USE OF A GLASS FIBRE MEMBRANE (GF/DVA) TOWARDS THE DEVELOPMENT OF A LATERAL FLOW ASSAY FOR DETECTION OF TRICLOSAN IN RIVER WATER

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We have developed a fibre-based assay for environmental monitoring of triclosan (TSC), a persistent freshwater pollutant, based on a competitive antibody-antigen recognition system. Typically nitrocellulose is used in such systems however to improve antibody binding we have used a glass fibre membrane or chemically modified cellulose to bind capture antibodies then perform visual detection via the competitive binding of TSC and HRP labelled TSC. Using the glass fibre membrane, GF/DVA we have successfully demonstrated an approach to visually detect TSC at environmentally relevant concentrations between 0.05 – 2.5 µg L^{-1}.

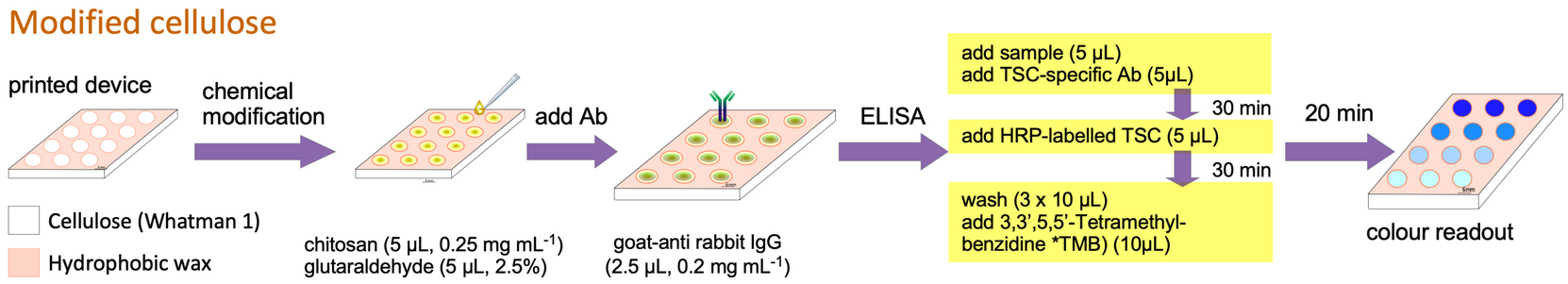
Context

- Potentially harmful compounds enter waterways from domestic sources, including **triclosan (TSC)**.[1]
- found in toothpastes and antibacterial soaps
- endocrine disruptor even at <ppb levels.
- may pose significant harm to aquatic organisms.[2]
  → **Currently monitoring of TSC is limited.**
- **Lateral-flow assays** employing antibody-antigen (Ab-Ag) recognition and colourimetric labels often employed in clinical point-of-care diagnostics [3]
  → Would be attractive for **environmental analysis**.
- Robust immobilisation of capture Ab critical.
- Physical absorption of Ab onto nitrocellulose (NC) can be inaffective .[4]
- Alternative = Chemically modified cellulose, *e.g.* chitosan cross-linked with glutaraldehyde (Chi-GA).[5]
- Glass fibers offer **flexible surface chemistry**, **favourable protein binding**, **fast wicking rates** (≥ 5x than NC), and increased thickness allows larger water samples to be handled.

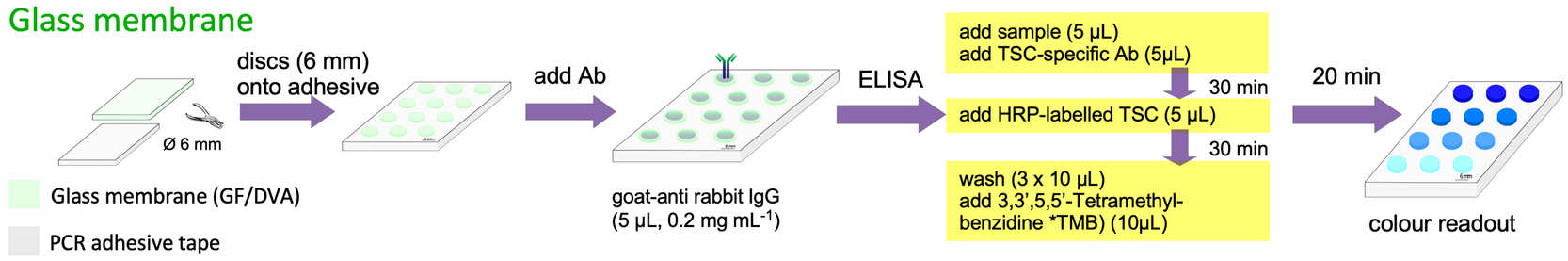
→ Investigate potentially very sensitive competitive ELISA for TSC on fibre devices, by adapting commercial microwell-based kit

