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Citation for published version (APA):

Rogosic, R., Lowdon, J. W., Heidt, B., Dillen, H., Eersels, K., Van Grinsven, B., & Cleij, T. J. (2019). Studying the Effect of Adhesive Layer Composition on MIP-Based Thermal Biosensing. *Physica Status Solidi A-applications and Materials Science*, 216(12), Article 1800941. <https://doi.org/10.1002/pssa.201800941>

## Document status and date:

Published: 01/06/2019

## DOI:

[10.1002/pssa.201800941](https://doi.org/10.1002/pssa.201800941)

## Document Version:

Publisher's PDF, also known as Version of record

## Document license:

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# Studying the Effect of Adhesive Layer Composition on MIP-Based Thermal Biosensing

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Molecularly imprinted polymer (MIP)-based thermal sensing has proven to be a very interesting tool for diagnostic purposes. However, many fundamental phenomena are not yet fully understood. In the following study, MIPs are imprinted with the new psychoactive substance methoxphenidine (2-MXP). Thermal detection of this compound in water is demonstrated for the very first time and the effect of varying the adhesive layer composition on the performance of the sensor is analyzed. Three different polymers are used to create a uniform adhesive layer. The surface coverage of MIPs on each of the layers as well as the heat-transfer properties are studied. The results of the study indicate that the chips coated with poly(vinyl butyral-co-vinyl alcohol-co-vinyl acetate) display a higher surface coverage and a lower thermal resistance value. This results in an improved effect size and therefore improves dynamic range of the sensor..

polymeric layer as illustrated in **Figure 1**. Larger entities such as mammalian cells get imprinted directly into this layer, creating a homogenous receptor layer directly onto the surface of the measurement chip.<sup>[16]</sup>

MIP particles for small molecules are often made in an external reaction vessel and need to be deposited onto the planar measurement electrode using an immobilization layer made by, e.g., spin coating or dip coating. In the latter case, it was recently demonstrated that it is possible to dip coat thermocouples with DNA and roll coat them with polymeric particles for the direct detection of molecules in aqueous media without the need of planar electrodes.<sup>[17]</sup> Although, this approach is promising, the smaller contact area of the thermocouple limits the sensitivity of the methodology. Therefore, in most cases

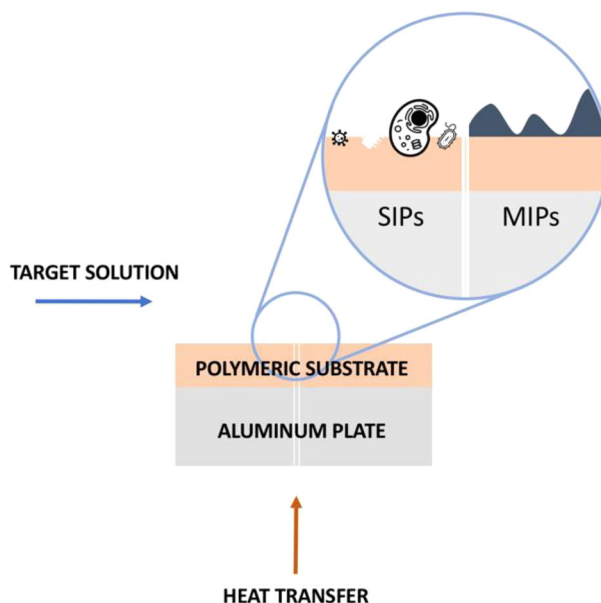
## 1. Introduction

Molecularly imprinted polymers (MIPs) are synthetic receptors that are designed to mimic the binding affinity natural receptors have for their target.<sup>[1,2]</sup> The wide applicability of imprinting technology, ranging from separation, and purification to point-of-care-diagnostics, can be attributed to the generic nature of the technology and the ability to mimic the affinity natural receptors have for their target. Other benefits include their low-cost and straightforward synthesis procedure and their thermal, chemical and long-term stability.<sup>[3–6]</sup> MIP-based biosensors have been made for small molecules as well as large macromolecular entities such as cells and pathogens.<sup>[7–12]</sup> The commercial potential of these sensors depends on the quality of the receptor layer but also on the readout method used for analyzing rebinding. In recent years, the authors of this article introduced a novel sensing technique based on analyzing thermal transport over a liquid interface. This low-cost and fast readout method, coined the heat-transfer method (HTM) has proven to be particularly useful in combination with synthetic receptors.<sup>[13–15]</sup> Traditionally, aluminum chips are coated with a

