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Cyanide-sensitive fluorescent probes

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Abstract

We characterize the response of several boronic acid containing fluorophores, which are widely used for sugar determination, towards aqueous cyanide. In two recent reports we have shown that boronic acid containing fluorophores can be used to sense aqueous cyanide through physiological safeguard levels. In this report we show that our new sensing mechanism is not just specific to our recently reported probes, but is indeed generic to the boronic acid moiety itself. Subsequently a wide range of cyanide-sensitive probes can now be realized, offering several modalities for fluorescence based cyanide sensing such as: intensity, lifetime, ratiometric, polarization and modulation fluorescence sensing.
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Keywords: Cyanide sensing; Boronic acid; Ratiometric fluorescence sensing; Colorimetric response; Poison; Physiological safeguard

1. Introduction

Cyanide is one of the most lethal poisons known [1–5]. The mechanism of human toxicity for cyanide is by absorption. Absorption occurs through the lungs, GI tract and even skin. The high toxicity of cyanide lies in its ability to inhibit oxygen uptake by cells, binding with the ferric iron in cytochrome oxidase, blocking the oxidative process of cells. Hence the tissues with the greatest requirement for oxygen, such as brain, heart and lungs, are the most affected by acute poisoning. While cyanide poisoning is rare, it can occur from smoke inhalation from both residential and industrial fires, and in those people who work in the metal industries [6,7].

Blood cyanide levels for healthy persons have been reported as being ≈0.3 μM using a gas chromatography method [7], with lethal cyanide blood levels for fire victims in the cyanide concentration range 23–26 μM [7,8], some 2 orders of magnitude higher than normal healthy blood levels. With such a small amount of cyanide able to produce acute cyanide poisoning, there has been a moderate amount of cyanide sensing literature [1–8]. Several chemical and physiochemical methods for the detection of cyanides, such as potentiometric, chromatographic, spectrophotometric, flow injection and electrochemical analysis have been used [1–10]. Yet with many cyanide approaches reported, there is still a requirement for simple, cheap and fast technologies to both detect and determine cyanide levels up to physiological lethal/safeguard levels, <20 μM [11,12].

In this paper we expand upon our previous report that cyanide sensing can be achieved using specific

Abbreviations: ANDBA, 9,10-bis[[*N*-methyl-*N*-(*o*-boronobenzyl) amino]methyl]-anthracene; BA, boronic acid; BAF and BAFs, boronic acid containing fluorophore/s; CSTBA, 4′-cyanostilbene-4-boronic acid; CT, charge transfer; Chalc 1, 3-[4′(dimethylamino)phenyl]-1-(4′-boronophenyl)-prop-2-en-1-one; DSTBA, 4′-dimethylaminostilbene-4-boronic acid; GI tract, gastrointestinal tract; PANSBA, 1-(4-boronophenylazo)-2-hydroxy-3,6-naphthalenedisulfonic acid disodium salt; PET, photo-induced electron transfer.

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boronic acid containing fluorophores [11] and show that boronic acid groups in general, readily complex cyanide (Scheme 1). This important find subsequently opens up the potential use of a vast arsenal of boronic acid containing fluorescent probes (BAFs) which have been developed over many years to sense glucose and other monosaccharides, where the boronic acid moiety is well known to readily bind these sugars [13–20]. Interestingly, the binding constants favor cyanide complexation by up to 3 orders of magnitude, (i.e. 3 times larger), so even extreme physiological hyperglycemic conditions would not interfere with physiological cyanide monitoring at safeguard levels, i.e. <20 μM cyanide.

