY **4** with 80 eq of other metal cations, mainly Al(III) and Cr(III), while Fe(III) was not analysed.[14] It can be reasoned that compound **4** is sensitive to tripositive metal cations, and a careful analysis of Figure 2 actually shows a small fluorescence quenching by Al(III) and In(III) in our experiments. However, the quenching effect of Fe(III) is much more evident and could have practical applications. To our knowledge, the behaviour of **3** as a ligand to metallic ions has never been addressed in the literature.

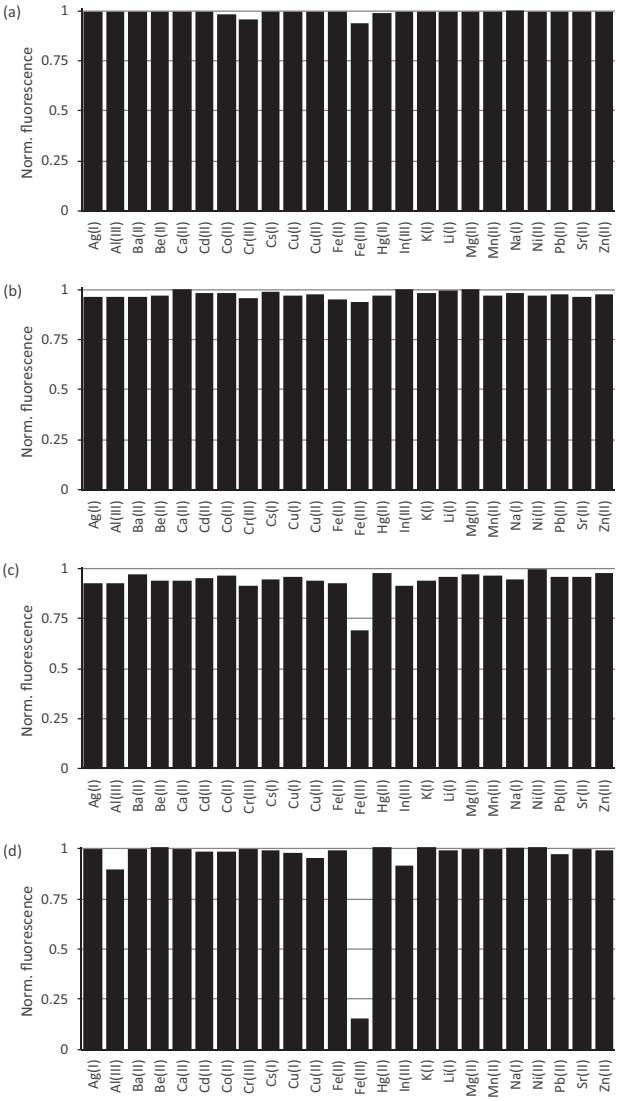

Figure 2. (From top to bottom) Effects of several cations on emission intensities of compounds **1**, **2**, **3** and **4**. Significant fluorescence quenching by Fe(III) can be observed for compounds **3** and **4**.

Stern-Volmer plots were obtained at 0, 25 and 50 ºC for the fluorescence quenching of **3** and **4** by Fe(III). For **3** (Figure 3, left) a good linearity was observed for the entire range of Fe(III) concentrations tested (0 to 1.0×10^{-3} mol L^{-1}), while for **4** (Figure 3, right), our analysis indicated loss of linearity for Fe(III) concentrations higher than 1.5×10^{-4} mol L^{-1} which can be reasoned as a result of saturation. At room temperature, quenching constants (K_{sv}) of compounds **3** and **4**, obtained from the slope of the Stern-Volmer plots, were 2.0×10^3 and