MIP particles are immobilized onto planar electrodes by means of an immobilization layer, often poly-vinyl-chloride (PVC) due to its wide-spread availability and low cost price.<sup>[18]</sup> This straightforward approach allows to stamp the particles into the adhesive layer or sink them into the layer by sedimentation. However, little is known about the effect of the adhesive layer on thermal transport properties, which might play a role in the effect size of observed phenomena and the limit-of-detection of the methodology. In this study, the authors compared the effect of using three different polymers; poly(vinyl butyral-co-vinyl alcohol-co-vinyl acetate) (Polymer 1), poly-vinyl-chloride (Polymer 2), and polystyrene-block-poly(ethylene-ran-butylene)-block-polystyrene (Polymer 3) on the effect size in a thermal measurement experiment. The different polymers were selected based on their good adhesive properties, transparency to allow visual examination and glass transition temperature for MIP adhesion. The effectiveness of the deposition method was examined by optical analysis after which the effect on thermal transfer measurements was studied. MIPs were made for the new psychoactive substance methoxphenidine (2-MXP) and their performance was assessed by comparing the response of a MIP to a non-imprinted reference (non-imprinted polymer or NIP). Thermal detection of this compound by bulk MIPs was demonstrated for the first time and the measurements indicate that the optimal deposition of MIP particles observed using poly(vinyl butyral-co-vinyl alcohol-co-vinyl acetate) co-polymer as an immobilization layer, also resulted in an improved effect size and linear range of the resulting biomimetic sensor.

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DOI: 10.1002/pssa.201800941



**Figure 1.** Concept of heat transfer method (HTM) combined with surface imprinted polymers (SIPs) and molecularly imprinted polymers (MIPs). The polymeric substrate has a crucial role in both applications, serving as functionalized surface in the SIPs and as adhesive in the MIPs, while in both cases being the active layer that vehicles the heat transfer across the sensor.

## 2. Results

### 2.1. MIP/NIP Surface Coverage

The particle distribution was analyzed using an optical microscope in reflection mode. The results are shown in **Figure 2** and were transformed for analysis of the surface coverage using Image J.

Samples were coated with an adhesive layer composed of poly(vinyl butyral-co-vinyl alcohol-co-vinyl acetate), poly(vinyl-chloride and polystyrene-block-poly(ethylene-ran-butylene)-block-polystyrene, which from now on will be denoted Polymer 1, 2, and 3, respectively, for reasons of simplicity. The average surface coverage and standard deviation were calculated for each of the polymers under study and plotted in **Figure 3**. A significant difference in particle deposition coverage was found among the three differently coated samples. The highest surface coverage was observed on the sample coated with Polymer 1, with a value of  $(80.07 \pm 2.14\%)$  in comparison to Polymer 3  $(70.83 \pm 1.37\%)$  and Polymer 2 with a value of  $(63.71 \pm 3.64\%)$ .