The origin of the cyanide response is due to the boronic acid group's ability to interact with bases such as CN^-, as shown in Scheme 1 [11,12], to form the tricyanide anion $R-B-(CN^-)_3$, which is an electron-donating group, the extent of which is dependent on the concentration of cyanide present and the electron-donating or withdrawing capabilities of R [11,12]. To show the generic application of the boronic acid moiety to cyanide sensing we have chosen to study different mechanisms, which have been previously used to induce spectral changes in the presence of sugar. In particular we have used dyes that show excited-state charge transfer (CT) [14,16–18], photo-induced electron transfer (PET) [21] and a probe based on a resonance interaction (RI) [22] (Fig. 1). In all cases, a strong response towards cyanide is observed, similar to that observed with monosaccharides, both further confirming our previous reports, as well as demonstrating the utility of the boronic acid group to complex and therefore sense aqueous cyanide.

2. Experimental

2.1. Materials

All chemicals were purchased from Sigma except for the fluorescent probes shown in Fig. 1, which were prepared as previously reported [14,16,18,21,22].

2.2. Methods

All solution absorption measurements were performed in a $4 \times 1 \times 1$ 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 0.1.

Stability (K_S—units μM^{-3} or $mol^{-3}\,dm^9$) and dissociation constants (K_D—units μM^3 or $mol^3\,dm^{-9}$) were obtained by fitting the titration curves with aqueous sodium cyanide to the relation:

$$I = \frac{I_{min} + I_{max} K_S[\text{cyanide}]}{1 + K_S[\text{cyanide}]} \quad (1)$$

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

3. Results and discussion

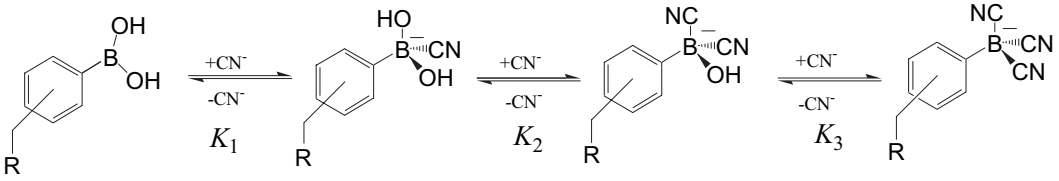
To demonstrate the utility of cyanide sensing using boronic acid containing fluorophores, it is informative to compare different sensing mechanisms, which have previously been employed to induce spectral changes in the presence of monosaccharides [13–22].

3.1. Charge transfer

CT is a versatile mechanism that can be applied to a large number of fluorophores, where the boronic acid group and an electron donor group are present on the same fluorophore. Here, the BA group [$R-B(OH)_2$] acts as an electron-withdrawing group. However, in the presence of cyanide and at an appropriate pH, the boronic acid group is present in its anionic form, namely $R-B-(CN^-)_3$ and is no longer an electron-withdrawing group. Hence spectral changes can be observed due to the perturbation of the charge transfer nature of the excited state.

3.1.1. Stilbene derivatives

Fig. 1 shows two stilbene derivatives which contain the boronic acid moiety. DSTBA, 4′-dimethylaminostilbene-4-boronic acid, which combines the electron-donating dimethylamino group with the electron-withdrawing boronic acid group and CSTBA, 4′-cyanostilbene-4-boronic acid, which combines the electron-withdrawing cyano group with boronic acid, in essence two probes



Scheme 1. Equilibrium involved in the interaction between the boronic acid group and cyanide.

