

Electronic Supporting Information (ESI) accompanying the paper:

“Communication between hydrogel beads via chemical signalling”

Ross W. Jagers and Stefan A. F. Bon^{*a}

^a S.Bon@warwick.ac.uk, Department of Chemistry, University of Warwick, Coventry, CV4 7AL, United Kingdom, www.bonlab.info

Materials

Alginic acid sodium salt (referred to as sodium alginate in this paper), calcium chloride hexahydrate (98%), calcium hydroxide ($\geq 95\%$, ACS reagent) urease from *Canavalia ensiformis* (Jack bean) type III (100K units), DL-dithiothreitol ($\geq 98\%$), silver nitrate ($\geq 99.0\%$) and urea (99.0 - 100.5%) were purchased from Sigma Aldrich. Acetic acid glacial and bromothymol blue, ACS reagent were purchased from Fisher Scientific.

Gel bead synthesis

Sodium alginate solutions were prepared by dissolving dry alginic acid powder in water at an appropriate concentration (5 or 10 wt. %) overnight. Once prepared, solutions were used within 3 weeks. 0.1 mol dm^{-3} aqueous solutions of silver nitrate were prepared and kept in darkness to prevent degradation. Once prepared, these were used within 1 week.

Urease beads

In the case of 1 and 5 g L⁻¹ urease beads, dry urease powder was dissolved into 5 wt. % sodium alginate solutions at appropriate concentrations. For lower concentration urease beads, 1 g L⁻¹ aqueous urease solutions were prepared, diluted as necessary, and combined with appropriate volumes of 10 wt. % sodium alginate solutions to produce solutions of a desired urease concentration. Bromothymol blue was added to each of these urease/sodium alginate solutions at a concentration of 2 mg mL⁻¹ and corrected to a pH of 3.5 using a 1 mol dm⁻³ aqueous solution of acetic acid.

Urease/silver beads

For beads containing both urease and silver nitrate, the above protocol (*urease beads*) was repeated. This was followed by the addition of an aliquot of 0.1 mol dm^{-3} aqueous solution of silver nitrate to produce solutions of a desired silver ion concentration. These solutions were kept in darkness to prevent degradation.

Silver beads

An aliquot of 0.1 mol dm^{-3} aqueous solution of silver nitrate was added to 5 mL of 5 wt. % sodium alginate solution to produce a solution of a desired silver ion concentration. Bromothymol blue was added at a concentration of 2 mg mL⁻¹ and corrected to a pH of 3.5

using a 1 mol dm⁻³ aqueous solution of acetic acid. The solution was kept in darkness to prevent degradation.

Dithiothreitol beads

0.08 g of dithiothreitol was added to 1 mL of 5 wt. % sodium alginate solution. Bromothymol blue was added at a concentration of 2 mg mL⁻¹ and corrected to a pH of 3.5 using a 1 mol dm⁻³ aqueous solution of acetic acid.

To form solid beads, these 4 alginate solutions were introduced to calcium ions, hereby ionically cross-link the alginic acid polymer chains to form millimetre sized beads with liquid cores. Beads make contact with calcium solutions for a few minutes, which is not long enough to cross-link throughout the bead. Alginate beads were cross-linked by dropping the alginate solution into a 0.1 mol dm⁻³ solution of calcium chloride hexahydrate from a pipette tip. For silver beads, a 0.1 mol dm⁻³ aqueous solution of calcium hydroxide was used as the calcium ion source (so as to prevent precipitation of silver chloride). Both solutions are corrected to pH 3.5 using a 1 mol dm⁻³ aqueous solution of acetic acid. Beads are rinsed in deionised water before use.

Communication experiments

For all experiments, gel beads were immersed in a petri dish containing 20 mL of deionised water and 2 mL of a 1 mol dm⁻³ aqueous solution of urea corrected to a pH of 3.5 using a 1 mol dm⁻³ aqueous solution of acetic acid.

Communication experiments were filmed on a Nikon D5100 camera with AF-S DX Micro NIKKOR 40mm f/2.8G Lens.

Full timings breakdown, calculated from supporting videos

Experiment	Figure	Onset of blue colour / seconds	
		Blank bead	Paired bead
1 g L ⁻¹ enzyme/silver bead	2	25	n/a
1 g L ⁻¹ enzyme + silver bead	3a	38	43
0.25 g L ⁻¹ enzyme + silver bead	3b	82	130
0.125 g L ⁻¹ enzyme + silver bead	3c	218	n/a
5 g L ⁻¹ enzyme/silver + DTT bead	4	n/a	42
0.125 g L ⁻¹ enzyme + silver + DTT bead	5	214	420