### 2.2. Effect of Adhesive Layer Composition on Thermal Properties of Solid-Liquid Interface

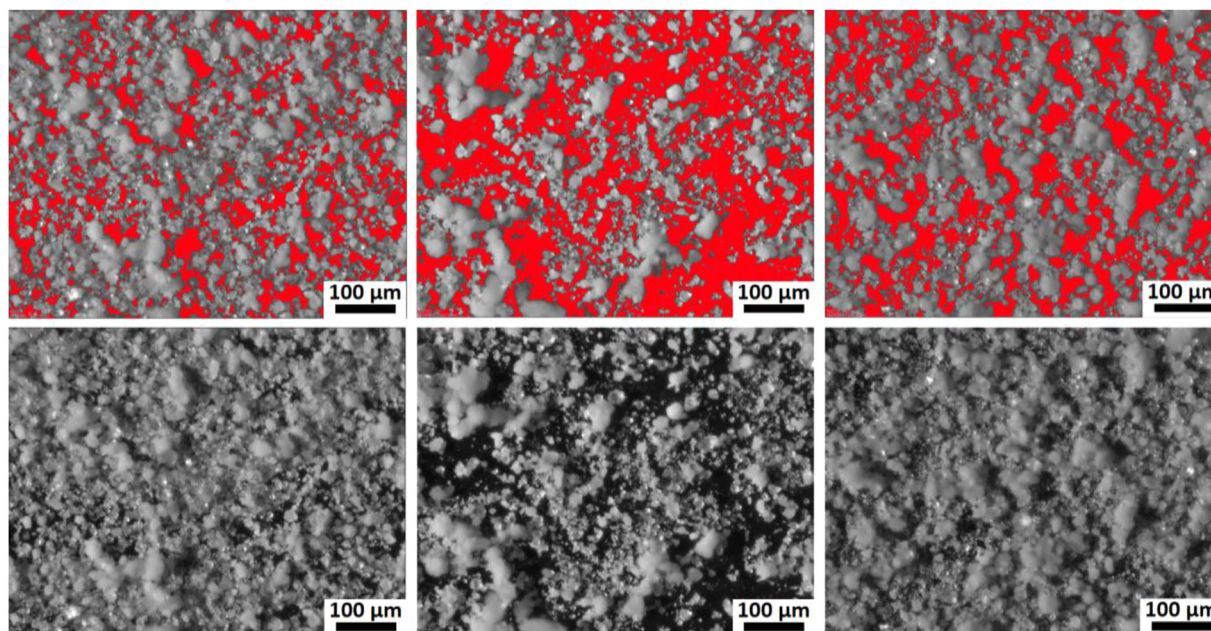
In order to have a better insight into the effect of using different adhesive layers on thermal transfer characteristics, background measurements (prior to MIP/NIP deposition) were performed for each of the polymers under study (**Figure 4**) on three identically prepared samples. After a stabilization period of 700 s with the measurement chamber filled with deionized (Milli Q or MQ) water, the interfacial thermal resistance was measured for each sample across a 100 s period as described in the

experimental section. As shown in **Figure 4a**, the lowest thermal resistance ( $R_{th}$ ) was shown for Polymer 1, with a value of  $2.59 \pm 0.12 \text{ }^\circ\text{C/W}$  while the highest reading was measured for Polymer 2, with a value of  $3.26 \pm 0.16 \text{ }^\circ\text{C/W}$ .

In addition, a kinetic study on temperature stabilization on the same samples was performed, starting from the temperature of  $28 \text{ }^\circ\text{C}$  for all three polymers. In this way, the percentages of increase in the curves at 15, 30, 60, and 90 s was analyzed with respect to the mean stabilized temperature calculated over a 100 second interval, reached after 700 s of stabilization (**Figure 4b**). All samples show a similar pattern although the samples using polymer 1 and 2 as coating appear to heat up a little bit slower, reaching  $95.7 \pm 0.17\%$  and  $95.8 \pm 1.2\%$  of the final temperature after 90 s. The dynamic thermal resistance of the Polymer 3-coated is a little bit lower and reaches  $99.3 \pm 1.5\%$  of the final stabilized temperature after 90 seconds.

### 2.3. Effect on 2-MXP Detection

The samples coated with different adhesive layers were used in an experiment to detect 2-MXP in aqueous media. MIPs were immobilized onto the three different polymers as described in the experimental section. After a 20 min stabilization time, the concentration of 2-MXP was gradually increased. For each addition, the thermal resistance was calculated over a period of 400 s. The response of a MIP-coated chip was compared to a non-imprinted reference chip. The effect size was determined by subtracting the NIP signal from the MIP signal and normalizing the value to the baseline. This measurement was repeated three times for each of the polymers under study. The results, summarized in **Figure 5**, demonstrate that using Polymer 1 and Polymer 3 lead to an increase in the effect size over most of the



