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Development of a Flexible MIP-Based Biosensor Platform for the Thermal Detection of Neurotransmitters

Kai Betlem[1], Michael P. Down[1], Christopher W. Foster[1], Shamima Akthar[1], K. Eersels[2], B. van Grinsven[2], T.J. Cleij[2], C.E. Banks[1], M. Peeters[1*]

1) Manchester Metropolitan University, Faculty of Science and Engineering, Div. of Chemistry & Environmental Science, John Dalton Building, Chester Street, M15GD, Manchester, United Kingdom. 2) Maastricht University, Maastricht Science Programme, P.O. Box 616, 6200 MD Maastricht, The Netherlands.

ABSTRACT

We have developed high affinity Molecularly Imprinted Polymers (MIPs) for neurotransmitters such as dopamine, noradrenaline and caffeine. These polymer particles are mixed within the bulk of screen-printed ink allowing masss-producible bulk modified MIP Screen-Printed Electrodes (MIP-SPEs) to be realised. We have explored different SPE supporting surfaces, such as polyester, tracing paper and household-printing paper. The performance of those MIP-SPEs is studied using the Heat-Transfer Method (HTM), a patented thermal method. With the combination of screen-printing techniques and thermal detection, it is possible to develop a portable sensor platform that is capable of low-cost and straightforward detection of biomolecules on-site. In the future, this unique sensor architecture holds great promise for the use in biomedical devices.

INTRODUCTION

Molecularly Imprinted Polymers (MIPs) are synthetic antibody mimics; similar to antibodies, they have high affinity for a chosen template molecule but their advantages include low-cost, superior chemical and thermal stability, and straightforward production process [1,2]. These polymeric receptors are widely used in purification and separation processes but have limited applications in the field of biosensors [3]. Key challenges in that area include difficulty to incorporate MIPs into suitable sensor platforms and the lack of low-cost and straightforward sensing strategies.

In this contribution, we will focus on using SPEs as sensor platforms since they are highly reproducible, can be used as disposable electrodes and their production process can be tailored with our in-house facilities. We were the first to report on novel and simple functionalization strategy for the direct incorporation of MIPs into SPEs [4]. These MIP-SPEs will be combined with the Heat-Transfer Method (HTM) as sensing strategy. HTM is a patented thermal analysis technique [5] that relies on evaluating the thermal resistance at the solid-liquid interface. This method has commercial potential but is not currently

suitable for mass-production due to various reasons, including the use of home-made design of the thermocouple device and flow cells.

We will address those issues by mass-producing MIP-SPEs for polymer particles designed for a variety of neurotransmitters and use of 3D-printing to develop and optimize flow cell design. This will overcome important hurdles that need to be taken for the commercial application of MIP-based thermal sensors, which holds great promise for future use in various areas such as biomedical devices or on-sight environmental analysis.

EXPERIMENTAL

MIP synthesis

MIPs for noradrenaline were produced as described in ref [4]. MIPs for caffeine were synthesized according to a protocol adapted from ref [6], using methacrylic acid and acrylamide as monomers in varying ratios.

For the selective recognition of dopamine, a range of polymers was produced to optimize specificity. Table 1 provides an overview of the chemicals and quantities that were used. Prior to the polymerisation, stabilisers in the monomer and crosslinker monomers were removed by filtering the solution over basic alumina oxide. Dopamine hydrochloride (1 mmol) was then dissolved with the functional monomers (2 mmol), ethylene gylcoldimethacrylate (10. mmol) into a suitable porogen (5 ml). Subsequently, the initiator azobisisobutyronitrile (50 mg AIBN) was added and the solution was purged with a gentle flow of nitrogen for ~5 min. The mixture was then polymerised under reflux at 60°C for 12h and was ground to obtain microparticles. Extraction was performed with various solvents, including a methanol/water mixture (12h), acetic acid combined with acetonitrile (48h) and finally methanol/water (12h). The extraction process was monitored by determining the amount of template in the filtrate. After extraction was complete, the polymers were ground to obtain microparticles and dried under vacuum for 12h. Particles were sieved to only obtain those with sizes smaller than <10 µm. Non-Imprinted Polymers (NIPs) were prepared in the same manner but without addition of the template. Binding to the MIP and NIP was determined by optical batch rebinding experiments, demonstrating equilibrium was reached after a binding time of 1h.

