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# Designed Boronate Ligands for Glucose-Selective Holographic Sensors

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**Abstract:** In this study, 2-acrylamidophenylboronate (2-APB) was synthesised and its ability to bind with glucose was investigated both in solution and when integrated into a holographic sensor. Multiple forms of 2-APB, resulting from the neighbouring effect of the amido group with the boronic acid through an intramolecular B–O-coordinated interaction, were shown to exist in solution by using multinuclear NMR spectrometry. It was found that 2-APB predominantly adopts a zwitterionic tetrahedral form at physiological pH values. The complex formation of 2-APB with glucose and lactate was investigated in DMSO; 2-APB favours binding with glucose rather than lactate and generates a five-membered-ring complex. Furthermore, a 2-APB-based holographic sensor displayed a significant response to glucose with little interference from lactate, and with no dependence on pH in the physiological pH range. These features suggest that the new ligand 2-APB is a potential candidate for the development of glucose-selective sensors.

**Keywords:** boronic acid · glucose · holography · pH · sensors

## Introduction

It is well known that boronates rapidly and reversibly form cyclic esters in alkaline media with diols such as glucose. A number of novel boronate analogues containing functional groups have been designed to improve their affinity to glucose at physiological pH values.[1–3] Such analogues have been included in glucose-responsive cyclodextrin, fluorescence, coloured, electrochemical, polymeric and imprinted receptors, although most of the novel boronate receptors reported still display a pH dependency in the physiological range, both in solution as well as in the sensors. Furthermore, interference from the metabolic end product, lactate, to boronate-based glucose sensors has rarely been mentioned.[4]

Holographic glucose sensors based on 3-acrylamidophenylboronate (3-APB) were recently developed in our group.[4] The holographic sensors are based on planar, small-volume polymer hydrogels containing silver halide, which acts as a holographic recording material. A reflection hologram is recorded within the polymer hydrogel matrix, producing an optical sensor that only reflects a narrow band of wavelengths when illuminated with white light. Holographic glucose sensors have been fabricated from 3-APB incorporated into acrylamide-based "smart" hydrogels that respond to glucose by changing their swelling state or degree of hydration upon glucose binding with 3-APB. The change in the volume of the polymer causes a change in the wavelength of the light diffracted by the holographic grating within the hydrogel. The narrow band of wavelengths diffracted is determined by the spacing between silver ($Ag^0$) nanoparticle fringes. Thus, the hologram acts as a monochromatic mirror, in that light with a wavelength of approximately double the distance between the fringes will emerge in phase at a specific angle and constructive interference occurs. The diffraction wavelength of the hologram is governed by Bragg's law. The results to date show that 1) The sensor displays reversible changes in diffraction wavelength as a function of monosaccharide concentration at physiological pH values; 2) maximum sensitivity to glucose is observed at a functional monomer concentration of 20–25 mol%;

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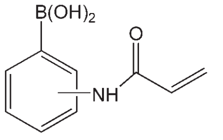
Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author. It contains selected $^1H$, $^{13}C$ and $^{11}B$ NMR spectra and FTIR spectra of 2-APB and 3-APB. It also contains more details of the synthesis of the copolymer films of 2-APB and 3-APB and the hologram construction.

3) the potential for using this holographic sensor to detect real-time changes in bacterial cell metabolism has been demonstrated by monitoring the vegetative metabolism of *Bacillus subtilis.* However, such boronate-based glucose sensors display strong interference from lactate. This feature makes them especially unsuitable for application in glucose-selective holographic sensors for monitoring tear fluid and bacterial culture media, because lactate is a metabolic by-product that is present at high concentrations in both these fluids. Thus, there is a need for upgrading the current 3-APB-based holographic glucose sensor into one that displays little or no response to lactate as well as a limited pH sensitivity to binding affinity within the physiological pH range. To achieve this, one solution is to design new ligands that can selectively bind with glucose and obviate lactate interference. It is known that the tetrahedral form of the boronic acid favours binding with glucose, whereas the trigonal form preferentially binds with lactate.[5] Thus, if it is possible to generate a tetrahedral boronate analogue as the dominant species at physiological pH values, then the interference from lactate is likely to be diminished. Such a tetrahedral form can be induced by designing boronic acids with lower $pK_a$ values or with intramolecular B–X bonds that confer a tetrahedral conformation at the boron centre through the neighbouring effect of an *ortho* group. The latter approach is more appealing, because it is believed that if a strong intramolecular B–X bond can be generated, then such boronic acids would bind with glucose even under mildly acidic conditions. Although a number of boronic acids with B–N bonds have been reported and there are concerns over the strength of the B–N bond,[1a,2b,6–7] very few of them have been further employed in the development of glucose sensors.[8] Acknowledging that an intramolecular B–O bond is much stronger,[9] it is believed that the B–O bond should maintain the boron centre in the favourable tetrahedral form across a wide range of pH, although little of the literature has discussed pH stability of the tetrahedral species and its binding affinity to glucose.

