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Wavelength–Ratiometric Probes for the Selective Detection of Fluoride Based on the 6-Aminoquinolinium Nucleus and Boronic Acid Moiety

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Herein we report a set of new water-soluble fluorescent probes (*N*-boronobenzyl-6-aminoquinolinium bromides, BAQBAs) sensitive to aqueous fluoride. These probes shows spectral shifts and intensity changes in the presence of fluoride, in a wavelength ratiometric and colorimetric manner, enabling the detection of fluoride concentrations at visible wavelengths, in the concentration range ≈1-300 mM. Although the sensing mechanism is different for fluoride as compared to the other halides, we have tested the utility of these probes towards the other halides, and the results reveal that the BAQBAs are in fact potential candidates towards the sensing of the *all* the halides, but in different concentration ranges. As the probes are based on the boronic acid moiety, which is a well-known fluoride and sugar chelator group, we have investigated the response of sugars (such as glucose and fructose, which are present in biological fluids and foodstuffs) as interferences in fluoride detection using these probes. Interestingly, the BAQBAs show a suppressed sugar response potentially allowing for the predominant fluoride sensitivity. In addition to physiological sugars, we also have assessed the response of aqueous halides as potential interferents, or indeed analytes to be sensed, and show that the new boronic acid containing probes respond well to aqueous fluoride in the presence of a high background of other species, such as in a biological cocktail of 50 mM Glucose, 50 mM aqueous Chloride and 5 mM Fructose.

KEY WORDS: Boronic acid containing fluorophores; fluoride; monosaccharides; halides.

INTRODUCTION

The development of selective and sensitive chemisesensors for anions is an important topic of current research [1]. The reason for this interest is the importance of the detection and quantification of anions in disciplines such as biology and environmental chemistry. Among the anion sensors developed, those displaying an optical signal are of special interest [2]. We have been involved in the development of optical-chemosensors/fluorosensors for various anions including hydroxyl, cyanide, etc., and currently we are interested in the development of fluoride selective sensors based boronic acid fluorophores, which show a wavelength ratiometric response with fluoride.

The importance of fluoride detection and quantification can quite simply be judged by the vast amount of

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ABBREVIATIONS: BA, boronic acid; BAF and BAFs, boronic acid containing fluorophore(s); BAQ, *N*-(benzyl)-6-aminoquinolinium bromide; BAQBA, *N*-(boronobenzyl)-6-aminoquinolinium bromide; FWHM, full width at half maximum; I_{min}, initial fluorescence intensity of BAQBA in the absence of sugar or fluoride; I_{max}, final plateau fluorescence Intensity of BAQBA in the presence of sugar or fluoride; I_0, fluorescence intensity in the absence of quencher; I_Q, fluorescence intensity in the presence of a quencher, Q; K_{sv}, Stern–Volmer quenching constants; LED, light emitting diode; TCSPC, time-correlated single photon counting.

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literature published over the last 20 or so years [3–7]. Fluoride is present in biological fluids and tissues and especially in bone and tooth. Fluoride is easily absorbed but is slowly excreted from the body, which can result in chronic poisoning, acute gastric and kidney disorders, dental and skeletal fluorosis and even death. Fluoride can be accurately determined using fluoride-ion selective electrodes [8], spectrophotometry [9], gas chromatography [10] and even colorimetrically using boronic acid chemistry [11,12], although such systems are poisoned by the presence of sugars such as by glucose or fructose. It is due to the well-known high affinity between diol containing compounds and the boronic acid moiety that has lead to the development of carbohydrate sensors [13–19] chromatographic materials [20] and many glucose sensors [21–27].

One particular halide sensing mechanism that has attracted significant attention has been the *quenching of fluorescence*, which was first described by George Stokes as early as 1869, when he observed the fluorescence of quinine in dilute sulphuric acid was reduced after the addition of hydrochloric acid, which is now commonly attributed to the dynamic quenching by aqueous chloride ions [3]. Subsequently, there have been many halide sensitive probes derived from quinine derivatives, most notably using the quinolinium nucleus, which display a modest sensitivity for chloride due to its relatively long lifetime when quaternised that affords for chloride collisional quenching [3]. While the quinoline nucleus is only sparingly water soluble, its quaternised products are readily water-soluble and have subsequently been the transduction elements in many chloride/halide sensors [3,28–30].

It is not just the physiological significance of chloride that drives workers to mostly report the chloride sensitivity of some fluorescent probes, but because the quenching of fluorescence is not a selective process, and any fluorophore quenched by chloride is also quenched by bromide to a greater extent and also by iodide to an even greater extent. Therefore, for dynamic quenching, the sensitivity of fluorophores to halide is well known to be $I^- > Br^- > Cl^-$. The explanation of this effect lies in the fact that the efficiency of intersystem crossing to the excited triplet state, promoted by spin-orbit coupling of the excited singlet fluorophore and halide upon contact, depends on the mass of the quencher atom, hence the expression "*heavy-atom effect*" is sometimes used [3,31]. It is for this reason that fluoride does not typically quench fluorescence. As such, traditional halide sensitive probes are not very sensitive to fluoride and are therefore not suited for detecting fluoride <50 mM [3]. Only a few fluorescent probes can be found in the literature which are sensitive to fluoride [31], all based on either the benzene or naphthalene backbone and therefore showing absorption in the deep UV

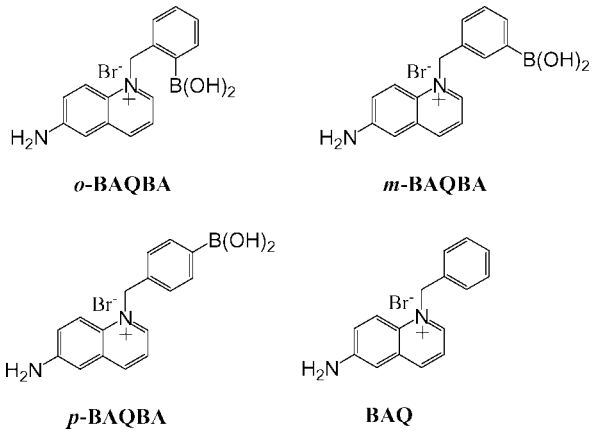

Fig. 1. Molecular structure of the boronic acid probes, *o*-, *m*- and *p*-BAQBA, and the control compound BAQ. BAQBA: *N*-(boronobenzyl)-6-aminoquinolinium bromide, BAQ: *N*-benzyl-6-Aminoquinolinium bromide.