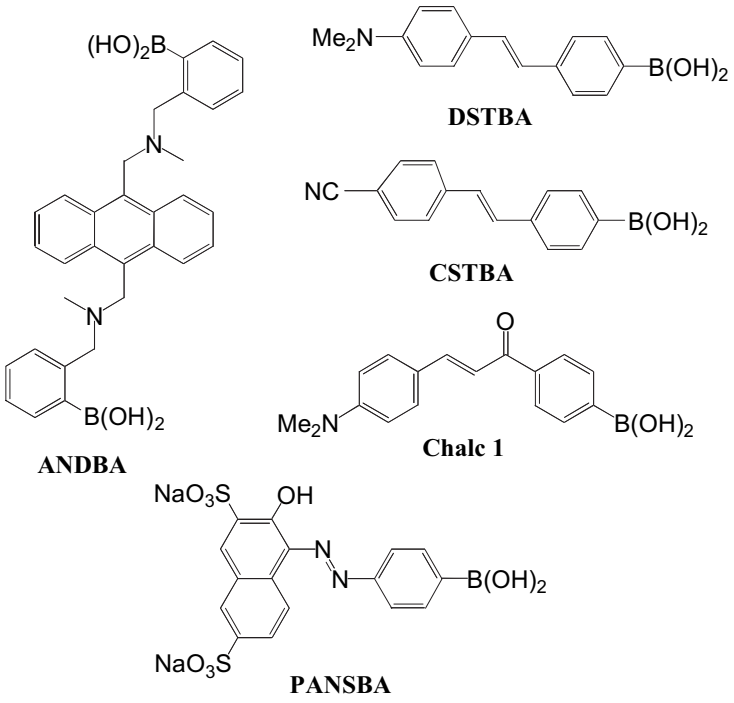


Fig. 1. Molecular structures of probes screened for their interactions with aqueous cyanide. DSTBA, 4′-dimethylaminostilbene-4-boronic acid; CSTBA, 4′-cyanostilbene-4-boronic acid; Chalc 1, 3-[4′(dimethylamino)phenyl]-1-(4′-boronophenyl)-prop-2-en-1-one; ANDBA, 9,10-bis[[*N*-methyl-*N*-(*o*-boronobenzyl)amino]methyl]-anthracene; PANSBA, 1-(4-boronophenylazo)-2-hydroxy-3,6-naphthalenedisulfonic acid disodium salt.

demonstrating both reduced and increased CT in the presence of aqueous cyanide.

Fig. 2 (top) shows the fluorescence emission spectra of DSTBA with increasing concentrations of aqueous cyanide. The emission spectra show a hypsochromic shift of about 40 nm and an increase in fluorescence intensity as the concentration of cyanide is increased. These dramatic and useful changes are also observed with monosaccharides [16] and can simply be explained here by the loss of the electron-withdrawing property of the boronic acid group following the formation of the anion cyanide bound form, $R-B-(CN^-)_3$, c.f. Scheme 1. We subsequently constructed the emission wavelength ratiometric plots based on the 450 and 515 nm intensity values (Fig. 2, bottom) where an almost linear response towards aqueous cyanide can be observed up to physiological safeguard limits, $<20\ \mu M$. Using Eq. (1), we estimated the dissociation constant to be $\approx 27\ \mu M^3$ (Table 1) as compared to a value of 98 mM for D-glucose and 2.5 mM for D-fructose, as previously reported by our laboratory [16].

The CSTBA stilbene derivative possesses two electron-withdrawing groups (Fig. 1). In the presence of cyanide, a 35 nm bathochromic shift, accompanied by a decrease in fluorescence intensity is observed (Fig. 3). This is opposite to that observed for DSTBA but similar to that reported for a sugar response [16,23]. This difference in behavior can likewise be attributed to an excited CT state present for the anionic form of CSTBA, where no CT states are observed for the neutral form of the boronic acid group [16]. This suggests that the anionic form of the boronic acid group can act as an electron-donating group. Similarly for DSTBA, we constructed the emission wavelength ratiometric plot based on the 390 and 455 nm emission intensity values (Fig. 3, bottom) where up to a 6-fold change in I_{390}/I_{455} can be observed in the cyanide physiological safeguard region.