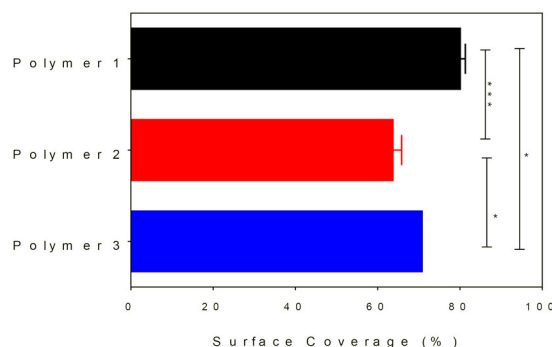
**Figure 2.** Coverage analysis of MIP/NIP particles: from left to right, samples coated with poly(vinyl butyral-co-vinyl alcohol-co-vinyl acetate) (Polymer 1), poly-vinyl-chloride (Polymer 2), and polystyrene-block-poly(ethylene-ran-butylene)-block-polystyrene (Polymer 3) as adhesive layer are shown respectively. In the bottom row the original images are shown while a threshold has been applied on the same images in the upper row to differentiate the substrate from the deposited particles.

concentration range in comparison to the polymer used in previous work.<sup>[18]</sup> In addition, the sensitivity seems to be improved which in turn leads to a more pronounced dynamic range and a more clear relationship between concentration and effect size.

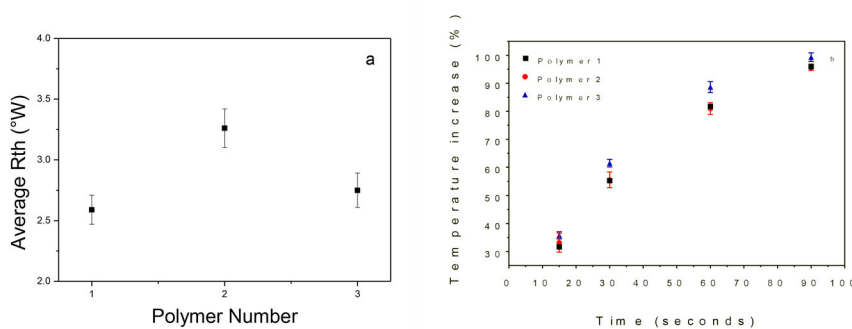
### 3. Discussion

The results shown in this paper confirm that the adhesive layer composition can have a significant effect on the performance of the resulting MIP-based sensor. The surface coverage is highly influenced by the type of polymer used for immobilizing the

MIP particles onto a planar electrode. The data in Figure 2 provide a very first empirical confirmation that layer composition can influence MIP immobilization efficiency. Analyzing the data with ImageJ and the statistical analysis in Figure 3 confirmed that the observed differences in surface coverage were indeed significant. These findings are in line with a previous study performed by Petri et al. in 2002.<sup>[21]</sup> Their study analyzed the morphology of different polymer films spin coated onto polar silica surfaces with atomic force microscopy and demonstrated that PVC in THF, polymer 2 in our study and the standard material used in previous studies, produces inhomogeneous layers. Polyvinyl butyral in THF on the other hand resulted in smooth, homogenous layers of polymer. The findings made by



**Figure 3.** Percentage of covered area in function of the polymer used as adhesive layer. Differences in surface coverage were analyzed using one-way analysis of variance (ANOVA) illustrating that the observed differences are significant (\* $p < 0.05$  and \*\*\* $p < 0.01$ ).



**Figure 4.** Thermal characterization of the substrates: a) Temperatures reached in the measurement chamber and the respective  $R_{th}$  of the three different polymers. b) Temperature dynamic curves of the differently coated samples.