(≈270 nm), which is not practical for many sensing applications [3,31,32]. Although water soluble sensor systems are deemed important, a few systems based on urea and thiourea based fluorophores were reported recently which show an optical response towards fluoride in organic media [33,34]. The new set of probes described in this paper are readily water soluble and indeed show a selective fluoride response.

Subsequently we have quaternized the halide sensitive 6-aminoquinoline heterocyclic nucleus with *o*-, *m*- and *p*-(bromomethyl)phenylboronic acid and benzyl bromide, to afford novel fluoride sensitive isomeric probes *o*-BAQBA, *m*-BAQBA, *p*-BAQBA, and a fluoride insensitive control compound BAQ, respectively. Molecular structures of the probes used in this study are shown in Fig. 1. The resultant boronic acid containing fluorophores (BAFs) show both an absorption and emission wavelength ratiometric response to fluoride at mM concentrations, providing a much better alternative to the low extent of fluoride collisional quenching which is inherent to most fluorophores. The origin of the fluoride response is due to the boronic acid group's ability to interact with hard bases such as F^-, as shown in Fig. 2, to form the trifluoroboronate anion [R-B($^-$)-F_3], which is an electron donating group, the extent of which is dependent on the amount of fluoride present, Fig. 2 [35]. This in turn interacts with the electron deficient quaternary heterocyclic nitrogen center of the quinolinium backbone, where the interaction is thought to provide, due to redistribution of the electronic densities of the fluorophore moiety, the wavelength shifts and intensity changes observed.

On going studies in our laboratory have shown that by replacing the 6-amino group on the quinolinium backbone

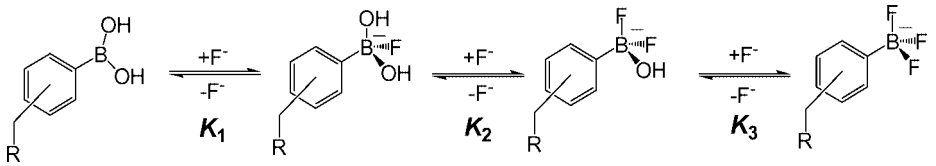

Fig. 2. Equilibrium involved in the interaction between the boronic acid group and fluoride.

with less efficient electron donating groups, e.g. —OCH_3, CH_3 etc, in essence making the nitrogen center relatively more electron deficient, the dual emission bands at 450 and ≈546 nm are lost for a single emission band at ≈450 nm, eliminating the possibility of a ratiometric response. In contrast, a greater electron deficient nitrogen center provides for a higher affinity for monosaccharides (data not shown) [36]. Hence our unique choice of backbone substituents affords a high fluoride affinity with relatively little sugar response, even in the case of fructose, which is well-known to have a high boronic acid affinity [21–27]. In this paper we characterize the fluoride response of BAQBAs in the presence of potential interferences such as sugar and other halides and, also the response towards the halides.

EXPERIMENTAL

Materials

All chemicals were purchased from Sigma. The preparation of BAQBAs, Fig. 1, has recently been reported [37].

Methods

All solution absorption measurements were performed in a 4*1*1 cm quartz cuvette (Starna), using a Cary 50 Spectrophotometer from Varian. Fluorescence spectra were similarly collected on a Varian Eclipse spectrofluorometer with solution optical densities less than 0.2 and $\lambda_{ex} = 358$ nm.

Stability (K_S) and/or dissociation constants (K_D) were obtained by fitting the titration curves with sugar or fluoride to the relation:

$$I = \frac{I_{min} + I_{max} K_S[\text{Analyte}]}{1 + K_S[\text{Analyte}]} \tag{1}$$

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

Stern-Volmer constants, K_{SV}, (M^{-1}) for aqueous halide, (Cl^-, Br^- and I^-) were obtained by plotting I_0/I_Q and τ_0/τ_Q as a function of halide concentration [Q], where I_0, τ_0 and I_Q, τ_Q are the intensities, mean lifetimes in the absence and presence of quencher, Q, respectively:

$$\frac{I_0}{I_Q} = \frac{\tau_0}{\tau_Q} = 1 + k_q \tau_0 [Q] \tag{2}$$

$$K_{SV} = k_q \tau_0 \tag{3}$$

Time-resolved intensity decays were measured using reverse start-stop time-correlated single-photon counting (TCSPC) with a Becker and Hickl gmbh 630 SPC PC card and a un-amplified MCP-PMT. Vertically polarized excitation at ≈372 nm was obtained using a pulsed LED source (1 MHz repetition rate) and a dichroic sheet polarizer. The instrumental response function was ≈1.1 ns, fwhm. The emission was collected separately at the magic angle (54.7°), using two long pass filters (Edmund Scientific), which cut off wavelengths below 416 and 546 nm respectively, allowing the lifetimes of the fluoride free and bound-forms to be realized. The use of a pulsed 372 nm LED provided for excitation near-to the isosbestic point at 358 nm, Fig. 3.

The intensity decays were analyzed in terms of the multi-exponential model:

$$I(t) = \sum_i \alpha_i \exp(-t/\tau_i) \tag{4}$$

where α_i are the amplitudes and τ_i the decay times, $\sum \alpha_i = 1.0$. The fractional contribution of each component to the steady-state intensity is given by:

$$f_i = \frac{\alpha_i \tau_i}{\sum_i \alpha_i \tau_i} \tag{5}$$

The mean lifetime of the excited state is given by:

$$\bar{\tau} = \sum_i f_i \tau_i \tag{6}$$

and the amplitude-weighted lifetime is given by:

$$\langle \tau \rangle = \sum_i \alpha_i \tau_i \tag{7}$$

The values of α_i and τ_i were determined by non-linear least squares impulse reconvolution with a goodness-of-fit χ^2_R criterion.