3.1.2. Chalcone derivatives

Chalcone derivatives, unlike the stilbenes, have the advantage of much longer wavelength emission, allowing their potential use with cheaper and longer wavelength laser or light emitting diode sources. For Chalc 1, the boronic acid group does not produce resonance forms with the electron-donating amino group [14,16]. The CT occurs between the dimethylamino group (electron-donating group) and the carbonyl group (electron-withdrawing group). Upon cyanide binding to the boronic acid group, a change in the electronic properties of the boronic acid group, both when free and bound to cyanide, directly leads to a change in the electronic density of the acetophenone moiety, noting that the boronic acid group is in resonance with the carbonyl group. The spectral changes observed in Fig. 4 (top) are very similar indeed to those obtained with sugar [16], further confirming the cyanide complexation interaction [11,12]. Additionally, Fig. 4 (bottom)

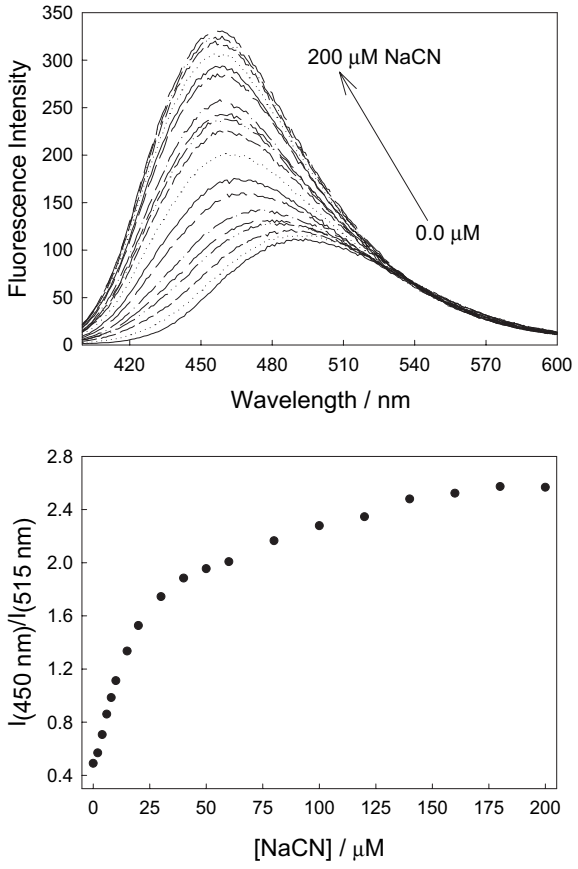


Fig. 2. Fluorescence emission spectra of DSTBA with increasing concentrations of aqueous cyanide (top) and the respective ratiometric plot using the intensities at 450 and 515 nm (bottom).

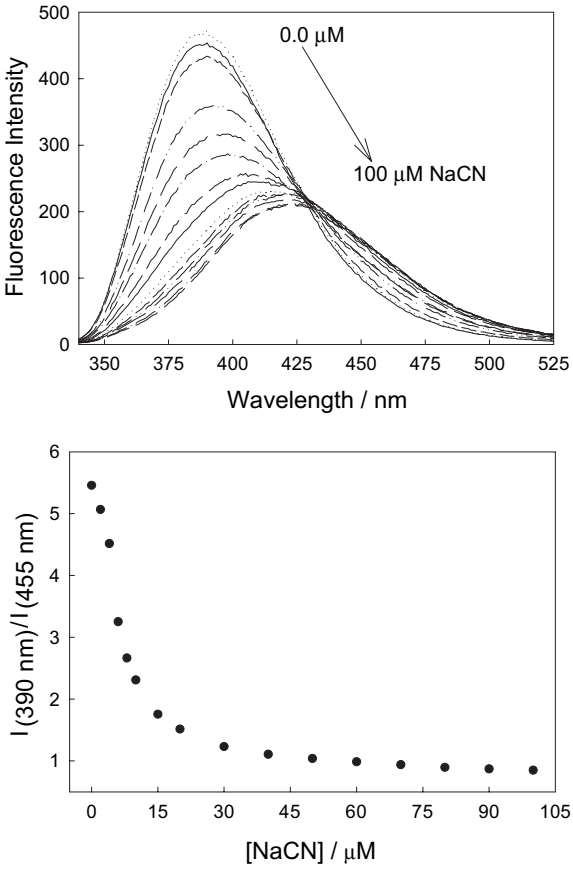


Fig. 3. Fluorescence emission spectra of CSTBA with increasing concentrations of aqueous cyanide (top), and the respective ratiometric plot using the intensities at 390 and 455 nm (bottom).

