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Wavelength-ratiometric near-physiological pH sensors based on 6-aminoquinolinium boronic acid probes

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Abstract

We describe the pH response of a set of isomeric water-soluble fluorescent probes based on both the 6-aminoquinolinium and boronic acid moieties. These probes show spectral shifts and intensity changes with pH, in a wavelength-ratiometric and colorimetric manner. Subsequently, changes in pH can readily be determined around the physiological level.

Although boronic acid containing probes are known to exhibit pH sensitivity along with an ability for saccharide binding/chelating, the new probes reported here are considered to be unique and show an unperturbed pH response, even in the presence of high concentrations of background saccharide, such as with glucose and fructose, allowing for the predominant pH sensitivity. The response of the probes is based on the ability of the boronic acid group to interact with strong bases like OH^-, changing from the neutral form of the boronic acid group, R-$B(OH)_2$, to the anionic ester, R-$B^-(OH)_3$, form, which is an electron donating group. The presence of an electron deficient quaternary heterocyclic nitrogen center and a strong electron donating amino group in the 6-position of the quinolinium backbone, provides for the spectral changes observed upon OH^- complexation. In addition, by comparing the results obtained with systems separately incorporating 6-methoxy or 6-methyl substituents, the suppressed response towards monosaccharides, such as with glucose and fructose, can clearly be observed for these systems. Finally we compare our results to those of a control compound, BAQ, which does not contain the boronic acid group, allowing a rationale of the spectral changes to be made.

Keywords: Wavelength-ratiometric pH sensing; Suppressed sugar response; Boronic acid probes

1. Introduction

The development of wavelength-ratiometric or lifetime based probes for the determination and/or quantification of a variety of analytes, offer intrinsic advantages for both chemical and biomedical fluorescence sensing [1,2]. Due to a variety of chemical, optical or other instrumental related factors, frequent calibrations of fluorescence intensity based sensing measurements are typically required. Unfortunately, while fluorescent probes are known to be useful in a variety of applications including fluorescence microscopy, fluorescence sensing, DNA technology, etc. most sensing fluorophores only display changes in intensity in response to analytes, and hence relatively few wavelength-ratiometric probes are available today [1,2]. Among these, a few wavelength-ratiometric probes for the sensing of pH, Ca^{2+}, Mg^{2+} Ag^+, Pb^{2+} and K^+ have been reported [3–9]. Subsequently there is great interest

Abbreviations: BA, boronic acid; BAF and BAFs, boronic acid containing fluorophore/s; BAQ, *N*-(benzyl)-6-aminoquinolinium bromide; *o*-, *m*-, or *p*-BAQBA, *N*-(2-, 3-, or 4-boronobenzyl)-6-aminoquinolinium bromide; HPTS, 1-hydroxypyrene-3,6,8-trisulfonate; LED, light emitting diode; SNAFL, seminaphthofluoresceins; SNARF, seminaphthorhodafluors; TCSPC, time-correlated single photon counting

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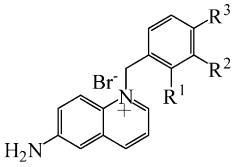


Boronic Acid Probe	R^1	R^2	R^3
***o*-BAQBA**	$B(OH)_2$	H	H
***m*-BAQBA**	H	$B(OH)_2$	H
***p*-BAQBA**	H	H	$B(OH)_2$
BAQ	H	H	H

Fig. 1. Molecular structure of BAQBA probes and the control compound BAQ.

in the development of new probes capable of sensing various analytes.