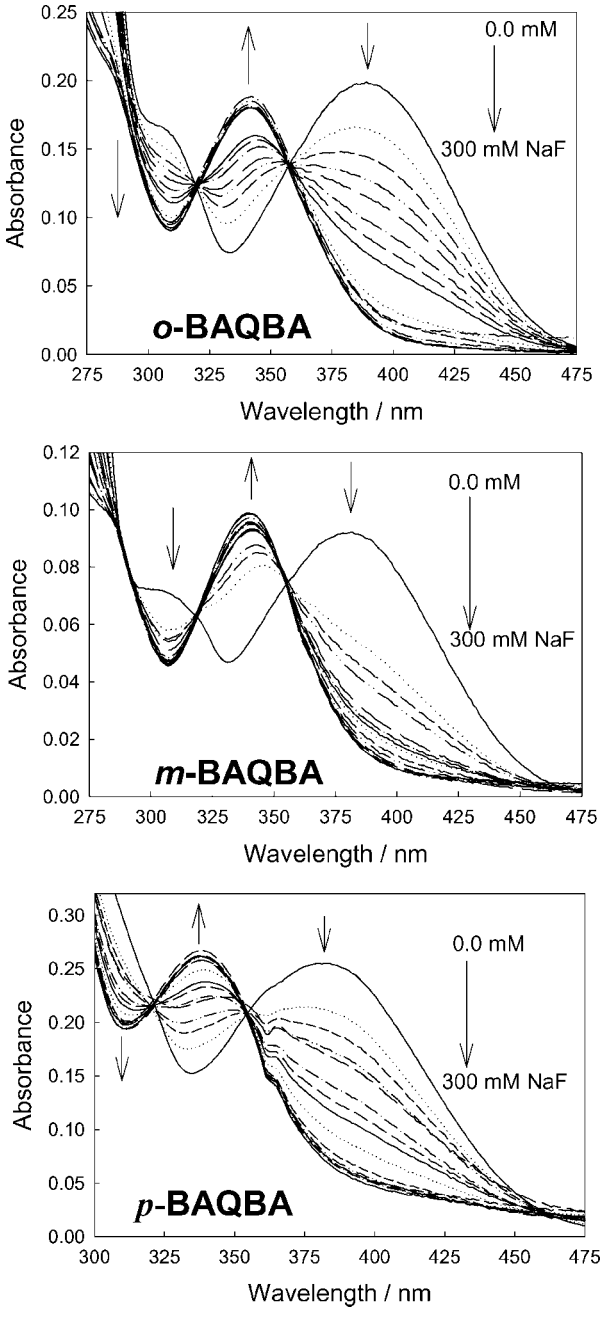

Fig. 3. Absorption spectra of *o*-, *m*- and *p*-BAQBA (*Top, Middle* and *Bottom*, respectively) in water with increasing concentrations of aqueous sodium fluoride.

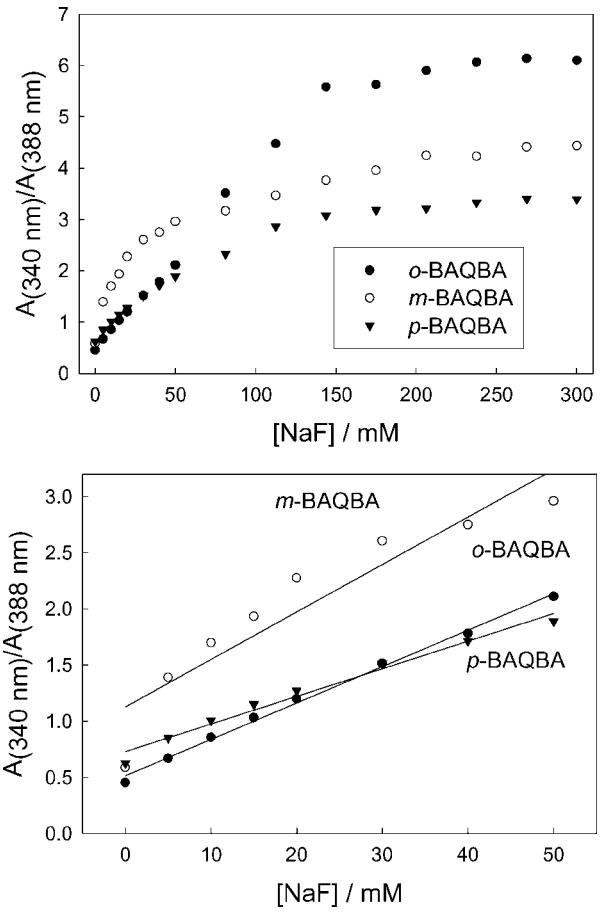

Fig. 4. Absorption wavelength ratiometric plots for BAQBAs with increasing fluoride concentrations based on the $A_{(340)}/A_{(388)}$ nm bands (*Top*), and the corresponding plots in the low fluoride concentrations range (*Bottom*).

RESULTS

Figure 3 shows the absorption spectra of *o*-, *m*- and *p*-BAQBA in water with increasing concentrations of fluoride. As the concentration of fluoride increases, the absorption band at ≈388 nm decreases while the band at ≈342 nm increases. We can also see a significant change in the 388 nm band with each 5 mM fluoride increment. As it is shown in the figure, all three isomers show very similar absorption spectral changes with increasing concentrations of fluoride. Subsequently, Fig. 4 shows the absorption wavelength ratiometric plot of the 342 and 388 nm bands for all three isomers. A linear response to fluoride was typically observed up to about 100 mM fluoride, Fig. 4 - bottom. The non-linear response thereafter is thought to be due to both the third order binding as depicted in Fig. 2, and the subsequent saturation of the probes. The response of *o*- and *p*-BAQBAs is similar, and shows about a 2 fold change in A_{342}/A_{388} with the addition of 50 mM fluoride. The *meta*-isomer shows a better initial response with about a 3 fold change in A_{342}/A_{388} by the same amount of fluoride. The dissociation constants for three isomers with fluoride obtained using Eq. (1) are shown in Table I.