shows the fluorescence intensity of Chalc 1 as a function of increasing cyanide concentration, normalized by the initial intensity in the absence of cyanide. An approximate 3.5-fold intensity change is observed with the addition of 10 μM CN^-, with a dissociation constant of 3.6 μM^3.

3.2. Photo-induced electron transfer

Photo-induced electron transfer is often used as a mechanism for fluorescence quenching in the development of many sensors [21]. The quenching is due to an electron rich amino group near the fluorophore. When the analyte of choice binds to the PET probe, then this new interaction with the nitrogen's lone pair dominates, removing the quenching, with an increase in fluorescence intensity observed. For glucose sensors, the mechanism is slightly different. In this case, changes in the acidity of the boron atom and the nitrogen atom in the presence of glucose are responsible for the intensity changes.

Table 1
Dissociation constants of the probes with cyanide in water

Probe	K_D (μM^3)
ANDBA	3.90
DSTBA	27.20
CSTBA	6.90
Chalc 1	3.60
PANSBA	7.95

Fig. 5 shows the interaction of an anthracene derivative, ANDBA, containing amino and phenyl boronic acid groups, with aqueous cyanide. This PET probe has been well characterized with regard to monosaccharides [21]. The addition of 10 μM cyanide almost completely quenches the ANDBA fluorescence, with a ≈15-fold, almost linear change in fluorescence intensity observed at 425 nm. This remarkable dynamic quenching range is most attractive here for physiological cyanide safeguard monitoring. In addition, ANDBA has been reported as a suitable fluorescence lifetime probe for glucose [21], which suggests its analogous use as a lifetime probe for cyanide. It is widely known that lifetime based sensing is preferred, as compared to intensity based sensing [24,25], as fluorescence lifetimes are generally independent of the probe concentration and intensity of the fluorescence signal, as well as fluctuations in the excitation source [24,25].

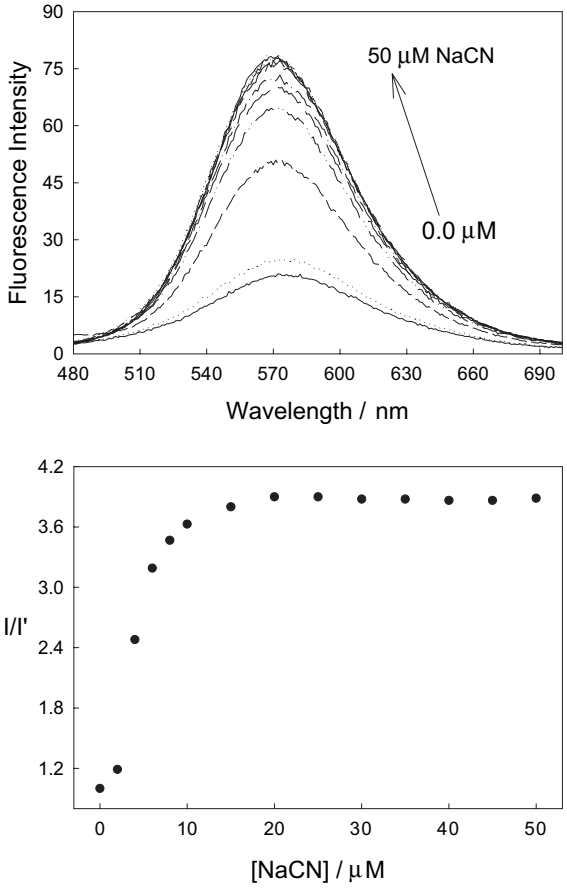


Fig. 4. Fluorescence emission spectra of Chalc 1 with increasing concentrations of aqueous cyanide (top), and the respective ratiometric type plot using the initial fluorescence intensity, I', at 570 nm in the absence of cyanide, and in the presence of increasing cyanide concentrations, I (bottom).