	Functional monomer (mmol)	Porogen
MIP 1	2-Hydroxyethyl methacrylate (HEMA, 2 mmol)	Methanol / water (80%, 20%)
MIP 2	Itaconic acid (2 mmol)	Methanol / water (80 %, 20%)
MIP 3	HEMA / Itaconic acid (1 mmol, 1 mmol)	Methanol / water (80 %, 20%)
MIP 4	HEMA (2 mmol)	DMSO
MIP 5	Itaconic acid (2 mmol)	DMSO
MIP 6	HEMA / itaconic acid (1 mmol, 1 mmol)	DMSO

Incorporating MIP particles into SPEs

Details about the functionalization procedure are described in ref [4]. A carbon-graphite ink formulation (C2000802P2 from Gwent Electronic Materials Ltd) was printed onto substrates with different substrates, including polyester and paper, and cured at 60°C for 30 min. The MIPs were incorporated into the bulk of the graphite ink on the basis of the weight percentage M_p and M_i, where M_p is the mass of the particulate, in this case the MIPs, and M_i is the mass of the ink formulation used in the printing process, *i.e.* = (M_p/M) x 100. 30% was chosen in this work because this is the balance between optimum MIP availability and conductivity of the ink. The particles are insulating and concentrations higher than 30% are not sufficiently conductive enough to be used in the screen-printing process. It was noted that the mixing of the MIPs with the graphite ink is strongly dependent on the hydrophobicity of the material.

Thermal measurements

MIP-SPEs were mounted into a home-made thermocouple device, which design is described in ref [5]. This was equipped with a automated NE500 syringe pump from ProSense (the Netherlands), allowing to control the inflow and flow rate of samples added to the flow cell. The flow cell was fabricated by 3D-printing and a technical drawing (*Solidworks 2016 3D CAD (SP4) Software package*) is provided in Figure 1.

The model was printer utilising a FORM 2 stereolithography (SLA) 3D-Printer from FormLabs (USA) with a layer height of 25 µm. The model was printed utilising FORM 2 Clear Resin (GPCL04), before being bathed and washed in isopropanol to remove any uncured polymer resin. The flow cell channels were cleared using a syringe of isopropanol to ensure no polymer resin would block or vary the diameter of the flow cell channels during the final cure. The printed flow cell was then fully cured in a UV post-print chamber to ensure the complete polymerization of the material and the stabilization of the mechanical properties.

A copper block is used to close off the flow cell and to serve as a heat sink. The temperature gradient is then determined as the difference between the temperature at which the copper is controlled (T_1) versus the temperature that is monitored in the liquid (T_2) by a thermocouple type K. In the design that we used the thermocouple is inserted at 1.7 mm above the electrode in the liquid, but with the 3D design it is possible to adjust this. The total volume of the flow cell is equal to 110 µL. The thermal resistance, R_{th}, is then defined as $(T_1-T_2)/P$. P is calculated is as the voltage squared over the internal resistance (22 Ohm). The voltage is monitored by the thermocouple device, temperature T_1 was kept constant at 37.00°C.

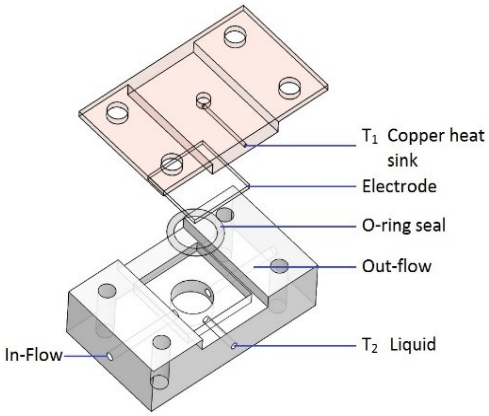

Figure 1. Technical drawing of the flow cell, demonstrating the in –and outlet, position of the thermocouples T_1 and T_2, O-ring used to seal off the sample and the copper block on top that acts as a heat sink. With eight connections to the thermocouple device, there is the possibility to develop an assay format.

RESULTS AND DISCUSSION

Binding affinity was determined by mixing polymer particles with solutions (phosphate buffered saline solutions of pH=7) of known neurotransmitter concentrations. After filtering off the MIP and NIP powders, the absorbance in the solution was determined and used to calculate how much had bound to the particles. To quantify the specificity of the MIP towards it target, the imprint factor (IF) is used which is defined as the amount bound to the MIP divided over the amount bound to the NIP at a certain concentration.

For the noradrenaline MIP, at pH=7 the solutions were unstable and the neurotransmitter oxidized. At pH=6, better results were obtained with a max binding of 25 µmol/g and an imprint factor of approximately 1.6.