Table I. Dissociation Constants, K_D, of *o*-, *m*- and *p*-BAQBA, with Glucose, Fructose and Fluoride

Probe	Glucose, K_D (mM)	Fructose, K_D (mM)	Fluoride, K_D (mM^3)
BAQ	—[a]	—[a]	—[a]
o-BAQBA	1.0	16	40.0 (140)[b]
m-BAQBA	17.1	22.2	10.8 (33.8)[b]
p-BAQBA	2.5	8.7	55.6 (88.5)[b]

[a]BAQ can not bind glucose, fructose or fluoride due to it not having a boronic acid group, Fig. 1.
[b]The data shown in parenthesis are obtained from the absorption ratiometric plots.

The fluorescence emission spectra of *m*-BAQBA in water with sodium fluoride is shown in Fig. 5 - Top. The fluorescence emission of all the BAQBAs show similar behavior, where the intensity of the band at 546 nm decreases while the emission band at 450 nm increases, noting the visible emission of the fluoride complexed form at 450 nm as compared to the red-shifted emission of the uncomplexed form at 546 nm. This colorimetric response can also be seen visually in Fig. 6, where the three vials from left to right contained 0, 50 and 300 mM fluoride respectively. For the data shown in Fig. 5 - top, we constructed the fluorescence emission ratiometric response based on $I_{(450)}/I_{(546)}$ nm bands, Fig. 5 - bottom. Using Eq. (1) we were again able to determine the dissociation constants for Fluoride, and the corresponding values are shown in Table I. Interestingly, the ratiometric response plots, Figs. 4 and 5, both show different dynamic sensing ranges, reflecting the differences in extinction coefficients and quantum yields of the fluoride unbound and bound forms respectively. The K_D values for *o*- and *p*-BAQBAs are similar while that of *meta*-isomer is relatively less. Interestingly, the K_D values obtained from Fig. 4 bottom were higher than that from Fig. 5, and this may be partially due to the data fitting, in light of the non-systematic increase, especially for *o*-BAQBA shown in Fig. 4 bottom, i.e. a deviation after 50 mM Fluoride.

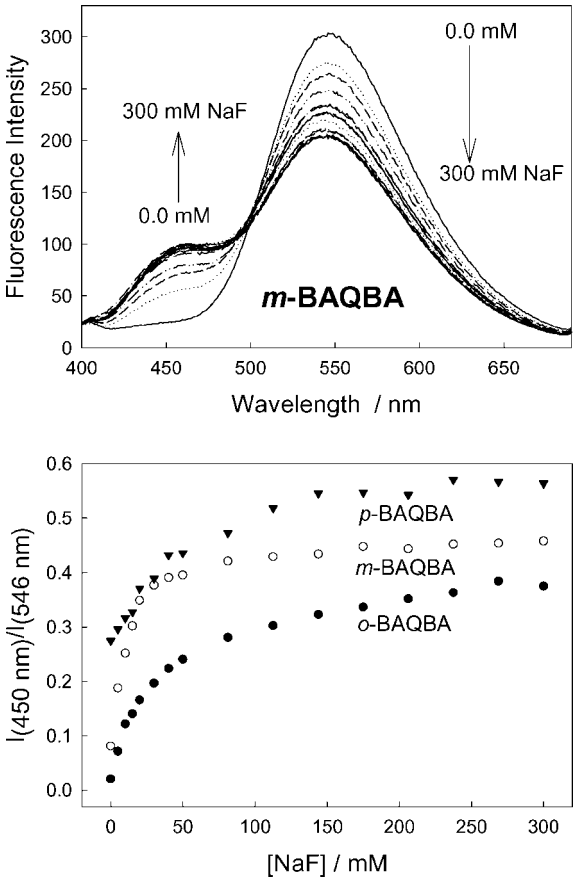

Fig. 5. Emission spectra of *m*-BAQBA in water with increasing concentrations of aqueous sodium fluoride (*Top*). $\lambda_{ex} = 358$ nm. The two other isomeric compounds show a similar emission response to aqueous fluoride. The respective wavelength ratiometric plots based on $I_{(450)}I_{(546)}$ nm bands (*Bottom*).

The affinity of boronic acid for diols is well known [13–27], hence for any fluoride sensor based on the boronic acid moiety, it is important to characterize the monosaccharide response. Subsequently, we tested the response of BAQBAs towards both glucose and fructose. A representative emission spectral response for *m*-BAQBA in pH 7 phosphate buffer with glucose and fructose is shown in Fig. 7. The corresponding I'/I plots at 546 nm with increasing sugar concentrations are shown in Fig. 7 - Bottom. The two other isomers show a very similar response towards glucose and fructose. As it can be seen from the figure, the BAQBAs show a similar affinity for fructose as compared to glucose. The reason for the increased glucose selectivity is not clear at the moment. Similarly to fluoride, we were able to determine the disossociation constants for the probes with glucose and fructose using Eq. (1), and the obtained values are shown in Table I.