In this paper, two boronates, 2-APB and 3-APB, have been designed, synthesised and characterised, and their boron geometries and binding affinity towards glucose and lactate have been investigated by using $^{1}$H, $^{11}$B and $^{13}$C NMR spectrometry, and IR and mass spectrometry. The pH titration results show that the tetrahedral forms are the dominant species present in 2-APB, whereas the trigonal and tetrahedral forms coexist in 3-APB, with the trigonal form being the dominant species at neutral pH values. A holographic sensor based on an acrylamide hydrogel, containing 2-APB as the glucose-responsive functionality, responded to the addition of glucose at physiological pH values. The observed contraction of the 2-APB-based hologram in the presence of glucose suggests that glucose cross-links proximal phenylboronate moieties due to the favourable tetrahedral form being the predominant species in the matrix, in contrast to the observed swelling of the 3-APB-based hologram in the presence of glucose. Furthermore, the 2-APB-based hologram displayed significantly less interference from lactate and a glucose-binding profile that was almost independent of pH over the physiological pH range, both of which are desired features for development of glucose-selective sensors.



3-APB: *m*-NHR
2-APB: *o*-NHR

## Experimental Section

**Materials**: All chemicals used were of analytical grade unless otherwise stated. 2-Aminophenylboronic acid and 3-aminophenylboronic acid were purchased from Avocado Research Chemicals. Acryloyl chloride was purchased from Sigma Chemical Co. All other chemicals related to hologram preparation and holographic measurement are the same as described in previous work.[4] All deuterated solvents, acids and bases were purchased from Cambridge Isotope Laboratories, Inc.

**Equipment**: A 400 MHz JEOL NMR instrument was used for NMR analysis. A glass pH microelectrode (combined with the NMR spectrometer) was supplied by ThermoOrion. A PerkinElmer FTIR spectrometer was used for collecting IR data. A UV exposure unit ($\lambda \approx 350$ nm, model no. 555-279) was purchased from RS Components. A frequency-doubled Nd:YAG laser ($E = 350$ mJ, $\lambda = 532$ nm, Brilliant B, Quantel) was used in hologram construction. Holographic measurements were performed by using an Avantes AVS-MC2000-2 reflectance spectrometer with AvaSoft 5 processing software (Knight Scientific).

**Synthesis of 2-acrylamidophenylboronic acid (2-APB)**: In an ice–water bath, a well-stirred solution of 2-aminophenylboronic acid•HCl (0.75 g, 4.4 mmol) in aqueous sodium hydroxide (5 N, 5 mL) was treated with drop-wise addition of acryloyl chloride (0.5 mL) over a period of about 10 min. After 30 min of stirring on ice, the reaction mixture was allowed to reach room temperature and was stirred for a further 2 h. The mixture was adjusted to pH 8 by using dilute HCl (0.1 M). The resulting beige-coloured precipitate was filtered off and washed with water and acetone. A fine, white powder was obtained in a yield of $\approx 50\%$ after drying. $^{1}$H NMR (400 MHz, [D$_6$]DMSO, 25 °C, TMS): $\delta = 12.08$ (s, 1H; NH), 7.63 (m, 1H; Ph–H), 7.54 (m, 1H; Ph–H), 7.28 (m, 1H; Ph–H), 7.13 (m, 1H; Ph–H), 6.33 (dd, $J_1 = 16.8$, $J_2 = 1$ Hz, 1H; =CH), 6.23 (quad, $J_1 = J_2 = 10$ Hz, 1H; =CH), 5.88 ppm (dd, $J_1 = 10$, $J_2 = 1$ Hz, 1H; =CH); $^{13}$C NMR (100.6 MHz, [D$_6$]DMSO, 25 °C, TMS): $\delta = 163.2$, 139.5, 133.7, 129.7, 128.7, 125.5, 117.1 ppm; $^{11}$B NMR (128 MHz, [D$_6$]DMSO, 25 °C, boric acid): $-5.63$ ppm; MS (ESI): $m/z$ (%): 191 (100) [$M^+$].