The optimized MIP mixture for caffeine detection, compromised of a 1:1 ratio of acrylamide and methacrylic acid, exhibited better specificity towards it target. A IF of 2.5 was achieved at pH=7, with a maximum binding capacity of 50 µmol/g. This is potentially because a combination of monomers is better able to bind targets if they have different hydrogen bond donor and acceptor groups on the molecule.

The results for dopamine are summarized in Table 2. To test the selectivity, the response of the MIP towards caffeine and noradrenaline was also measured and only minimal binding (max 15 µmol/g) was observed.

Table 2. Binding capacity and specificity of MIPs developed for dopamine

	Max binding capacity (µmol/g)	Imprint Factor (IF) -
MIP 1	49.0	1.1
MIP 2	60.8	1.6
MIP 3	110.0	1.9
MIP 4	41.0	1.1
MIP 5	58.0	1.5
MIP 6	95.0	1.9

The polymers produced with solely HEMA had the lowest amount of binding compared to the other monomer compositions. A potential explanation is that HEMA offers only one charged group for interaction, while itaconic acid has a higher charge density to increase interactions between monomer and template. The combination of itaconic acid and HEMA yielded in the highest binding and IF. The porogen did not seem to have a significant effect on template binding.

The produced MIP-SPEs were produced with a 30% mass of particulate vs ink ratio. MIPs were visible (Scanning Electron Microscopy) on the surface of the SPE but it was difficult to quantify since there was little contrast between the graphite based ink and the polymer. In the future, it might be worth considering adding a (fluorescent) label onto monomer or initiator to provide accurate values of the surface coverage. After stabilizing in a buffered solution, solutions with increasing concentration of neurotransmitter were added. The temperature in the set-up was kept at 37.00°C to mimic body conditions. After addition of a solution that was kept at room temperature, a spike in the thermal resistance is observed after which the signal gradually goes down. This corresponds to the feedback loop of the set up that will adjust the temperature back to its original value. For the NIP-SPEs, after stabilization the signal will then return to its original baseline value. For the MIP-SPEs, gradual increases in the thermal resistance are observed which is due binding of the template in the porous structure of the MIP that blocks heat-transfer through the surface (described in pore-blocking model, ref 6). A typical example of a heat-transfer measurement is shown in Figure 2. Dose-response curves are constructed to determine the limit of detection. For noradrenaline, it was not possible to determine this due to instabilities of the signal in phosphate buffers. For the caffeine MIP, it was estimated to be around ~50 nM, while for the dopamine MIP this was around 25 nM in buffered solution. Limit of detection depends on affinity of the MIP towards its target, but also towards how compatible the polymers are with the ink that is used in the screen-printing process. The limit of detection was at least an order of magnitude better compared to when electrochemical (Cyclic Voltammetry) measurements were performed on the functionalized SPEs.

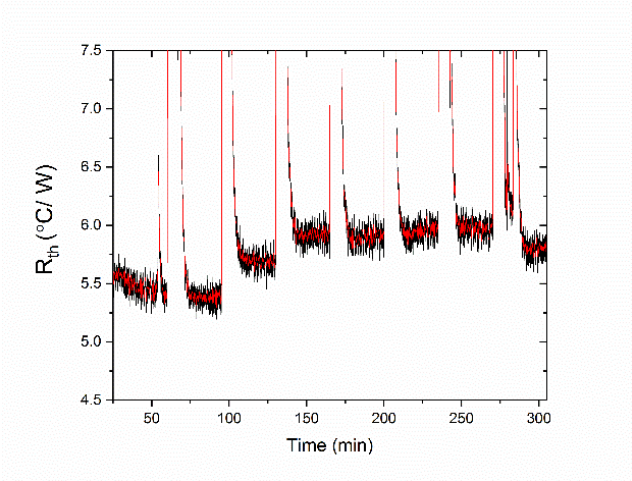

Figure 2. Thermal resistance for the MIP-SPEs printed for caffeine in time. The sensor was stabilized in PBS for 50 min, followed by additions of the following solutions with concentrations 0.05, 0.1, 0.15, 0.25, 0.5, 0.75 mM. The red line shows a percentile filter (average 10 points), which reduces LoD to ~25 nM.

CONCLUSIONS

MIPs for the neurotransmitters noradrenaline, caffeine and dopamine were developed. These were integrated into SPEs by direct mixing of the polymer particles with the ink, which is a fast and scalable production strategy. It was possible to determine neurotransmitter levels in the nanomolar regime using thermal detection, which is promising fast and low-cost sensing strategy with high commercial potential.

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