With a very slight sugar response evident, we tested the ability of BAQBAs to sense aqueous fluoride in the presence of both 100 mM glucose and 100 mM fructose. Fig. 8-Top Left shows the absorption spectra of *m*-BAQBA in the presence of 100 mM glucose with increasing fluoride concentration. Figure 8-Top Right, Bottom Left and Right shows the ratiometric plot for *o*-, *m*- and *p*-BAQBA, in water respectively, having either 0 or 100 mM glucose or fructose. We typically see little interference on the fluoride response, especially with the *ortho*-isomer in the presence of sugar, <100 mM. Even at higher fluoride concentrations, the BAQBAs respond well in the presence of sugar. It should be noted that the concentrations of glucose

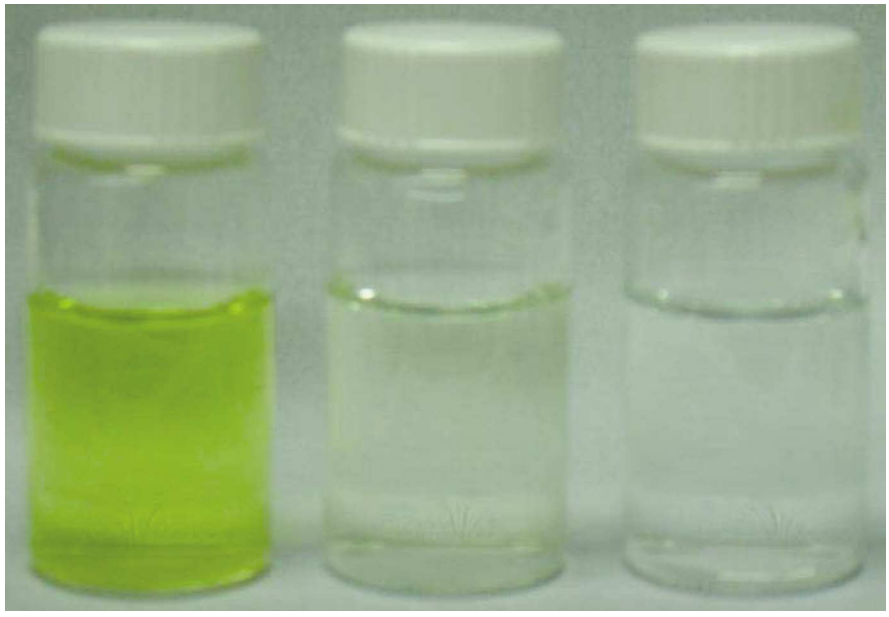

Fig. 6. Photograph of three vials containing equal concentrations of *m*-BAQBA and 0, 50 and 300 mM fluoride, *left* to *right*, respectively. Very similar findings were observed for all three isomeric boronic acid probes.

and fructose in blood for a normal healthy person are 2–8 mM and ≈1 mM respectively, and in food products can be much higher [3]. The apparent deviations from the trends shown at ≈60 mM fluoride in Fig. 8 - Bottom is not a measurement artifact, but is indeed attributed to the complex binding of sugars and anions with boronic acid.

While the physiological fluoride levels for healthy people have been reported to be as low as 20–60 μg/L [4], it is the ability to track elevated fluoride levels that has been the focus of much research, as high fluoride levels can lead to poisoning by the blockage of enzymatic functions [3]. We do not expect these new fluorescent probes will sense aqueous fluoride, 2 orders of magnitude below its dynamic sensing range, nor are fluoride levels during poisoning likely to increase 1000-fold. However, these probes do offer the unique opportunity to ratiometrically or colorimetrically determine fluoride levels in the 1-300 mM range in the presence of sugars, and as we will later show, in the presence of other halides also. To the best of our knowledge, these probes are amongst the most sensitive reported for fluoride to date [3], and may subsequently therefore find applications for food products or waste waters, where substantially elevated F^- levels may be present and adverse to human health.

Figure 9 shows fluorescence emission spectra for *m*-BAQBA for increasing fluoride concentrations, in the presence of a 100 mM sugar background. By comparing with the response in water (no sugar) we can clearly see that the presence of sugar has little effect on overall fluoride response of the probes, especially for *o*-BAQBA, again reflecting the boronic acid moiety's higher binding affinity for F^- as compared to monosaccharides.

In addition to fluoride we tested the response of BAQBAs towards aqueous chloride, bromide and iodide, as the quinolinium nucleus is well-known to be susceptible to heavy atom type collisional quenching [3]. As expected the steady-state Stern-Volmer constant for iodide was the largest, with bromide and chloride very similar and substantially smaller than for iodide, Table II. We additionally measured the lifetime/s of *o*-BAQBAs in the presence of halide to determine the dynamic quenching components, Table II. Interestingly the dynamic K_{SV} values were slightly smaller, ≈27, 0.4 and 0.3 M^{-1} for I^-, Br^- and Cl^- respectively, suggesting a small, but measurable, halide static quenching component. A very similar finding has recently been reported by Geddes *et al*. [30] for other quinolinium type fluorophores, and this was attributed to the presence of the halide counter ion. The dynamic quenching constants for the *meta*- and *para*-isomers were not measured but are quite simply thought to reflect similar findings as compared to the *ortho*-isomer.

Our time-resolved studies have revealed that *o*-BAQBA is bi-exponential in Millipore water with lifetimes of ≈1.99 and 3.28 ns, with amplitudes of 0.675 and 0.325 respectively, c.f. Eq. (4), Table III. Both the mean