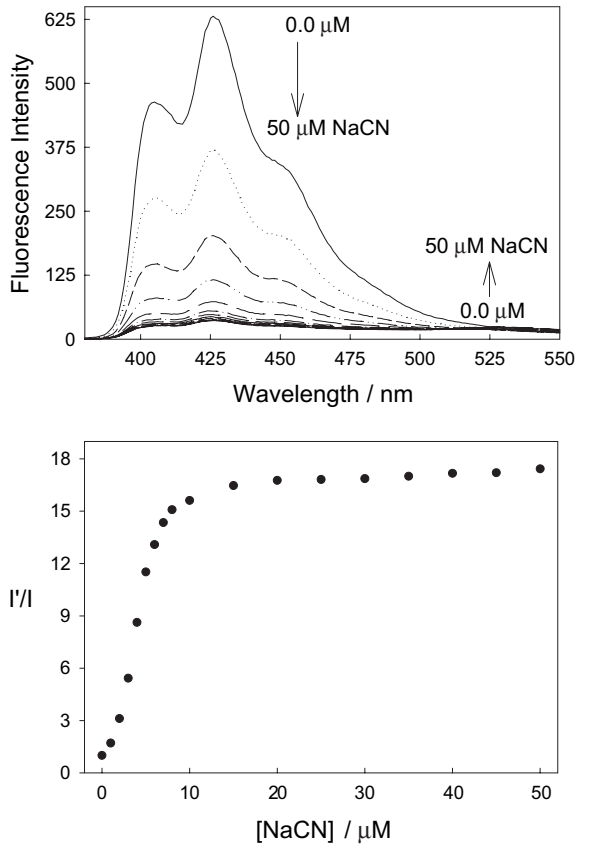


Fig. 5. Fluorescence emission spectra of ANDBA with increasing concentrations of aqueous cyanide (top) and the respective ratiometric type plot using the initial fluorescence intensity, I', at 425 nm in the absence of cyanide, and in the presence of increasing cyanide concentrations, I (bottom).

3.3. Resonance interaction

Finally to asses the utility of BAFs for cyanide sensing we also considered the use of azo-type dyes which have been reported as being ideal "Color Chemosensors" for monosaccharides [22]. Again, a change in the electronic properties of the boronic acid between its neutral form (no cyanide) and anionic form (with cyanide) accounts for the spectral changes observed. Fig. 6 (top) shows the changes in absorption spectra for increasing cyanide concentrations, where the small absorption changes are enough to be detected visually (Fig. 7) making for a colorimetric type response towards aqueous cyanide. These changes are very similar to those also reported for monosaccharides [22]. While the absorption ratiometric plot (Fig. 6, bottom) shows a relatively smaller dynamic sensing range as compared to others discussed here, the utility of this probe clearly lies in its visual response. It should be noted that the color change is thought to be due to the conformational change of the boron atom between its neutral and anionic forms, i.e., the boronic acid group is an electron-deficient Lewis Acid having an sp^2-hybridized boron atom and a triangular conformation, while the anionic form is an electron rich sp^3-boron atom with a tetrahedral geometry. Despite the changes between the electron-withdrawing and donating properties of the boronic acid group, the effect of intramolecular charge transfer is thought to be weak [22].

4. Conclusions

In this paper we have characterized several known probes towards aqueous cyanide. These probes have previously been used to complex and therefore sense monosaccharides by a variety of different sensing mechanisms. Given that cyanide induces spectral changes similar to that of sugars, we can conclude that cyanide complexation with boronic acid does not appear specific to just certain classes of fluorophores. This important find expands on our recent reports of cyanide-sensitive probes based on the boronic acid moiety [11,12], and suggests the widespread application of BAFs to cyanide sensing.

