Designed Boronate Ligands for Glucose-Selective Holographic Sensors

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**Synthesis of 2-APB**

A well-stirred, cooled solution of 2-aminophenylboronic acid•HCl (0.75 g, 4.4 mmol) in aqueous sodium hydroxide (5N, 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 stirred for a further 2 h. The mixture was adjusted to pH 8 using dilute HCl (0.1 M). The resulting beige coloured precipitate was filtered off and washed with water and acetone. A fine and white powder was yielded with (~50%) after dried. $^1$H NMR (d-DMSO, ppm): 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 (m, 1H, =CH), 6.23 (m, 1H, =CH) and 5.88 (m, 1H, =CH). $^{13}$C NMR (d-DMSO, ppm): 117.05, 125.53, 128.73, 129.70, 133.72, 139.52 and 163.18. MS (ESI): 191.

A mixture of 2 and sodium lactate, MS (ESI): 381.1427 for $C_{15}H_{16}BNO_7Na$ +2H;

A mixture of 2 and glucose, MS (ESI): 334.6298 for $C_{15}H_{18}BNO_7$. 

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**FT-IR spectrum of 3-APB**

![FT-IR spectrum of 3-APB](image)
$^1$H NMR spectrum of 2-APB in DMSO

$^{13}$C NMR spectrum of 2-APB in DMSO
FT-IR spectrum of 2-APB

$\text{H NMR spectra of 2-APB and 2-APB+ glucose in DMSO}$
$2$ in DMSO

$2$ & glucose in DMSO

$^{11}$B NMR spectra of 2-APB and 2-APB + glucose in DMSO

$^1$H NMR of 2-APB and lactate in DMSO
2 & lactate in DMSO

2 in DMSO

$^{11}$B NMR of 2-APB and lactate in DMSO

$^{11}$B NMR of 2-APB and sodium acetate in DMSO
Synthesis of Co-polymer Films

3-APB based copolymer films:
The appropriate quantity of each monomer was dissolved to give a pre-polymer solution with the required molar ratios. In each case, the amount of cross-linker, MBA, was kept constant at 1.5 mol %. The monomer 3-APB (12 mol %) was dissolved in DMSO containing 2% (w/v) of the photo-initiator DMPA. The solid to solvent ratio of monomer to DMSO was kept constant throughout at 0.452:1 (w/v). A 100 µl droplet of monomer solution was pipetted onto the polyester surface of an aluminised polyester sheet sitting on a glass plate. A glass microscope slide which had been treated with 3-(trimethoxysilyl)propyl methacrylate (Mayes et al., 1999) was then gently lowered, silane-treated side down, onto the monomer mixture. Films were polymerised by UV initiated free radical reaction at room temperature for 30 min. Polymerised films were peeled off the polyester sheet whilst submerged in deionised water. Prior to hologram construction, the edges of each film were cleaned with a scalpel blade to remove any excess material.

2-APB based copolymer films:
The monomer 2-APB (20 mol %) mixed with 5.0eq of glucose was dissolved in DMSO containing 2% (w/v) of the photo-initiator DMPA. The solid to solvent ratio of monomer to DMSO was kept constant throughout at 0.452:1
(w/v). A 100 µl droplet of monomer solution was pipetted onto the polyester surface of an aluminised polyester sheet sitting on a glass plate. A glass microscope slide which had been treated with 3-(trimethoxysilyl)propyl methacrylate (Mayes et al., 1999) was then gently lowered, silane-treated side down, onto the monomer mixture. Films were polymerised by UV initiated free radical reaction at room temperature for 60 min. Polymerised films were peeled off the polyester sheet followed by exhaustively washing with water. The binding complex was converted to the free 2, and thereby releasing the favorable tetrahedral species present in the copolymer matrix. We term the process im printing photo-polymerization.