**Synthesis of 3-acrylamidophenylboronic acid (3-APB)**: 3-APB was synthesised according to a method previously reported.[4]

**Preparation of copolymer films and hologram construction**: The 3-APB-based copolymer films were made according to a method previously described.[4]

*Preparation of 2-APB-based copolymer film*: The monomer 2-APB (20 mol %) mixed with glucose (5.0 equiv) was dissolved in DMSO containing the photoinitiator 2,2-dimethoxy-2-phenylacetophenone (2 % w/v). The solid-to-solvent ratio of monomer/DMSO was kept constant throughout at 0.452:1 (w/v). A droplet (100 µL) of monomer solution was pipetted onto the polyester surface of an aluminised polyester sheet sitting on a glass plate. A glass microscope slide, pretreated with 3-(trimethoxysilyl)propyl methacrylate, was then gently lowered, silane-treated-side down, onto the monomer mixture. Films were polymerised by means of a UV-initiated free-radical reaction at room temperature for 60 min. Polymerised films were peeled off the polyester sheet and then exhaustively washed with water. Consequently, the binding complex was converted to the free 2-APB, thereby releasing the favourable tetrahedral species present in the copolymer matrix. The process is termed “imprinting photopolymerisation”.

**Hologram construction**: The holograms were constructed according to a method previously described.[4]

**$^{11}$B NMR spectroscopic pH titration**: A boronate (2-APB or 3-APB; ≈3 mg) was dissolved in $D_2O$ (0.5 mL), and the pH was adjusted to a desired value by using DCl or NaOD (≈0.1 M) and measured by using a microelectrode pH probe. $^{11}$B chemical shifts of samples with different pH values at room temperature were collected and compared with boric acid as a standard reference.

**Monitoring holographic responses**: The holographic sensors were interrogated by using a procedure described in previous work.[4]

**Holographic pH titration**: Holographic pH titration was performed by equilibrating the 2-APB- or 3-APB-based hologram samples at each pH value and then recording the diffracted peak wavelength. For each pH value, 1 mL of the appropriate buffer was added and removed from the cuvette three times to ensure the buffering capacity of the hologram was cancelled out. Thereafter, the buffer (1 mL) was left in the cuvette and the hologram was allowed to equilibrate. The wavelength was recorded before the buffer was removed and the sample was adjusted to the next pH value. The following pH buffer systems were used: phosphate (pH 2), formate (pH 3), acetate (pH 4 and 5), 2-morpholinoethanesulfonic acid (MES; pH 6 and 7), tris(hydroxymethyl)methylamine (Tris; pH 7.4 and 8), *N*,*N*-bis(2-hydroxyethyl)glycine (Bicine; pH 9), 2-(*N*-cyclohexylamino)ethanesulfonic acid (CHES; pH 10) and piperidine (pH 11 and 12); all of the buffers prepared had a constant concentration (10 mM) and a fixed ion strength (154 mM).