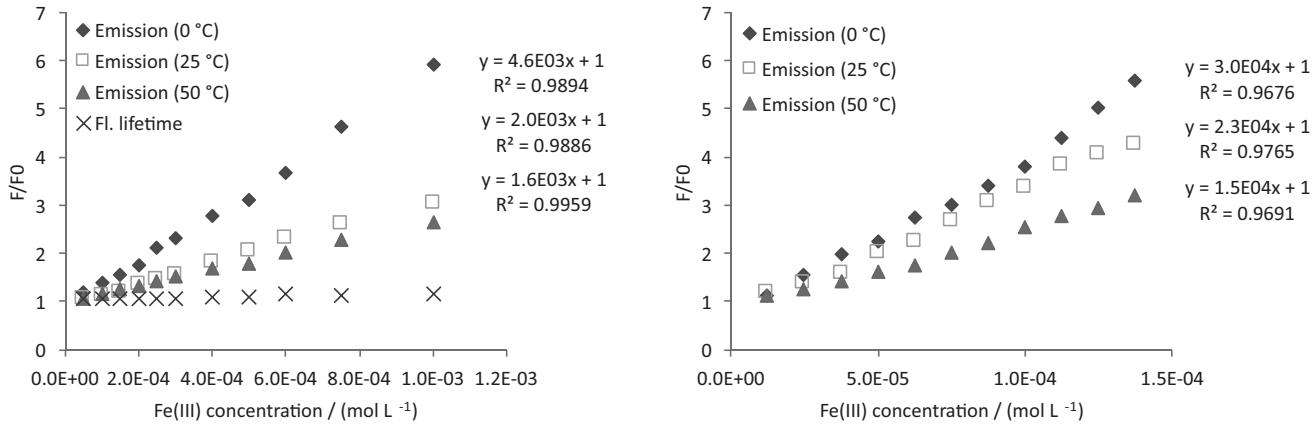

Figure 3. (Left) Stern-Volmer plots obtained for the emission of compound **3** in three temperatures (0, 25 and 50 ºC), and for the fluorescence lifetime at 25 ºC. (Right) Stern-Volmer plots obtained for the emission of compound **4** in three temperatures (0, 25 and 50 ºC). The Stern-Volmer plot for the fluorescence lifetime could not be studied for this compound due to the very fast fluorescence lifetime and low fluorescence intensity.

2.3×10^4 L mol^{-1}, respectively, which reflects the higher sensitivity of compound **4**.

The inverse relation between the slope of the fitted curves and temperature, the short fluorescence lifetimes and its changelessness in growing concentrations of Fe(III) are consistent with a static quenching process and suggests the formation of a non-fluorescent complex between these BODIPYs and Fe(III). The stoichiometry of the such complexation was studied by the Job method, and for both compounds the convergence of the regression lines were roughly near a molar fraction of 0.5, which indicates a 1:1 stoichiometry (Supplementary Information). Regarding the quenching of **4**, one might propose that a redox reactivity leading to the *o*-benzoquinone could occur, however addition of ethylenediaminetetraacetic acid (EDTA) to the Fe(III)-quenched BODIPY solution results in total restoration of initial fluorescence. Additionally, the *o*-benzoquinone BODIPY is known to be highly fluorescent instead.[12]

pH effects

Titration of **3** and **4** dissolved in Britton-Robinson buffer with 30% (v/v) DMSO in the range of pH 1 to 13 shows that both compounds are pH-sensitive. Fluorescence of **3** was almost absent in acidic pH, presumably due to protonation of the nitrogen in the pyridine substituent and the turning point could be followed visually. The titration curve (Figure 4) shows an inflection near pH 4 (pKa 3.3), and stable emission intensity from pH 5 to 13, similarly to what has been previously described.[15] As for compound **4**, deprotonation of the first catecholic hydroxyl was related to lower intensity in fluorescence emission. The deprotonation of the second hydroxyl only occurred in very basic solution (over pH 12) and could be observed as an intense fluorescence emission enhancement. The titration showed that emission was stable between pH 2 and 6. The first inflexion point seemed to be between pH 7 and 8 (pKa 7.1) while the second inflexion point was over pH 12 and pKa could not be calculated.

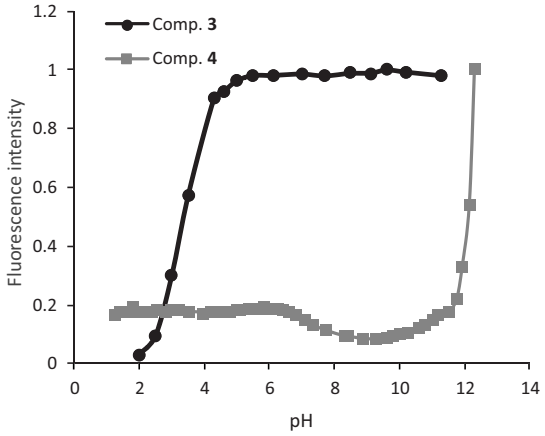

Figure 4. Influence of pH over intensity of fluorescence emission for compounds **3** and **4**. pH dependency was observed for both compounds.