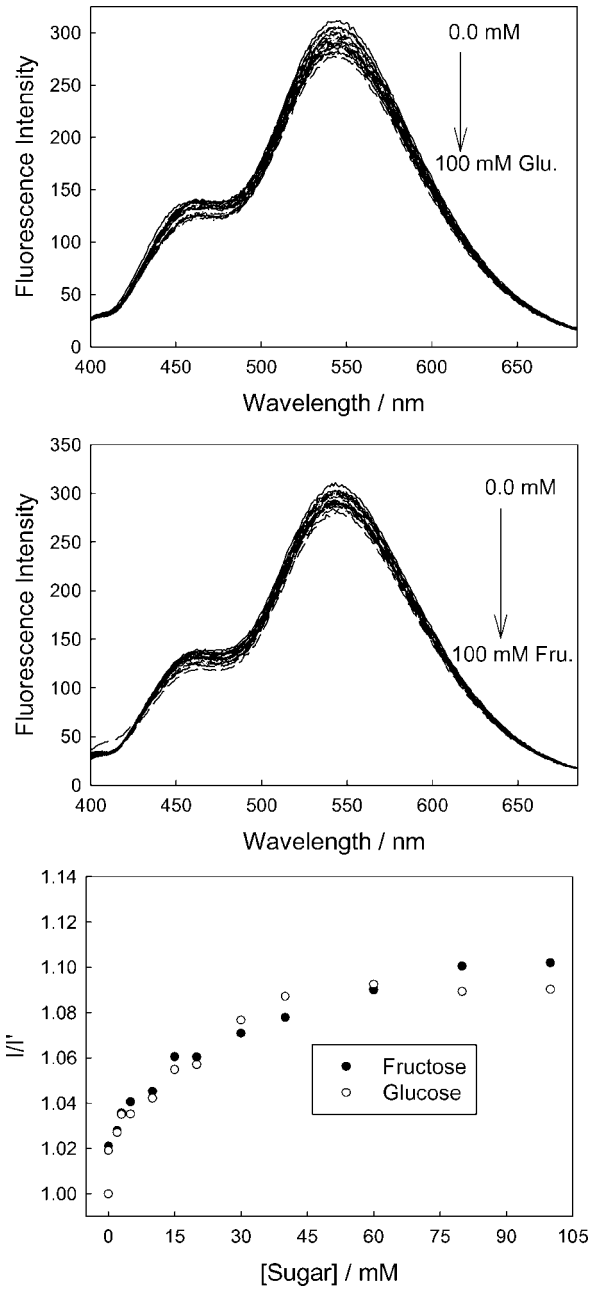

Fig. 7. Emission spectra of *m*-BAQBA in pH 7.5 phosphate buffer with increasing concentrations of glucose (*Top*) and fructose (*Middle*), and the corresponding I'/I plots (*Bottom*), where I' and I are the intensities in the absence and presence of sugars, respectively.

and amplitude weighted lifetimes were found to be 2.56 and 2.41 ns respectively. Interestingly, the low response of BAQBA towards aqueous halide can be attributed to its reduced mean lifetime as compared to other quinolinium type fluorophores [3,28–30], noting the weak response towards aqueous Cl^-, which could be particularly advantages when using BAQBA in physiological or environmental fluids.

We measured the lifetimes of *o*-BAQBA separately through two long pass filters, 416 and 546 nm, to investigate the lifetime changes during fluoride complexation. Table III shows that the lifetime of the uncomplexed fluoride probe form, i.e. visualized using the 546 nm long pass filter, remains biexponential upon fluoride addition, where the mean lifetime changes from 2.52 → 2.32 ns by the addition of 300 mM NaF. However, when we consider the lifetime of both bound and unbound forms using the 416 nm long pass filter, we find the intensity decay data is best described by a 3-exponential function with a short component now evident, which increases in amplitude (α_1) as the concentration of fluoride is increased. This short component, <200 ps, is attributed to the fluoride bound form, shown after 358 nm steady-state illumination at 450 nm in Fig. 5. As expected the amplitude weighted lifetime changes notably, from 2.41 → 1.84 ns, an ≈25% change. This suggests the possibility of lifetime based fluoride sensing using these new fluorescent probes. Similar results were found for all three BAQBA probes.

Finally, we tested the response of BAQBAs towards aqueous fluoride in the presence of a constant 50 mM glucose, 5 mM fructose and a 50 mM chloride background concentration, Fig. 10. The ratiometric plot of the I_{450}/I_{546} fluorescence emission bands shows the utility of these probes in that they can readily determine fluoride concentrations in a high quenching background of potential interferents. Similarly, the absorption ratiometric response based on A_{342}/A_{388} of all three probes with fluoride is shown to be ≈ 4–6-fold with 300 mM fluoride, Fig. 10 - inserts.

DISCUSSION

It is widely recognized that ratiometric or lifetime-based methods offer intrinsic advantages for both chemical and biomedical sensing [31,38]. Fluorescence intensity measurements are typically unreliable away from the laboratory and can require frequent calibration/s due to a variety of chemical, optical or other instrumental related factors [31,38]. Unfortunately, while fluorescent probes are known to be very useful for many applications, such as in fluorescence microscopy, DNA technology and fluorescence sensing, most sensing fluorophores only display changes in intensity in response to analytes, and relatively few wavelength ratiometric and lifetime based probes are available [31,38]. Some useful wavelength ratiometric probes are available for pH, Ca^{2+}, and Mg^{2+} [39,40] but the probes for Na^+ and K^+ generally display

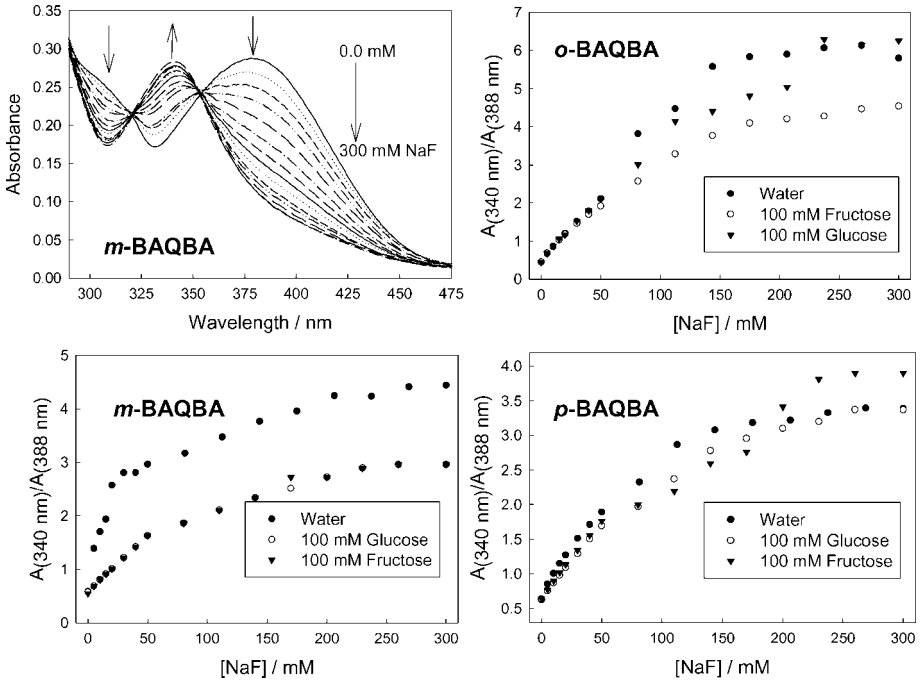

Fig. 8. Absorption spectra of *m*-BAQBA in water having 100 mM glucose with increasing concentrations of NaF (*Top left*). The corresponding wavelength ratiometric plots based on $A_{(340)}/A_{(388)}$ nm bands for *o*-, *m*- and *p*-BAQBA in water having either 100 mM glucose or fructose with increasing concentrations of NaF (*Top right*, *Bottom left* and *Bottom right*, respectively).

