

# The development of molecularly imprinted polymers for sensor and colorimetric assay applications

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## Summary

The presented thesis investigates the uses of MIPs in various sensing applications, drawing close attention to the possibility of using MIPs in low-cost sensors and assay formats. More specifically, sensing arrays were investigated that would prove beneficial to the in-the-field-testing of illicit or controlled substances.

**Chapter 1** introduces the idea of molecular sensing and the different methods developed for the analysis of molecular species. This builds the bigger sensing picture, directing the reader towards the use of molecularly imprinted polymers in sensing platforms.

**Chapter 2** conducts a deep dive into the current status quo of molecularly imprinted polymers, drawing specific attention towards low-cost sensing platforms and assay formats. The review paper shows how MIPs have been applied to the sensing to a whole range of molecular species on a research level, though struggle to be applied in a commercial setting. One of the reasons discussed hinges on the reliability and reproducibility of MIPs, which in turn also questions the associated sensing platforms that utilize these synthetic receptors.

**Chapter 3** seeks to offer a solution to the reliability and reproducibility questions were raised in the previous chapter. To this end, a method of directly grafted molecularly imprinted polymeric receptor layers is highlighted. The methodology discussed enables the synthesis of receptor layers across the surface of an aluminium substrate, thus allowing the measurement of the targets presence via thermal impedance. The selected target for the experiments was the New Psychoactive Substance (NPS) 2-methoxyphenidine, demonstrating that MIPs could be used to detect newly found substances of concern for law enforcement. The presented results demonstrate a superior limit of detection (LoD) of the sensor compared to traditionally generated receptor layers. Correlating the thickness of the newly grafted layers to the LoD achieved.

**Chapter 4** developed the idea of sensing controlled substances further, by designing a sensing method that is not reliant on electrical transducers. The proposed dye displacement assay demonstrated how a MIP could be modified by preloading a dye that can be displaced to the surrounding medium in the presence of the target molecule. The resulting assay demonstrated a selectivity

towards the NPS target, and showed a vibrant colour transformation that allowed for naked-eye detection of the chosen compound. The research builds a relationship between the imprint factor (IF) of a MIP and the binding capabilities (BF) of a dye molecule, aiming to generate a mathematical probability of the dye being displaced by the target substrate. Various other facets of the sensor are investigated, optimising the incubation time, and amount of MIP required to generate a colorimetric response.

**Chapter 5** builds further on the concept of dye displacement assays, replicating the process for a more traditional drug of abuse (amphetamine). This work highlights how the concept can be adapted to other compounds and not just the model compound introduced in the previous paper, thus lending to its potential for commercial viability. Various dye molecules (crystal violet, basic blue, pararosaniline, methyl orange and phenol red) are tested for suitability as a displaceable dye indicator, with crystal violet proving the most suitable. The sensor is shown to displace dye in a quantitative manner, relating the amount of amphetamine present to the amount of dye displaced. The research culminates in the use of the sensor in spiked urine, providing similar amount of dye displaced in both urine and PBS.

**Chapter 6** expands on the knowledge base previously built in the prior two chapters, and extends the use of dye displacement assays towards the detection of antibiotics. In this chapter, the rational design of a MIP towards the detection of amoxicillin is outlined, proposing various monomer: crosslinker pairings for the binding of the antibiotic in aqueous medium. These compositions were implemented in monolithic bulk imprinted polymers, with the best composition (MAA/EGDMA) being carried forwards to the development of MIP particles by an emulsion polymerization approach. The more elegantly synthesized particles showed greater homogeneity in both terms of size and morphology over their monolithic counterparts, leading to their use in the ensuing assay. Mordant orange was selected as the dye to be pre-loaded onto the MIP for the assay after a study with itself, malachite green and crystal violet. The produced sensor was then subjugated to amoxicillin, cloxacillin, and ampicillin, enabling the sensitivity and selectivity of the assay to be evaluated.

Finally, the results are critically analyzed in **Chapter 7**. In this chapter, the research findings are debated, drawing attention to the windfalls and pitfalls of each of the developed sensors.