Due to the pH sensing characteristics of compounds **3** and **4** it is clear that pH must play an important role in their Fe(III) sensing capabilities. Additionally, Fe(III) is an acidic cation, and can form sparingly water-soluble hydroxides in basic medium. The effect of pH in the analytical characteristics of compounds **3** and **4** was further studied in systems with constant pH. Titration of **3** and **4** with $FeCl_3$ was realised in three systems with constant pH. While the titration curve obtained for **3** (Figure 5, top) was barely affected by pH (with a K_{sv} between 1.0×10^3 and 2.0×10^3 L mol^{-1}), compound **4** was highly influenced by pH (Figure 5, bottom). Moreover, at pH 6, this compound was very sensitive to fluorescence quenching by Fe(III) ($K_{sv} = 1.5 \times 10^4$ L mol^{-1}) while this

sensitivity was diminished at pH 2 and 4 (K_{sv} = 1.7 × 10^3 and 2.1 × 10^3 L mol^{-1}, respectively). This behaviour can be explained by the deprotonation of one cathecolic hydroxyl at pH 8 which seems to enhance the chelating properties of **4**. This pH-dependency might explain the lack of linearity of the titration curve obtained in the experiments without pH control, indicating that analytical application of such compounds should be realised in buffered systems.

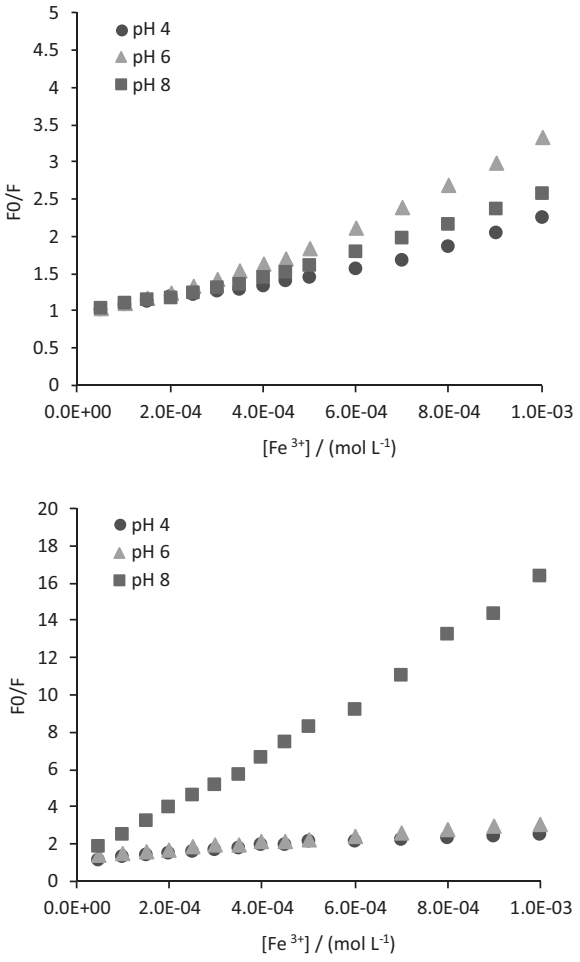

Figure 5. (Top) Stern-Volmer plots for the fluorescence quenching of **3** by $FeCl_3$ in three pH values showing a relative pH-independency. (Bottom) Stern-Volmer plots for the fluorescence quenching of **4** by $FeCl_3$ in three pH values. A significantly higher inclination at pH 6 reflects the higher sensitivity of the probe in this condition.

Conclusions

Herein we showed the synthesis and cation-recognition of four tetramethyl BODIPY fluorescent dyes. As expected, the BODIPY core itself (**1**) was not a good cation sensor, however the fluorescence of meso-substituted dyes containing a pyridyl (**3**) or a catecholyl (**4**) moiety were highly supressed by Fe(III) cations, while fluorescence of thienyl-substituted dye **2** was not influenced by any of the studied salts. The application of both compounds as analytical tools for the analysis of Fe(III) seems to be feasible depending on the analyte concentration. BODIPY**3** possesses a high fluorescence brightness, however, it is not highly sensitive to Fe(III), while the opposite happens with **4** - low fluorescence brightness associated with high sensitivity to Fe(III) cation. Additionally, pH was shown to be a very important parameter for the analytical performance of **4**, and acetate buffered solution at pH 6 seemed to be an optimal condition in our assays. Finally, it seems feasible that BODIPY-based ratiometric probes for Fe(III) could be obtained by the substitution of pyridyl and catecholyl groups in other ring positions.

Supplementary Information

Supplementary data (synthetic procedures, spectroscopic description, absorption and fluorescence spectroscopy and fluorescence quenching plots) are available free of charge at http://jbcs.sbq.org.br as PDF file.

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