The requirements of pH sensing have driven the development of several notable dyes in the past such as fluorescein [10–13], HPTS (1-hydroxypyrene-3,6,8-trisulfonate) [14–17], SNAFL (seminaphthofluoresceins) and the SNARF (seminaphthorhodafluors) [1,18] pH probes. While these probes are widely used and amongst the main pH probes used today, they were historically developed due to the requirement for visible wavelength excitation, noting the expense and complexity of past UV excitation sources [1]. However, blue and UV, laser diode and light emitting diode sources are now readily available, allowing the possibility of using ratiometric probes at shorter wavelengths, which was previously not considered practical [1]. Subsequently, in a previous communication we described a wavelength-ratiometric pH sensor based on the boronic acid derivative 6-aminoquinolinium bromide (*o*-BAQBA) [19]. In this full paper we extend and characterize a full set of isomeric boronic acid containing quinolinium probes, Fig. 1, which shows spectral shifts and intensity changes as a function of pH, in both a ratiometric and colorimetric manner, enabling pH to be sensed at near-physiological levels. These probes are readily water-soluble, are simple to synthesize, and work in both an excitation and fluorescence emission ratiometric manner. It is worth noting that both fluorescein and HPTS can only be used in an excitation wavelength-ratiometric manner, with only one emission band observed at ≈510 nm [1]. With an isosbestic point at around 358 nm these probes can be readily used in a fluorescence ratiometric manner using a simple UV LED for excitation.

Additionally, these probes, having a boronic acid group, show suppressed sugar response unlike the 6-methoxy or 6-methyl quinolinium probes published previously by authors [20,21]. Although an explanation for the suppressed sugar response of these probes is not yet clear, the use of an amino group in the 6-position here is unique, potentially enabling for practical pH sensing in physiological fluids.

2. Experimental

2.1. Materials and preparation of the pH sensitive probes

All chemicals were purchased from Sigma–Aldrich at the highest purity available. Bromomethylphenylboronic acids were either purchased from Combiblocks or prepared from the corresponding methylphenylboronic acids using *N*-bromosuccinamide and a peroxide initiator as described in the literature [22]. The boronic acid containing fluorescent probes *o*-, *m*- and *p*-BAQBA and a control compound BAQ, were conveniently prepared using the following generic one step synthetic procedure, described below for the control compound BAQ. The corresponding *o*-, *m*-, or *p*-boronobenzyl bromides are employed instead of benzyl bromide to obtain the isomeric boronic acid derivatives *o*-, *m*- and *p*-BAQBA, respectively, Fig. 1. Equimolar amounts of 6-aminoquinoline and benzylbromide were dissolved in 10 mL dry acetonitrile in a 25 mL round-bottomed flask equipped with a magnetic stirrer. The reaction mixture was allowed to stir under an inert atmosphere for 24 h at room temperature. During this time a quantitative amount of quaternized salt was precipitated as a yellow solid. The solid product was recovered by filtration, washed several times with dry acetonitrile, and then dried under vacuum for 12 h. The obtained compounds were further purified using preparative TLC (silica gel, 20% methanol in dichloromethane). The absorption and emission spectral properties of the probes in water are shown in Table 1.

2.1.1. Analytical data for the compound BAQ

^{1}H NMR (D_2O), δ (ppm): 6.2 (s, 2H), 7.2–7.5 (m, 5H), 7.8 (m, 2H), 8.0 (d, 1H), 8.2 (d, 1H), 8.8 (m, 1H) and 9.1 (d, 1H). HRMS (FAB+, H_2O), *m*/*e*: calculated: 235.1235 (M^+), found: 235.1291 (M^+).

2.1.2. Analytical data for the compound o-BAQBA

^{1}H NMR (D_2O), δ (ppm): 6.5 (s, 2H), 7.1 (s, 1H), 7.4–7.5 (m, 2H), 8.0–8.3 (m, 4H), 8.5 (d, 1H), 8.95 (d, 1H) and 9.2 (d, 1H). HRMS (FAB+, H_2O), *m*/*e*: calculated: 279.1299 (M^+), found: 279.1305 (M^+).

Table 1
Spectral properties in water and dissociation constants, K_D, of *o*-, *m*- and *p*-BAQBA and control compound with glucose and fructose in pH 7.0 phosphate buffer

Probe	λ_{abs} (max) (nm)	λ_{em} (max) (nm)	Dissociation constants, K_D (mM)	
			Glucose	Fructose
[a]BAQ	391	546	–[a]	–[a]
o-BAQBA	381	546	1.0	16
m-BAQBA	381	546	17.1	22.2
p-BAQBA	398	560	2.5	8.7

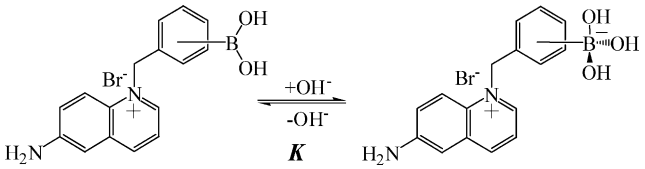
[a] BAQ cannot bind glucose or fructose due to it not having a boronic acid group, Fig. 1.