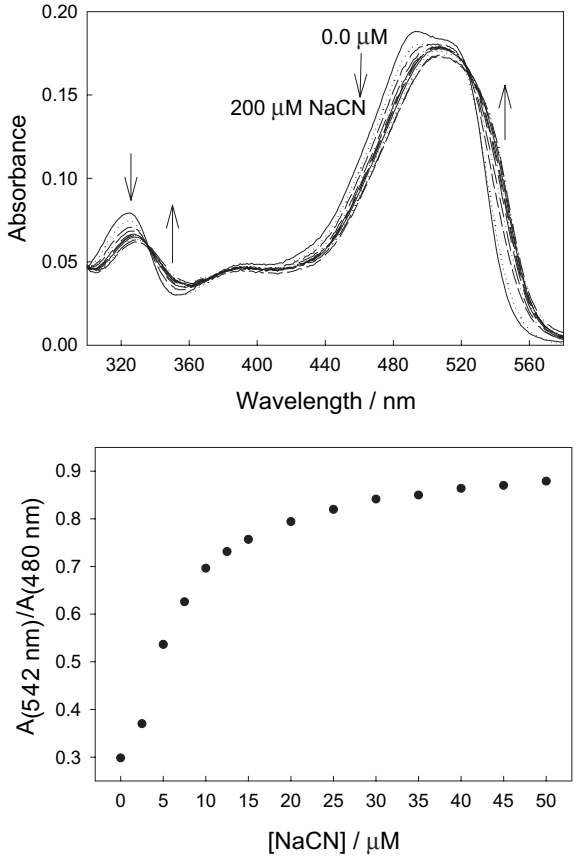


Fig. 6. Absorption spectra of PANSBA with increasing concentrations of aqueous cyanide (top) and the respective ratiometric plot using the intensities at 542 and 480 nm (bottom).

With regard to physiological cyanide sensing and safeguard, other anions such as OH^- [26] and F^- [27,28] are also known to complex with boronic acid. However, fluoride levels in blood are very low, and not thought to vary much, ≈20–60 μg/L [28], while the concentration of OH^- in blood is $\approx 10^{-7}$ M, with most physiologies not experiencing any notable changes in pH. Finally, with regard to the sensing mechanism of boronic acid with cyanide, a recent report by us has suggested that traditional dynamic fluorescence quenching [29] has no or little effect on probe fluorescence at these μM cyanide concentrations of interest [30], further confirming our boronic acid–cyanide complexation interaction for sensing.

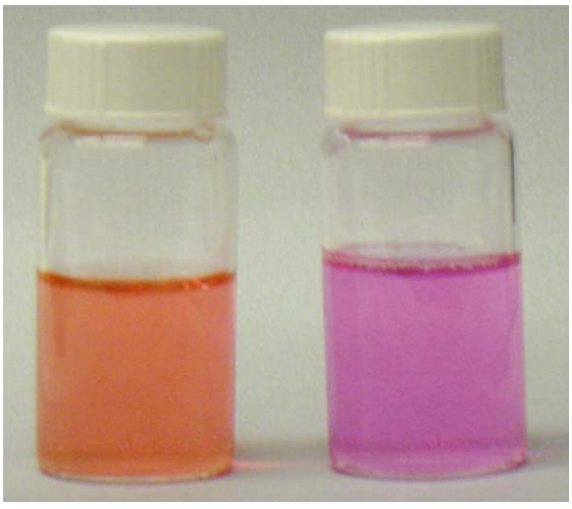


Fig. 7. Photograph of two vials containing equal concentrations of PANSBA with both 0 and 50 μM NaCN (left and right, respectively).

Acknowledgement

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