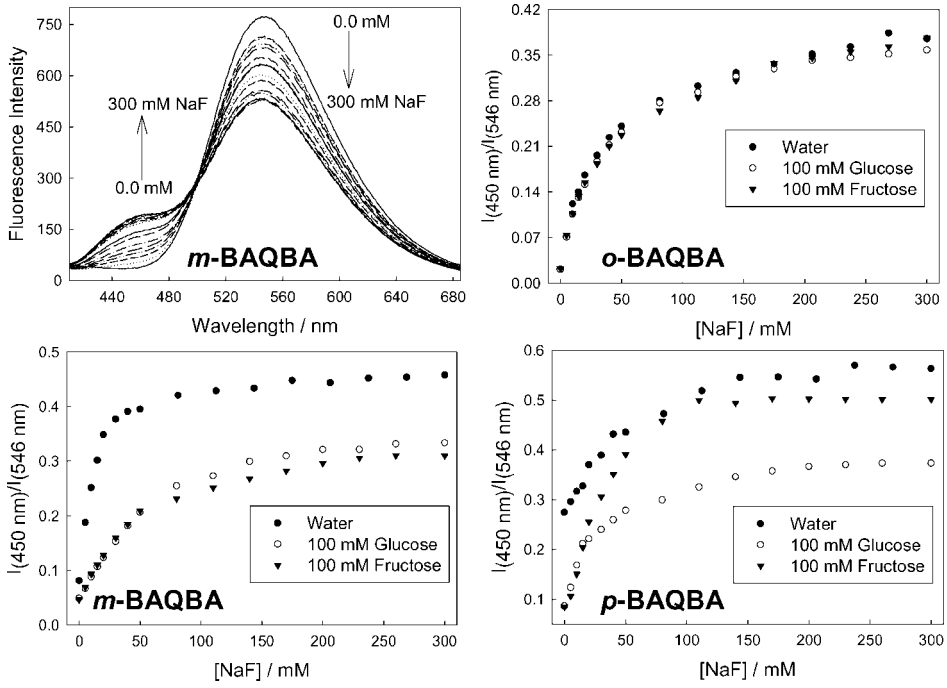

Fig. 9. Emission spectra of *m*-BAQBA in water having 100 mM glucose with increasing concentrations of NaF (*Top left*). $\lambda_{ex} = 358$ nm. The corresponding wavelength ratiometric plots based on $I_{(450)}/I_{(546)}$ nm bands for *o*-, *m*- and *p*-BAQBA in water having either 100 mM glucose or fructose with increasing concentrations of NaF (*Top right, Bottom left* and *Bottom right*, respectively).

Table II. Stern–Volmer constants, K_{SV} (M^{-1}), for *ortho-*, *meta-* and *para*-BAQBA with sodium halides

Halide	*o*-BAQBA	*m*-BAQBA	*p*-BAQBA
Cl^-	1.0	<0.3	1.1
Br^-	1.4	0.3	1.4
I^-	34.0	34.5	33.4

small spectral shifts and negligible lifetime changes and are subsequently inadequate for quantitative sensing measurements. Dynamic quenchers such as O_2 and the halides usually occur with a change in intensity and lifetime but without an emission spectral shift. In addition, due to its low mass, the fluoride ion is not an effective collisional quencher and only a few probes are known to be quenched by fluoride in the low mM concentration range [3].

In this paper we have shown that the boronic acid group can be relatively selective towards fluoride. Since the boronic acid group also interacts with other strong bases such as hydroxyl ions [37], BAQBAs are likely to also have a pH dependent response, although at pH 7 such as in physiological fluids or waste waters, the interaction is low (the concentration of $OH^- = 10^{-7}$ M) and environmental and physiological safeguard sensing applications do not experience notable pH changes.

While the many boronic acid containing fluorophores that have been synthesized to date [21–27] and are likely to show a fluoride response, only a few will demonstrate spectral shifts and intensity changes, affording for ratiometric sensing, while even fewer will demonstrate an additional poor/non-sugar response. Further, BAQBAs offer the additional possibility of lifetime based halide sensing (collisional quenching). Hence BAQBA offers the attractive opportunity for both ratiometric and lifetime based sensing of aqueous halide at neutral pH, in the mM halide concentration range. Moreover, because halide collisional quenching is an excited-state based process and fluoride complexation with boronic acid is a ground state binding process, one has the future opportunity to potentially now simultaneously measure both fluoride (ratiometrically) and one other halide (lifetime based sensing), using one excitation wavelength, e.g. a 372 nm LED, and by monitoring 2 emission wavelengths, given the 94 nm emission band separation. It should be noted that fluoride will indeed collisionally quench quinolinium fluorescence, but not notably in the 10's of mM fluoride concentration range. Such an approach may remove the requirement of needing 2 fluorescent probes, each with different K_{SV}'s, for the simultaneous measurement of 2 halides, as first described by Wolfbeis and co-workers [41].