## Results and Discussion

**Equilibria of 2-APB and 3-APB in solution**: Boronates 2-APB and 3-APB were synthesised from aminophenylboronic acids with good yields, then fully characterised by using multinuclear NMR spectrometry, IR spectrometry and high-resolution mass spectrometry (HRMS).[4,10] Our previous work demonstrated that a 3-APB-based hologram responds to both glucose and lactate; thus, utilising this sensor to monitor glucose levels in biofluids is problematic, because it is likely to suffer interference from lactate, which is a frequent metabolic byproduct. Therefore, the boronate 2-APB was designed to improve the utility of the 3-APB-based hologram. By moving the acrylamide group from the *meta* to the *ortho* position, the neighbouring group participation of the amido group through an intramolecular B−O-coordinated interaction occurs, resulting in the tetrahedral form of 2-APB being the dominant species. A number of boronic compounds containing *ortho* substituents, most of which contain B−N interactions, have been reported, and research into their binding affinity to analytes has been mainly based on a change in fluorescence or colour,[11] rather than on the geometry at the boron centre. The latter is a crucial factor that influences both their affinity for and selectivity towards glucose, because the boron geometry is closely related to the electron density on the boron atom as well as to that on the substituents attached to the boron centre. Consideration of such effects helps to explain why a variety of structures exist in a particular boronic acid, and hence their variable affinity towards different analytes. Therefore, investigating the structures of 2-APB in solution should enable a greater understanding of the behaviour of the 2-APB-based holographic sensors.

The $^{11}$B NMR chemical shift is sensitive to the type of substituents attached to, as well as to the amount of negative charge residing on the boron centre. Within a series of compounds bearing a similar substitution pattern at the boron atom, the $^{11}$B chemical shift values may be used to deduce their hybridisation states, and thus result in structural identification. Structure determination using $^{11}$B NMR spectrometry has been applied to many different types of boron-containing compounds.[12] Hence, in order to gauge the strength of the B−O bond of the intermediate, the boron geometry of 2-APB relative to 3-APB was explored by using $^{11}$B NMR spectroscopic pH titration (Figure 1). The titration curve of 3-APB shows a pH-dependent interconversion between the trigonal and tetrahedral forms. Below pH 6, the major species of 3-APB is the trigonal form ($\delta$ = 11.0 ppm); above pH 10, the tetrahedral form is dominant ($\delta$ = −15.3 ppm); between pH 6 and 10, a broad peak is observed, which shifts from $\delta$ = 11.0 to −15.5 ppm and suggests the presence of a mixture of the two species due to a fast exchange on the $^{11}$B NMR timescale. A p$K_a$ value of 8.87 for 3-APB was determined from the curve. It was no surprise that 2-APB behaved differently, with two species present at $\delta$ = −13.50 and −15.56 ppm, over the whole pH range. The latter form exists at pH > 11.0 and corresponds to species **C**, which possesses the normal tetrahedral structure (Scheme 1) that has been observed in 3-APB. An interesting fact is that over the wide pH range between 2.0 and 10.0, only one $^{11}$B NMR peak at $\delta$ = −13.50 ppm was observed, which is most likely due to a contribution from the boron di-heterocyclic tetrahedral zwitterionic adducts **A** and **B**. It is believed that the acrylic amido group is active enough to add readily and reversibly to the Lewis acid site at the boron centre in a process that generates zwitterionic adduct **A**. Similar zwitterionic boronic adducts have been reported previously.[9d] With increasing pH, the deprotonation of species **A** on N4 occurs, to generate species **B**, although $^{11}$B NMR analysis is unable to differentiate them because

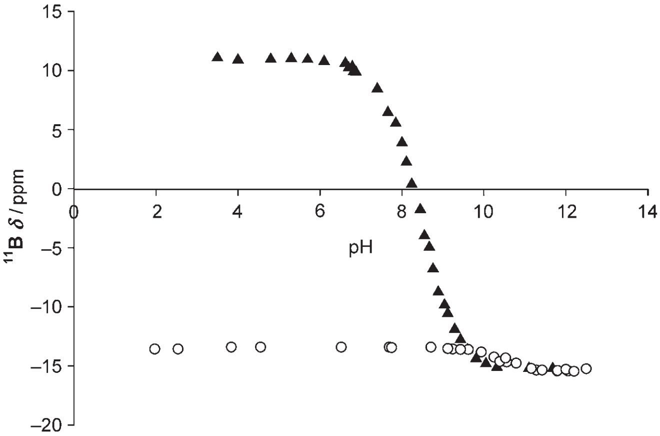
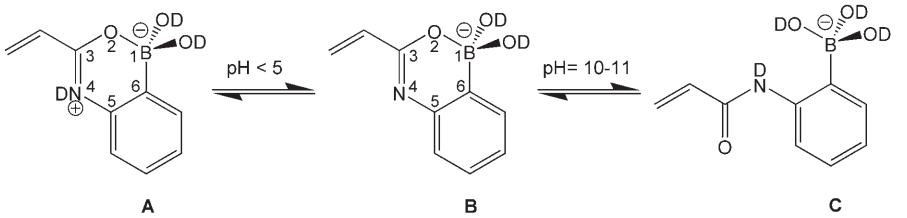