Device manufacture

Chemically modified cellulose

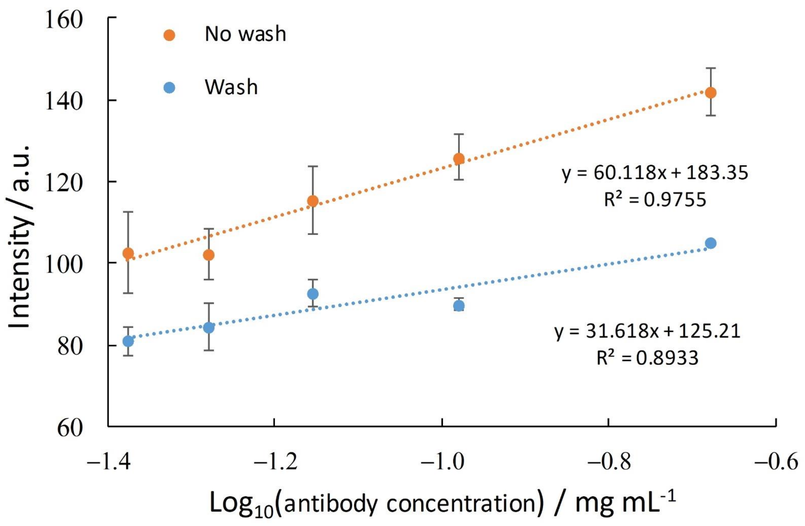


Glass membrane



Visualisation of Ab immobilisation

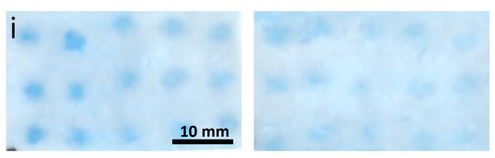
Ab binding visualised using Coomassie Blue Safe stain.



- 1 µL Ab deposited and dried on glass membrane at 0.2-0.04 mg mL^{-1}.
- Stained for 10 min, then de-stained with water for 10 min.
- Staining intensity decreased with wash step, however some Ab remained bound.

GF/DVA membrane

No wash step | Wash step

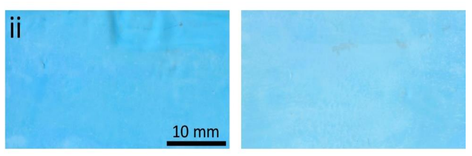


0.2, 0.1, 0.07, 0.05, 0.04 mg mL^{-1} antibody concentration

→ Ab immobilisation successful

NC membrane

No wash step | Wash step

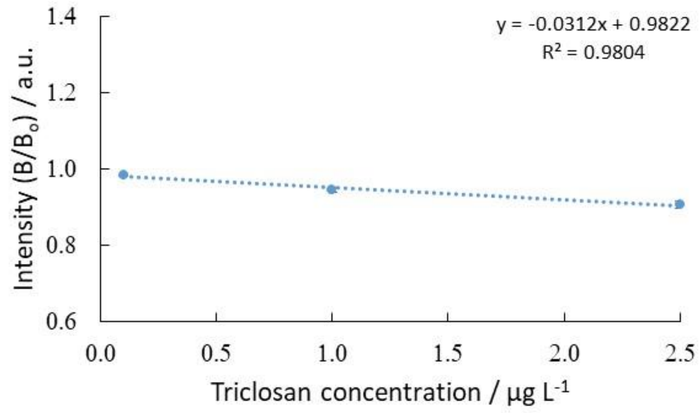


0.2, 0.1, 0.07, 0.05, 0.04 mg mL^{-1} antibody concentration

→ Ab immobilisation unsuccessful

Detection of TSC on membranes

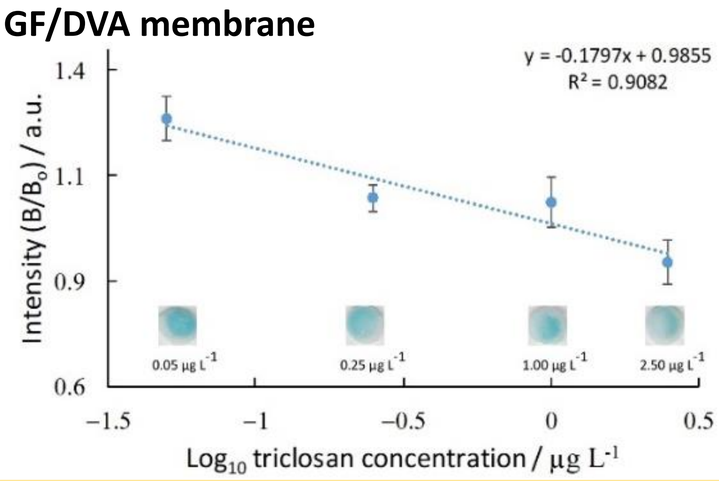
Chi/GA modified cellulose



- ELISA performed on modified cellulose using 1/10th of volumes for standard assay.
- TSC concentrations 0 – 2.5 µg L^{-1} (n = 3).
- 7% loss of colour intensity between 0 and 2.5 µg L^{-1} TSC.

→ Limited difference between 0 and 2.5 µg L^{-1} TSC

GF/DVA membrane



- ELISA performed on GF/DVA membrane using 1/10th of the volumes for standard assay.
- TSC concentrations 0 – 2.5 µg L^{-1} (n = 4).
- 26% loss of colour intensity between 0 and 2.5 µg L^{-1} TSC.

→ Clear difference between 0 and 2.5 µg L^{-1} TSC

Conclusions

- Robust binding of Ab to nitrocellulose membrane, the most commonly used method, was unsuccessful for this system at any Ab concentration.
- Successful Ab binding achieved using chemically modified cellulose and glass membranes, providing different options for development of a fibre-based ELISA for TSC.
- Detection of triclosan at sub-ppb levels, as found in the environment, via competitive ELISA performed on GF/DVA membrane shows potential to develop this into on-site detection for monitoring of TSC.
- Future work: development of simple to operate lateral flow assay for frequent on-site field analysis.

Acknowledgments

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