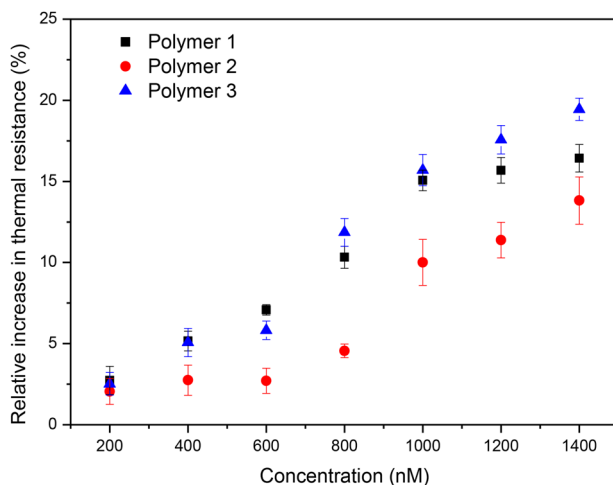
Petri et al. provide a potential explanation as Polymer 1, the polymer that gave the highest surface coverage, is actually a copolymer of polyvinyl butyral dissolved in toluene, which offers a more homogenous immobilization platform for the MIP particles.

The thermodynamic properties of Polymer 1 and Polymer 3 also seem to be beneficial in comparison to the previous standard, Polymer 2. Both polymers show a much lower static thermal resistance in comparison to Polymer 2. Surprisingly, this effect is absent in the dynamic thermal analysis where only Polymer 3 seems to heat up a bit faster in comparison to the other polymers. However, the effect is only small and the setups limited sensitivity requires further study to draw any meaningful conclusions. More importantly, the increased MIP surface coverage and lower baseline thermal resistance seem to be the most important parameters when actually analyzing the different samples in an analytical setting. The data in Figure 5 do not only demonstrate that it is possible to use bulk 2-MXP MIPs for thermal detection purposes, a finding that to our knowledge have never been demonstrated before, but also

confirm that the composition of the adhesive layer influences the performance of the sensor. The average effect size over the entire concentration range is higher for the samples coated with Polymer 1 and Polymer 3 and, more importantly, the sensitivity of the resulting sensor is improved, thereby increasing the linear relationship between concentration and effect size over a wider concentration range.

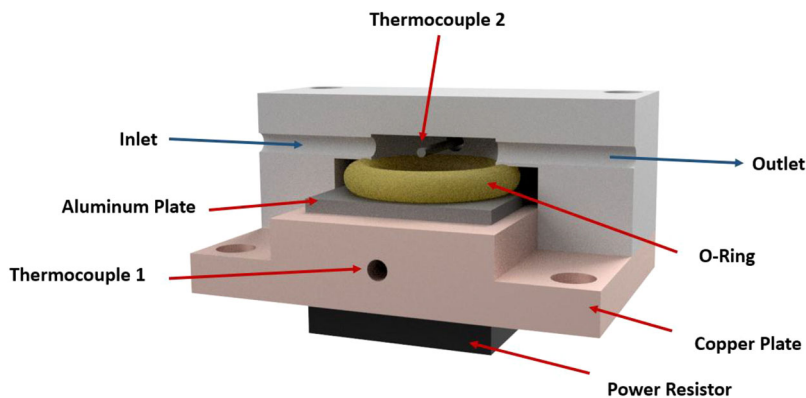
#### 4. Conclusion

The results in this paper indicate that it is possible to detect the new psychoactive substance 2-MXP in a quantitative manner using the previously successful combination of bulk MIPs with a thermal readout unit. The easy synthesis process of the receptor particles and the fast, user-friendly readout open the door for low-cost detection platforms that enable legislators to identify new psychoactive substances in an easier and faster manner. The findings in this paper also illustrate the effect of the composition of the adhesive layer used for MIP immobilization on the surface



**Figure 5.** Effect size of the  $R_{th}$  of samples characterized by different substrates:  $R_{th}$  is measured as  $R_{thMIP} - R_{thNIP}$  where  $R_{th}$  is calculated as  $(T_{Heater} - T_{Chamber})/P$ , with P being the power delivered by the power resistor in order to stabilize the system. For each addition of 200 nM 2MXP solution, the system is left to stabilize for 20 minutes and the mean value of  $R_{th}$  is calculated over the last 400 seconds. All data were normalized on the baseline.