Figure 1. $^{11}$B NMR spectroscopic pH titration curves of 3-APB (▲) and 2-APB (○) in $D_2O$ (2–5 mg mL$^{-1}$) at 23 °C. DCl (0.1 M) and NaOD (0.1 M) were used for the titration.



Scheme 1. Equilibrium forms of 2-APB in $D_2O$.

their boron geometries are the same and the NH peak is exchanged by $D_2O$. However, pH titration of the 2-APB-based hologram provides significant evidence to strongly support the equilibrium between species **A** and **B**, as presented in Scheme 1. On increasing the pH to above 10, species **B** is converted quantitatively into species **C**, because a strong nucleophilic reagent ($HO^-$) is able to open the boron di-heterocycle by breaking the intramolecular B–O bond.

It is known that the zwitterionic adducts are the preferred form in polar and proton-donor solvents.[13] Interestingly, the $^{11}$B NMR spectrum of 2-APB in [D$_6$]DMSO exhibits a broad peak at $\delta = -5.30$ ppm, corresponding to a quasitetrahedral form instead of the zwitterionic tetrahedral species **A** or **B** in water (Scheme 1). In the quasitetrahedral form, the boron centre is not fully negatively charged like species **A** or **B**, because a weakly coordinated intramolecular B–O interaction occurs between the amide carbonyl and the boron. Furthermore, by increasing the temperature, a second species at $\delta = 9.30$ ppm appears, suggesting that the trigonal form is generated due to a cleavage of the weak intramolecular B–O bond (Figure 2). The presence of such a B–O bond in the quasitetrahedral form was also confirmed in the $^1$H NMR spectrum, in which the N–H resonance was observed at $\delta = 12.2$ ppm at room temperature. The NH signal is shifted upfield slightly and becomes broader with increasing temperature, suggesting that two species are present at higher temperature. Similar N–H peaks have been reported previously.[14] FTIR data revealed that there are very strong, relative to 3-APB, intermolecular hydrogen bonds among the OH groups present in the solid state of 2-APB and that the tetrahedral boron geometry of 2-APB remains even in the solid state.[10]

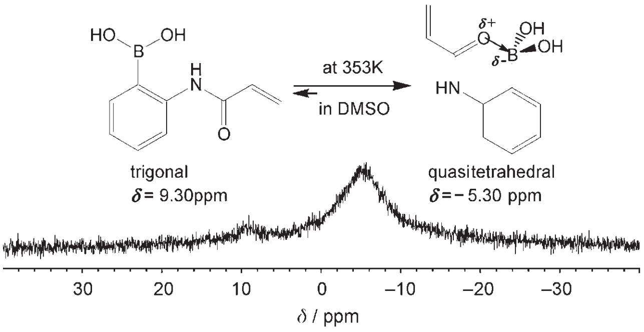


Figure 2. $^{11}$B NMR spectrum of 2-APB in [D$_6$]DMSO at 80 °C.

**Binding affinity of 3-APB and 2-APB in solution**: The binding of 3-APB with glucose at pH > 9 was clearly confirmed by using $^{11}$B NMR spectrometry because peaks of the free 3-APB and the complex were easily recognised at around $\delta = -13.5$ and $-10$ ppm, respectively. However, the binding of 3-APB with lactate occurs in acidic and neutral media, for example, its complex was observed at $\delta = -11.8$ ppm at pH 6.5. It is believed that the five-membered-ring ester complexes are formed in both additions; the significant response to both glucose and lactate has been previously observed in the 3-APB-based holographic sensor.[4]

Binding of 2-APB with glucose and lactate was carried out in [D$_6$]DMSO owing to the poor solubility of 2-APB in aqueous solutions except at high pH (>10). The $^1$H NMR spectrum shows significant changes in the chemical shifts of 2-APB observed after addition of glucose; for example, two NH peaks appear in the mixture instead of one NH peak in free 2-APB.[10] Furthermore, a fragment with $m/z = 334.6298$ was observed in the mass spectrum of the mixture, corresponding to the complex. These observations led us to the conclusion that 2-APB binds with glucose in [D$_6$]DMSO. However, no change was observed in the $^{11}$B NMR spectrum, suggesting that the complex containing the five-membered ring with glucose remains in the same quasitetrahedral form as free 2-APB (Figure 2).