2.1.3. Analytical data for the compound m-BAQBA

^{1}H NMR (D_2O), δ (ppm): 6.2 (s, 2H), 7.3–7.5 (m, 2H), 7.6 (s, 1H), 7.7 (d, 1H), 7.9 (d, 1H), 8.0 (m, 2H), 8.2 (d, 1H), 9.0 (d, 1H) and 9.25 (d, 1H). HRMS (FAB+, H_2O), *m/e*: calculated: 279.1299 (M^+), found: 279.1302 (M^+).

2.1.4. Analytical data for the compound p-BAQBA

^{1}H NMR (D_2O), δ (ppm): 6.2 (s, 2H), 7.2 (d, 2H), 7.7 (d, 2H), 7.8 (t, 1H), 8.0–8.2 (m, 3H), 9.0 (d, 1H) and 9.15 (d, 1H). HRMS (FAB+, H_2O), *m/e*: calculated: 279.1299 (M^+), found: 279.1297 (M^+).



Scheme 1. Equilibrium involved in the interaction between the boronic acid group and OH^-.

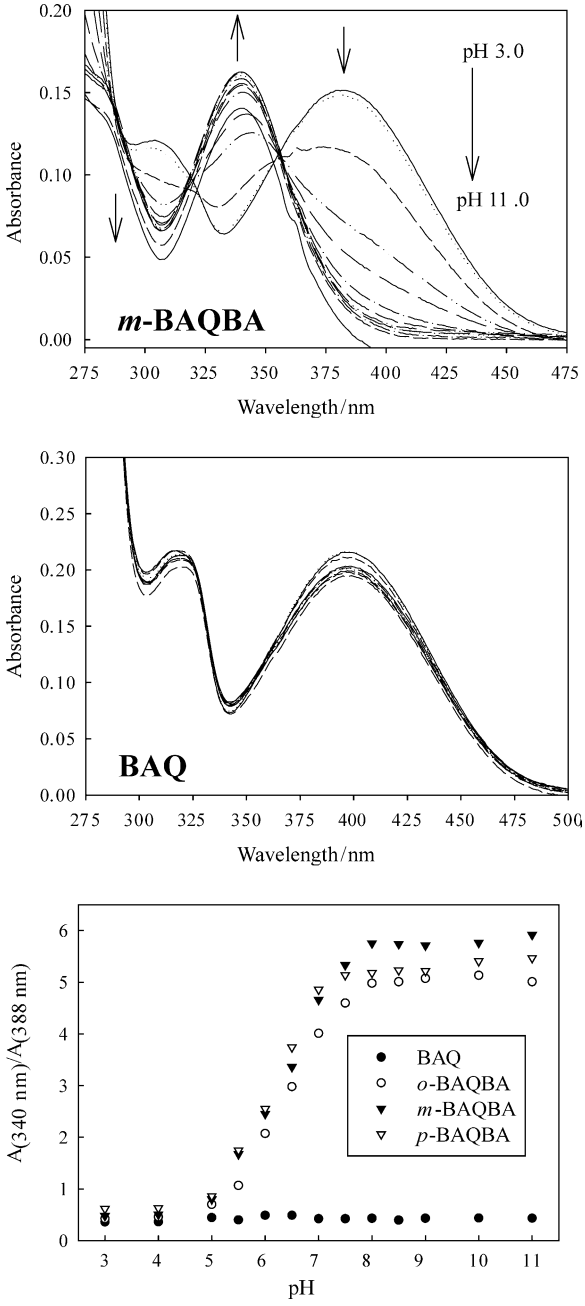


Fig. 2. Absorption spectrum of *m*-BAQBA (top) and BAQ (middle) with increasing pH and the corresponding absorption ratiometric plots obtained based on A_{340}/A_{388} bands (bottom).