Table III. Multiexponential Intensity Decay of BAQ and *o*-BAQBA

Compound	[Fluoride] (mM)	τ_1 (ns)	α_1	τ_2 (ns)	α_2	τ_3 (ns)	α_3	$\bar{\tau}$	$\langle\tau\rangle$	χ^2
BAQ	0	2.48	1	—	—	—	—	2.48	2.48	1.10
	(416 nm)[a]									
	0[b]	1.99	0.675	3.28	0.325	—	—	2.56	2.41	1.01
	30[b]	1.27	0.230	2.58	0.770	—	—	2.41	2.28	1.26
	60	0.14	0.110	1.99	0.700	3.96	0.190	2.66	2.16	0.97
	90	0.18	0.120	1.80	0.510	3.01	0.370	2.44	2.05	1.06
	120	0.17	0.150	1.83	0.530	3.06	0.320	2.42	1.97	1.15
	150	0.14	0.150	1.56	0.380	2.81	0.470	2.39	1.93	0.95
	200	0.18	0.180	1.85	0.590	3.33	0.230	2.42	1.89	0.98
	250	0.18	0.200	1.83	0.610	3.59	0.190	2.45	1.83	1.06
	300	0.22	0.210	1.92	0.670	4.19	0.120	2.49	1.84	1.24
o-BAQBA										
	(546 nm)[a]									
	0	2.00	0.620	3.07	0.380	—	—	2.52	2.41	0.89
	30	1.86	0.540	2.89	0.460	—	—	2.45	2.33	0.90
	60	1.93	0.720	3.41	0.280	—	—	2.53	2.34	0.95
	90	1.67	0.420	2.73	0.580	—	—	2.40	2.28	1.08
	120	1.70	0.510	2.89	0.490	—	—	2.44	2.28	1.15
	150	1.73	0.580	2.99	0.420	—	—	2.43	2.26	1.02
	200	1.54	0.410	2.67	0.590	—	—	2.35	2.21	1.04
	250	1.57	0.500	2.78	0.500	—	—	2.34	2.18	1.00
	300	1.59	0.520	2.77	0.480	—	—	2.32	2.16	1.10

[a] Long pass filters.

[b] No notable improvement in fit could be obtained using a 3-exp function. Similar values were also found for the *meta-* and *para*-BAQBA probes.

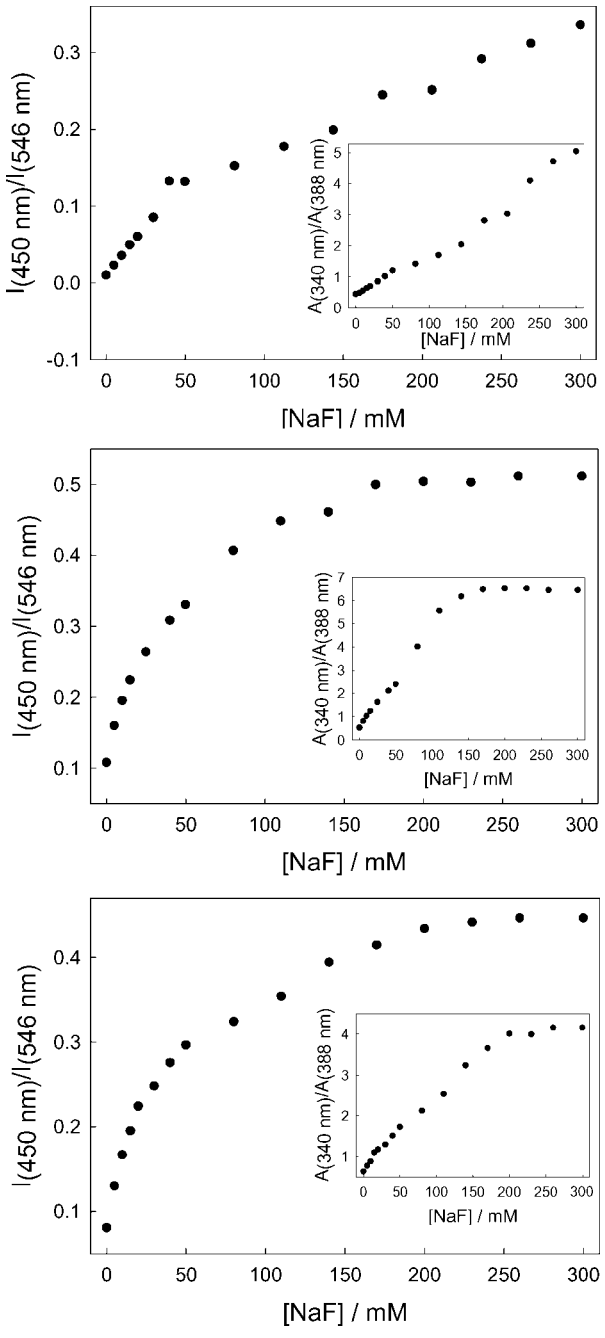

Fig. 10. Emission ratiometric plot for *o*-, *m*- and *p*-BAQBA in physiological fluid-like cocktail (50 mM glucose, 50 mM sodium chloride and 5 mM fructose) with increasing concentrations of sodium fluoride (*Top*, *Middle* and *Bottom*, respectively). The insert figures show the respective absorption ratiometric plots in the same concentration range.

CONCLUSIONS

We have reported the ratiometric and colorimetric response of moderately fluorescent and water soluble boronic acid containing fluorophores towards aqueous fluoride, in the presence of other potential sensing interferents known to bind-to boronic acid. These new probes display a suppressed sugar response, brought about by the amino substituent in the 6-position of the quinolinium backbone. While not shown here, by replacing the 6-amino group with other relatively less electron donating groups, one does not observe the long wavelength 546 nm fluorescence emission band, eliminating the potential for ratiometric type sensing. In addition, a greater sugar response (affinity) is observed by introducing either CH_3, —OCH_3, and H groups into the 6-position. Hence the unique combination of the amino group in the 6-position, coupled with the boronic acid moiety and a quinolinium nucleus, affords for a unique halide sensitive probe, sensitive to fluoride, chloride, bromide and iodide in the mM concentration range, which is only slightly perturbed by sugars at a constant pH. The *meta*-isomer showed the greatest affinity for fluoride with a K_D of 10.8, which is thought due to the combined through space/bond interactions as compared to the other two isomers. Little, if any, steric effects are thought to play a role in the K_D vales determined for fluoride.

In this paper we have described the utility of this probe by its response to fluoride in the presence of other potential analytes/interferents, and the future opportunity for both simultaneous ratiometric and lifetime based analyte sensing.

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