Titration of 2-APB with sodium lactate shifts the $^{11}$B signal upfield from $\delta = -5.6$ (broad) to $-10.8$ ppm (sharp),[10] suggesting that the boron configuration is converted from the quasitetrahedral to the tetrahedral form. To consider the structural features of lactate, the effect of the carboxylic anion on the conversion was first investigated by titration with sodium acetate. The $^{11}$B NMR spectrum shows that the broad peak at $\delta = -5.6$ ppm displays no significant shifts after adding sodium acetate to free 2-APB, suggesting that the carboxylic anion does not affect the boron stereocentre because the intramolecular B–O interaction in the quasitetrahedral form blocks the anion from attacking the boron stereocentre. On the other hand, it was found that in methanol, 2-APB exists in a zwitterionic tetrahedral form ($\delta = -11.85$ ppm; Figure 3); such an effect of methanol on similar boronic acids with B–O bonds has been reported previously.[14] Hence, the conversion of the boron stereocentre is due to ester formation with the α-hydroxyl group of lactate,

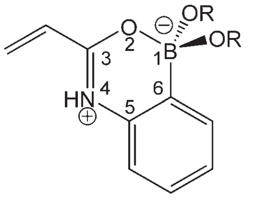


Figure 3. Zwitterionic tetrahedral structure of 2-APB in [D$_4$]methanol ($R = CH_3$) and 2-APB with lactate in [D$_6$]DMSO ($R = CH_3CH(OH)COONa$).

which plays a similar role to methanol (Figure 3), rather than due to the formation of a five-membered-ring lactate complex as observed with 3-APB. Investigations in solution suggest that boronic acid 2-APB is able to possess variable structures under different conditions (pH, solvent or temperature) because of the neighbouring effect of the substituents on the boron stereocentre, and can consequently generate a variety of affinities. In terms of the development of glucose-selective sensors, the tetrahedral forms may be the preferred species because they are stable and insensitive to pH; furthermore, the coordinated B–O interaction is believed to be strong enough to eliminate lactate interference.

**Holographic pH titration of 2-APB and 3-APB**: With a thorough understanding of the behaviour of 2-APB in solution established, an acrylamide-hydrogel-based hologram containing 2-APB as the responsive functionality was fabricated by means of photopolymerisation. The pH titration curves of the 2-APB and 3-APB holograms were recorded in order to evaluate the effect of pH on the holographic response (Figure 4). As the pH was increased, the 3-APB-based hologram was found to swell in the pH range from ≈7.5 to 12, resulting in a diffraction-wavelength shift of $\lambda \approx 160$ nm. The swelling can be explained by the generation of negative charges within the matrix due to the conversion from the trigonal to the tetrahedral form of 3-APB, in agreement with the pH titration results in solution (Figure 1). In the 2-APB-based hologram, however, substantial matrix swelling with a wavelength shift of $\lambda \approx 330$ nm was observed in the pH range from 8 to 11, which is believed to result from the ring opening in a conversion from species **B** to **C**, rather than a charge effect occurring in the 3-APB-based hologram. This ring-opening effect (Scheme 1), verified in solution, plays the opposite role to the cross-linking effect in polymer matrices; hence, it is reasonable to generate such a large shift. Between pH 6 and 7.5 there is a relatively static response to pH, because species **B** is the dominant form present in the hydrogel matrix. On continuously decreasing the pH from 5 to 3, a significant contraction ($\lambda \approx 50$ nm) was observed, due to the fact that species **B** with a negative charge on B1 is able to be protonated as the pH value falls and is converted to the zwitterionic species **A**, which possesses a negative charge on B1 and a positive charge on N4. The generation of species **A** neutralises the charge within the matrix, thus a contraction is observed. The holographic pH titration curve proves that the 2-APB-based hologram is relatively independent of the pH in the physiological pH range, and its reactivity in hydrogels is comparable to that in solution, both of which are important features for further application of this sensor.