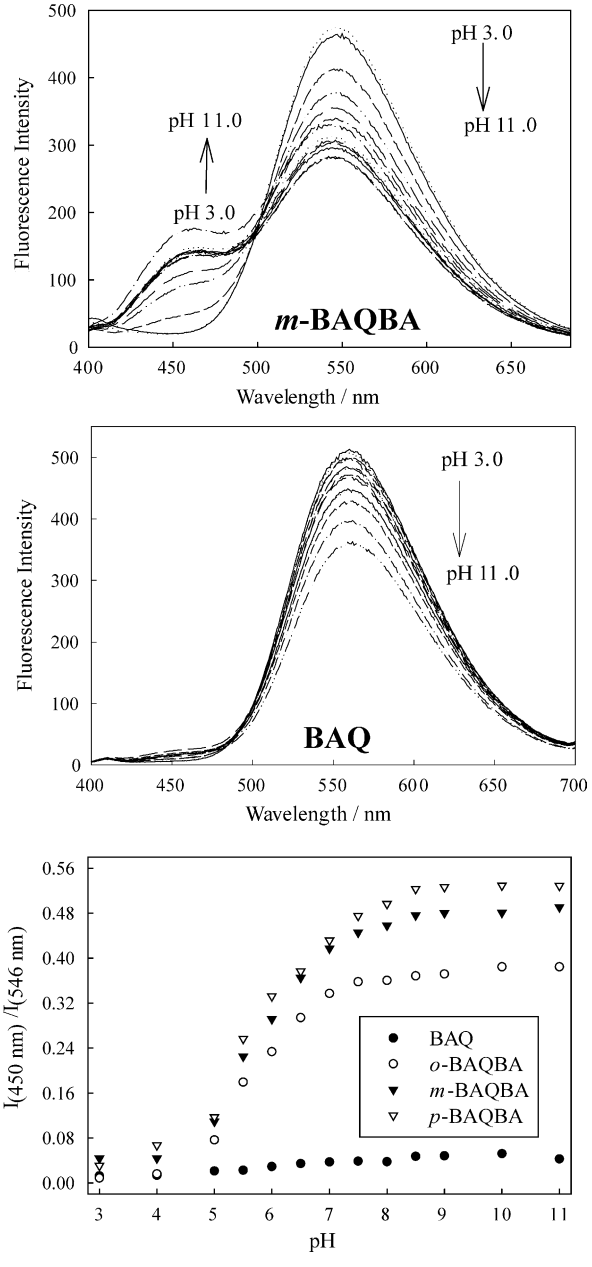


Fig. 3. Fluorescence emission spectra of *m*-BAQBA and BAQ with increasing pH, top and middle, respectively, and the corresponding ratiometric response based on the I_{450}/I_{546} bands.

2.2. Methods

All steady-state fluorescence measurements were undertaken in 4 cm × 1 cm × 1 cm fluorometric plastic cuvettes, using a Varian Cary Eclipse fluorometer, and all absorption measurements were performed using a Varian UV–vis 50 spectrophotometer.

2.3. Data analysis

Titration curves with pH were determined in buffer solution: pH 3 and 4 acetate buffer; pH 5–9 phosphate buffer and pH 10 and 11 carbonate buffer. Titration curves were fitted and pK_a (p$K_a = -\log_{10} K_a$) values were obtained using the relation:

$$I = \frac{10^{-\mathrm{pH}} I_{\mathrm{acid}} + K_a I_{\mathrm{base}}}{K_a + 10^{-\mathrm{pH}}} \quad (1)$$

where I_{acid} and I_{base} are the intensity limits in the acid and base regions, respectively.

Stability (K_S) and dissociation (K_D) constants were obtained by fitting the titration curves, with sugar, using the relation:

$$I = \frac{I_{\mathrm{min}} + I_{\mathrm{max}} K_S[\mathrm{sugar}]}{1 + K_S[\mathrm{sugar}]} \quad (2)$$

where I_{min} and I_{max} are the initial (no sugar) and final (plateau) fluorescence intensities of the titration curves, where $K_D = 1/K_S$.