**Hologram Construction**

Holograms were constructed using the diffusion method as described by Blyth et al. (1999). The polymer films were placed face-down onto 400 µl of 0.2 M silver nitrate for 2 min. Excess solution was wiped off and the film was dried in a stream of warm air. Under red safe lighting, the slides were placed film side up in 40 ml of 4 % (w/v) KBr and 0.05 % (w/v) ascorbic acid in 1:1 methanol:H₂O (v/v) with 1 ml of 0.1 % (w/v) QBS in methanol and agitated for 1 min. The films were rinsed under distilled water and immersed polymer side down into the hologram exposure bath (Mayes et al., 1999; Marshall et al., 2003) containing PBS, pH 7.4 for 10 min. The whole area of the slide was then exposed to three single pulses from a frequency-doubled (532 nm) Nd:YAG laser. Figure x shows the exposure set up. The filtered laser beam from Nd:YAG laser source enters an achromatic lens ensuring production of images free of chromatic aberrations. The laser beam is reflected off one mirror onto another which is situated at the bottom of the exposure bath. The laser beam interferes with light returning from the mirror in the exposure bath. This forms a standing wave patterns in the acrylamide polymer with the silver fringes spread half a wavelength apart. The acrylamide-phenylboronic acid containing is situated on top of the microscope glass slide. This sits upon a small spacer which prevents it from lying flat along the mirror. This avoids the end hologram from diffracting the incident light at
precisely the same angle as the incoming white light. The slide was removed from the exposure bath and agitated for about 10 s in freshly made Saxby developer containing solution A (40 g/l ascorbic acid and 6 g/l 4-methylaminophenol in distilled water) and solution B (100 g/l anhydrous Na$_2$CO$_3$ and 30 g/l NaOH in distilled water). The holograms were developed by immersion in an agitated solution with a 4:1 (v/v) A:B developer solution. The time spent in the developer was typically around 10 s or until a holographic image emerged. The developed hologram was rinsed under distilled water and immersed in stop solution (5 % (v/v) acetic acid) for 30 seconds. After rinsing under distilled water, the film was immersed for 5 min in agitated 20 % (w/v) sodium thiosulphate to remove any undeveloped silver and rinsed in methanol and then distilled water.

**Holographic pH titration**

pH titration was performed by equilibrating and collecting the diffracted peak wavelength of the hologram at each pH. For each pH, 1ml 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, 1 ml of buffer was left in the cuvette and the hologram was allowed to equilibrate before the buffer was removed and replaced by the next pH. The following pH buffer systems were used: phosphate (pH 2), formate (pH 3), acetate (pH 4 and 5), MES (pH 6 and 7), Tris (pH 7.4 and 8), Bicine (pH 9), CHES (pH 10) and piperidine (pH 11 and 12). Each buffer was brought to the desired pH by drop wise addition of HCl or NaOH with the use of an electrode pH probe. For each solution, the buffer components were used at a concentration of 10 mM and the final ionic strength of the solutions were fixed to 154mM using NaCl. Salt and buffer solutions were prepared in ultra pure deionised water.

**Monitoring Holographic Responses**
Single strips of 2-APB based hologram, about 8 mm wide were cut from a slide and inserted into a 4 ml plastic cuvette with the film side facing inward. A buffer (1 ml) was added and the cuvette left for hours to equilibrate at 30°C in a temperature controlled cuvette holder. A reflection spectrophotometer was used to measure the wavelength of light reflected from a white light source by the hologram as determined by spacing of the fringes within the polymer.

The response of the holographic sensors to D-(+)-glucose in a buffer was tested. 0.1 M stock solution of glucose was equilibrated for overnight to ensure the relative proportion of glucose isomers present in the buffer. The response of the sensor to the addition of six 20 µl aliquots of the stock solution to the cuvette was recorded. Between each addition, the sensor was allowed to equilibrate to a stable diffraction wavelength. A 2x5 mm magnetic follower (Fisher) and stirrer arrangement was used to ensure constant agitation. The response to lactate and a mixture of glucose with 10 mM of lactate were also tested in a similar manner.
Glucose Response of 2-APB based Hologram at Variable pH Values
Glucose response of 3-APB based hologram at variable pH

![Graph showing glucose response at different pH values](image-url)