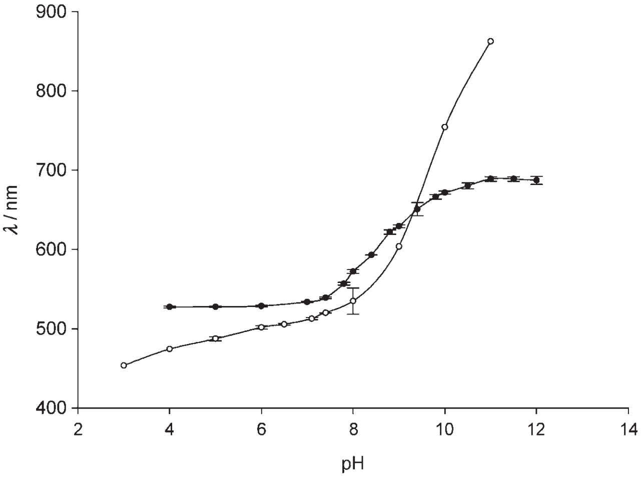


Figure 4. pH titration curves of 3-APB-based (12 mol %; ●) and 2-APB-based holograms (20 mol %; ○).

**Response of 2-APB-based holograms**: The response of the 2-APB-based holograms to glucose and lactate was also investigated using the methods reported previously.[4] The 2-APB-based holograms showed a significant contraction in response to glucose at physiological pH (7.1), whereas no significant response to lactate was observed (Figure 5). Furthermore, no significant change was observed in the overall response to an addition of glucose in the presence of lactate (10 mM) compared to the response in the absence of lactate. These results suggest that the interference from lactate is inconsequential for the 2-APB-based hologram in aqueous media. This may result from the predominance of the tetrahedral forms of 2-APB within the hydrogel matrix, because the tetrahedral forms favour yielding a cyclic ester with glucose via *cis*-diols, rather than complexing with lactate. Compared with a similar situation in 3-APB-based holograms,[4] it is apparent that employing the new ligand 2-APB significantly diminishes lactate interference. It is also interesting that the two holographic sensors behave differently towards glucose. The blueshift observed in the 2-APB-based hologram indicates that the hydrogel matrix contracts upon the addition of glucose, which is due to the formation of a 2:1

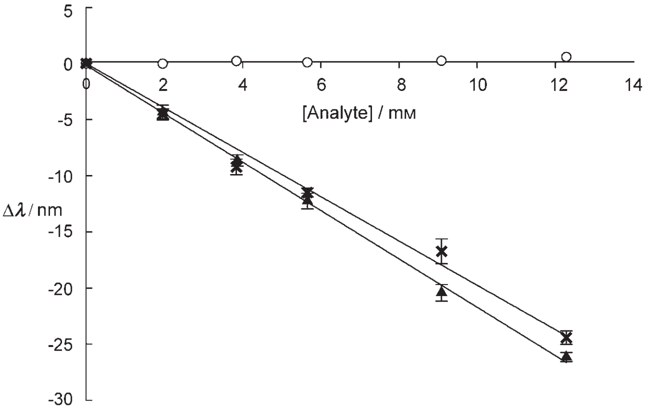


Figure 5. Response of a 2-APB hologram (20 mol %) in phosphate buffer solution (pH 7.1) to lactate (○), glucose (×) and glucose mixed with lactate (10 mM) (▲).

complex between 2-APB and glucose generating a cross-linking effect. A similar cross-linking effect through two binding sites on glucose with boronic acids has been proposed previously.[15] In contrast, hydrogel swelling is observed in the 3-APB-based hologram on addition of glucose. It is known that in the equilibrium of 3-APB there is a coexistence of trigonal and tetrahedral forms, which favour binding with lactate and glucose, respectively. In the physiological pH region, the trigonal species is dominant, and the 2:1 complex is less likely to occur due to the only small percentage of tetrahedral species present. As a result, the cyclic 1:1 complexes with glucose or lactate are predominantly generated in 3-APB-based holograms. Consequently, the response of 3-APB-based holograms to both glucose and lactate was swelling, because a charge effect in the hydrogels plays a key role through the generation of the negatively charged 1:1 complexes.