3. Results and discussion

Fig. 2 shows the change in absorbance for both *m*-BAQBA (top) and BAQ (middle) as a function of pH. As the pH increases the absorption band at about 388 nm decreases (for BAQBAs), while the band at 340 increases. We can see significant changes in both the bands as the pH is altered. In contrast, BAQ shows only a slight decrease in absorption as the pH is increased, which is attributed to the lack of a boronic acid group, Fig. 1, which is well-known to complex strong bases such as the hydroxyl ion [23], Scheme 1. Similarly, the two other probes show very similar absorption spectral changes towards pH. Subsequently, Fig. 2(bottom) shows the absorption wavelength-ratiometric plots for the three isomeric BAQBAs and BAQ based on the A_{340}/A_{388} bands. The obtained pK_a values are in the range of 6.5–6.7 for all three probes, which is appropriate for near-physiological pH measurements. In contrast BAQ, as ex-

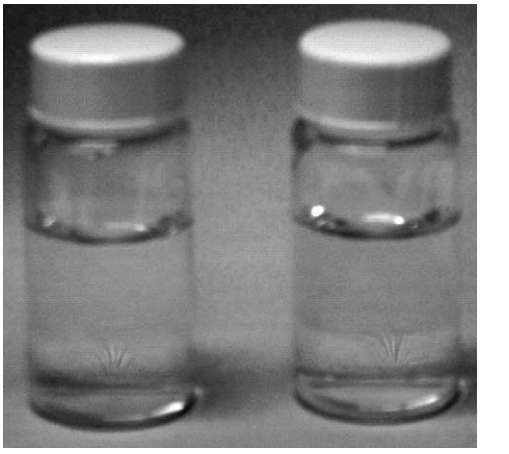


Fig. 4. Photograph of two vials containing equal concentrations of *m*-BAQBA in pH 5.0 and 8.0 phosphate buffer left and right, respectively.

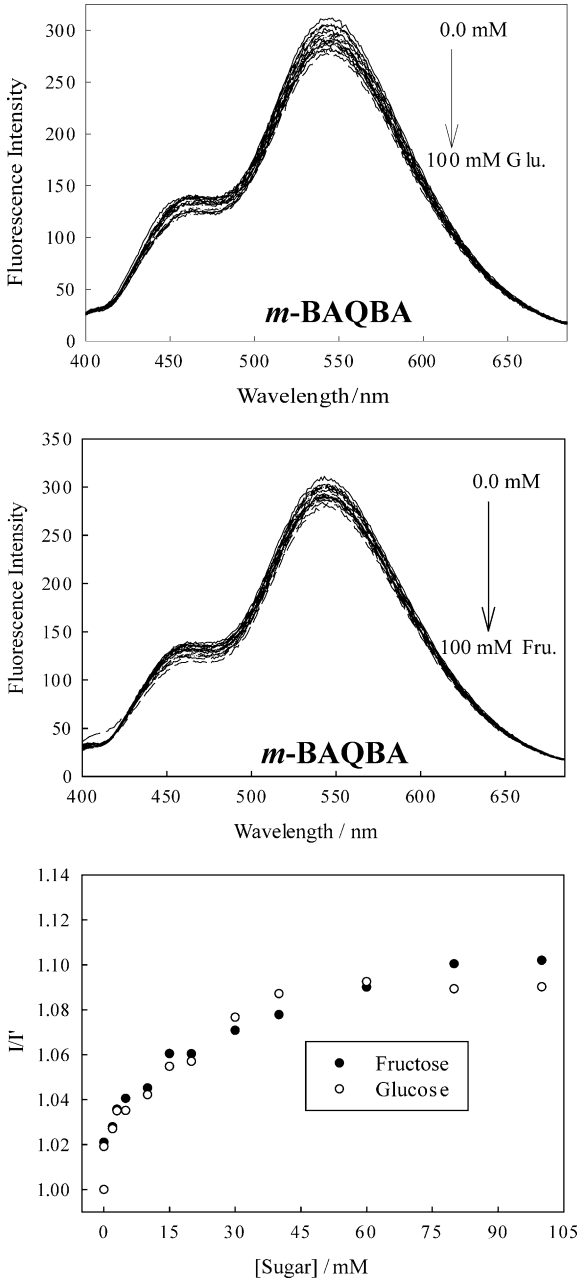


Fig. 5. Fluorescence emission spectra of *m*-BAQBA with increasing sugar concentrations, top and middle, and the corresponding normalized plots, where I and I' are the intensities in the presence and absence of sugar, respectively, bottom.

pected, shows relatively very little response to changes in pH.