Additionally, the binding affinity to glucose in the 2-APB-based holograms is relatively independent of the buffer pH in the physiological region pH 5.8–7.8, which is shown by the fact that the gradients of the calibration curves are almost identical (Figure 6).[10] This relatively static response to pH across the physiological pH in the holographic pH titration curve suggests that species **B** is present in the matrix. In contrast, the response of the 3-APB-based holograms to addition of glucose varies significantly across the same pH range (Figure 7). The holographic pH titration curves indicate that the equilibrium of 3-APB shifts dramatically over this pH range; the percentage of the tetrahedral form increases and hence the binding to glucose changes accordingly.

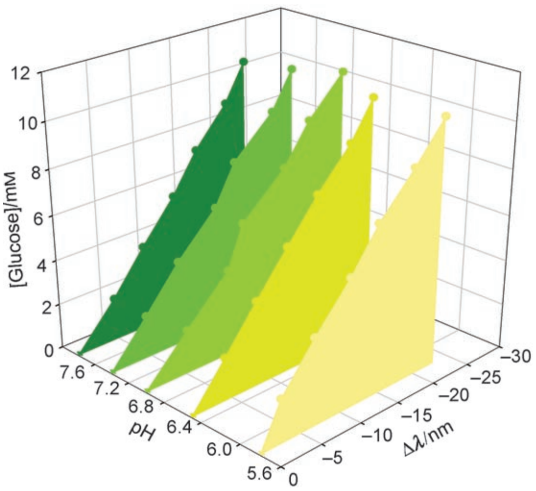


Figure 6. Glucose responses of a 2-APB-based hologram (20 mol %) at various physiological pH values: —●— pH 5.8, $y=-2.08x$ ($R^2=0.9867$); —●— pH 6.5, $y=-2.21x$ ($R^2=0.9991$); —●— pH 7.0, $y=-2.39x$ ($R^2=0.9929$); —●— pH 7.4, $y=-2.27x$ ($R^2=0.9968$); —●— pH 7.8, $y=-2.24x$ ($R^2=0.9973$).

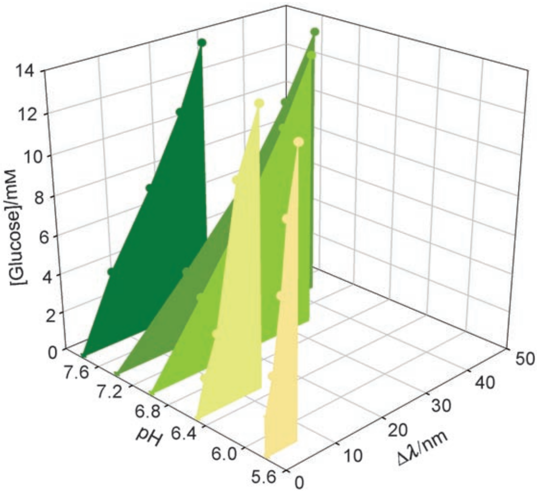


Figure 7. Glucose responses of a 3-APB-based hologram (12 mol %) at various physiological pH values: —●— pH 5.8, $y=0.44x$ ($R^2=0.969$); —●— pH 6.5, $y=0.87x$ ($R^2=0.9886$); —●— pH 7.0, $y=2.30x$ ($R^2=0.9823$); —●— pH 7.4, $y=3.40x$ ($R^2=0.9805$); —●— pH 7.8, $y=2.01x$ ($R^2=0.9964$).

## Conclusion

In conclusion, the acrylamide-hydrogel-based holographic sensor containing 2-APB as the glucose-binding functionality displays a significant response to glucose at physiological pH values with only minor interference from lactate. Furthermore, the binding affinity of 2-APB in the hydrogels to glucose is pH independent. The current results suggest that the boronate ligand 2-APB shows great potential for the development of glucose-selective holographic sensors for use in physiological media.

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