The fluorescence emission spectra of *m*-BAQBA shows similar wavelength-ratiometric behavior, Fig. 3(top), where $\lambda_{ex} = 358$ nm, i.e. at the isosbestic point. As the pH increases we typically see a decrease in fluorescence intensity of the 546 nm band, which is the uncomplexed or acid form, while the band at 450 nm increases (complexed or ester form). In contrast, BAQ shows a simple decrease in fluorescence intensity as the pH is increased, Fig. 3(middle), which is attributed to the known quenching of the quinolinium nucleus by the hydroxyl ion at high pH [24].

For the data shown in Fig. 3 we constructed the fluorescence emission ratiometric response, Fig. 3(bottom). It is interesting to compare the dynamic sensing range towards pH shown in both Figs. 2 and 3. Clearly a greater change is observed for the ratiometric absorption measurements, reflecting the differences in extinction coefficients and quantum yields of the OH^- unbound and bound forms, respectively. Further, a change in pH readily leads to a most notable change in color, as evident by the changes in the absorption spectra

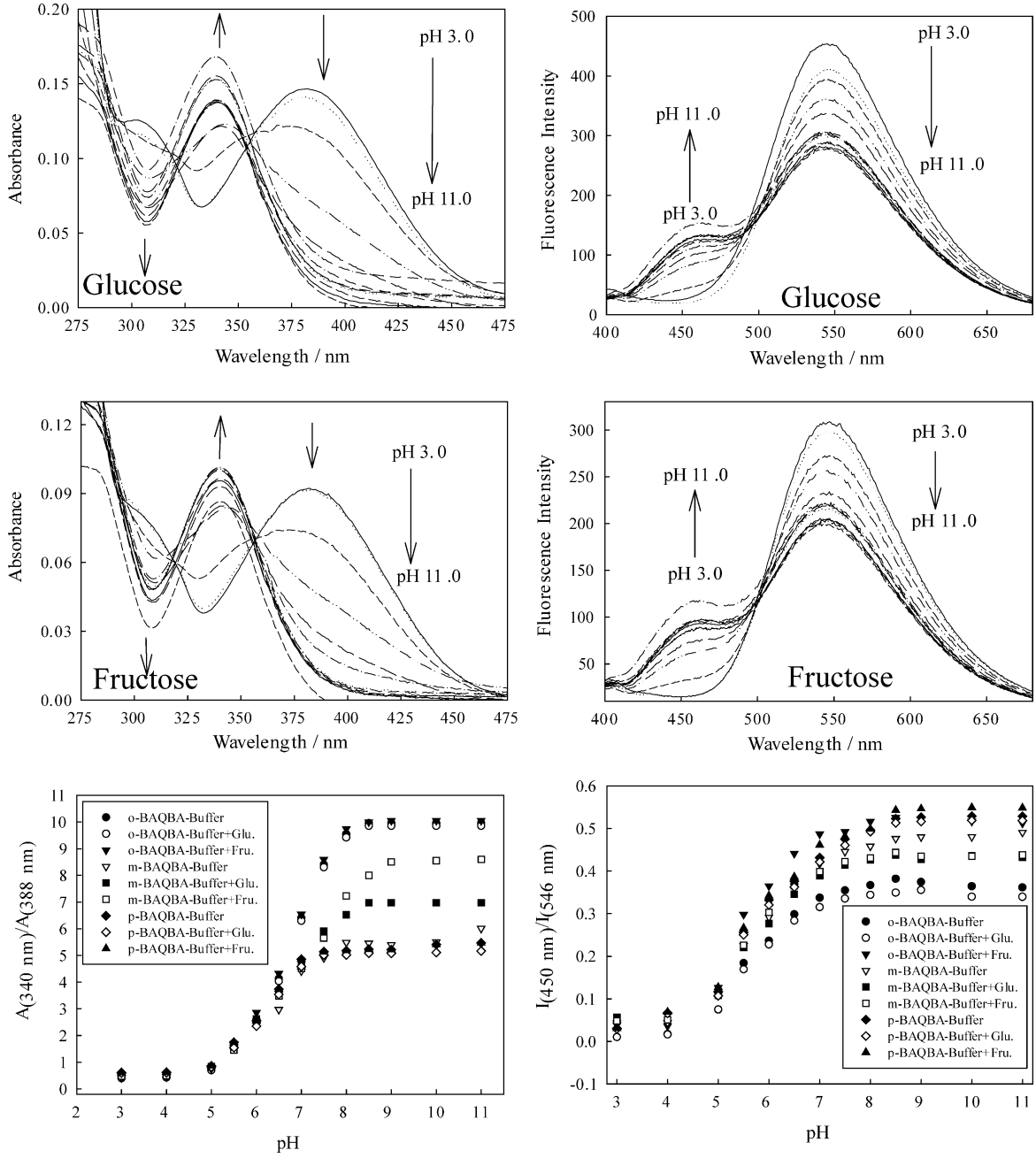


Fig. 6. Absorption and fluorescence emission ($\lambda_{ex} = 358$ nm) for *m*-BAQBA in the presence of glucose, top, fructose, middle, and the respective ratiometric plots for all three isomers both with and without sugars, bottom panels.

in Fig. 2(top) suggesting that the isomeric probes could be widely used as colorimetric type probes. Fig. 4 shows a photograph of two vials containing equal concentrations of *m*-BAQBA in pH 5.0 and pH 8.0 phospahate buffer, Fig. 4(left and right), respectively. One can see a noticeable color change from yellow in acidic media to colorless in basic pH solutions.

The affinity of boronic acid for diols is well-known [23–27]. Subsequently, we tested the response of BAQBA towards both glucose and fructose. No response was observed, as expected, for BAQ (data not shown). A comparison of Fig. 5(top and middle), shows a similar affinity of BAQBA for both fructose and glucose. While the emission spectra of BAQBA show similar bands, in the presence of OH^-, i.e. at 450 and 546 nm, the bands do not show increasing and decreasing intensities in the presence of sugar, but instead simply show an overall intensity decrease as sugar concentration increases. Subsequently, Fig. 5(bottom) shows the response curves to sugars normalized by the response of BAQBA in the absence of sugar, I'. Using Eq. (2), we were able to determine the binding (stability) constants for glucose and fructose respectively, Table 1. In contrast these binding constants can be tuned much higher by replacing the 6-amino group to a less efficient electron donating group, or indeed, electron withdrawing groups as mentioned earlier [20,21].

With a slight sugar response evident (relevant to similar structures but with different substituents [20,21]), we tested the ability of BAQBA to sense pH in the presence of 50 mM glucose and 50 mM fructose, Fig. 6. As we can see, the boronic acid containing quinolinium type fluorophores respond well towards pH in the presence of the sugar interferents. Fig. 6(bottom left and right) shows the ratiometric plots, where we can see that 50 mM sugar has little effect on the probe's overall response to pH, by comparing with the buffer (no sugar) titration curve. While a 50 mM fructose background has a slightly greater effect (lower K_D) as shown in Fig. 6(bottom), it should be noted that fructose levels in blood are typically 10–100-fold lower for a healthy person than used here, hence BAQBA is likely to be suitable for physiological pH measurements.

4. Conclusions

We have characterized three new water-soluble probes, BAQBAs, with regard to pH. These new probes respond well to changes in pH around 7. In addition we have shown that the pH can readily be determined in a background of 50 mM sugar allowing their potential use in physiological type measurements, in both an excitation and emission ratiometric manner and also colorimetrically. While other boronic acid containing fluorophores are well-known to be pH sensitive, these probes show a reduced sugar response as compared to phenyl boronic acid. These combined features make these probes ideal probes for near-physiological pH determination.

Acknowledgements

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