**Figure 6.** Section view of experimental setup: two thermocouples are used to control the temperature of the copper plate and monitor the temperature in the chamber, respectively.

coverage with significant differences observed between the different polymeric adhesive layers under study. The composition also influences the static thermal resistance, an essential parameter of the thermal detection methodology. Optimal results were achieved using Poly(vinyl butyral-co-vinyl alcohol-co-vinyl acetate), allowing for a higher degree of MIP immobilization which in turns positively influences the correlation between effect size and target concentration and the binding capacity of the sensor chip by improving the limit-of-detection of the resulting sensor. The lower thermal resistance values further improve the dynamic range of the resulting MIP-based biosensor as the relative effect size increases. In this way, the results demonstrated in this paper, open up the possibility for further follow-up studies that examine these phenomena in more detail to come up with optimal adhesive layer composition tailor-made for the envisioned analytical applications.

## 5. Experimental Section

**Sample Preparation:** Poly(vinyl butyral-co-vinyl alcohol-co-vinyl acetate), MW 90K-120K (Sigma Aldrich) was dissolved in THF at 6% (w/w). Poly(vinyl-chloride), MW 90 K (Sigma Aldrich) was dissolved in tetrahydrofuran (THF) (Sigma Aldrich) at 4% (w/w). Polystyrene-block-poly(ethylene-ran-butylene)-block-polystyrene, MW 118K (Sigma Aldrich) was dissolved in toluene (Sigma Aldrich) at 2% (w/w). Flat, polished 10 × 10 (mm) aluminum plates with 0.3 mm thickness were cleaned in acetone and air-dried. 1 mL of each solution was spin-coated on the aluminum plates at 3000 RPM for 1 min.

**Experimental Set-Up:** A similar experimental setup to the one used in the work by Grinsven et al. was used for the experimental measurements.<sup>[13]</sup> Briefly, polymer-coated aluminum chips were placed on top of a copper block (thin polymer layer facing up), that was heated by a 22 Ohm power resistor (Vishay, Farnell), soldered onto the bottom part of the copper heat provider. The temperature of the copper block was carefully monitored using a type K thermocouple (TC Direct), inserted into the copper via a 0.6 mm wide hole on one of the lateral sides, reaching to the middle of the block (**Figure 6**).

A software-driven proportional–integral–derivative (PID) controller keeps the block at 37 °C, controlling a 9V supply to the power resistor. The whole set-up is contained in a 3D printed plastic casing. The setup is characterized by a custom-made lid that uses a rubber O-ring to create a cylindrical sealed chamber on top of the treated aluminum. The lid features three channels that reach to the cylindrical chamber: two are used

as inlet and outlet for the sample injection while the third allows for a precise placement of a type K thermocouple. The thermocouple is glued in place, thus allowing monitoring the temperature inside the chamber (**Figure 6**).

**MIP Synthesis:** MIP and NIP particles were obtained using the same method described by Lowdon et al.<sup>[19–20]</sup> Briefly, MIPs were synthesized using methacrylic acid (MAA) as monomer and 2-MXP as template. A mixture of 1.82 mmol MAA and 0.17 mmol 2-MXP was dissolved in 3 mL of dimethylsulfoxide (DMSO). Following, Ethylene glycol dimethacrylate (EGDM, crosslinker molecule, 3.64 mmol), 4,4-azobis (4-cyanovaleric acid) (initiator, 50 mg) were added. After five minutes of sonication the mixture was purged with N<sub>2</sub> to remove any oxygen from the mixture and polymerization was initiated by heating the mixture to 65 °C for 12 h. The obtained polymer was ground and sieved to obtain microparticles with sizes between 25 and 50 μm. The template 2-MXP was then removed from the powders by continuous Soxhlet extraction with 50/50 mixtures of acetic acid and methanol and a 50/50 mixtures of methanol and water. Non-Imprinted Polymers (NIPs) were synthesized in a similar manner but without the presence of the template molecule. Finally, MIP and NIP powders were dried overnight in an oven at 100 °C.

**MIP/NIP Immobilization:** The deposition method was adapted from the work of other authors and adapted to be used with the new polymers.<sup>[18]</sup> Aluminum chips were coated with polymer by spin coating, following, 10 mg of the MIP/NIP powder was deposited on the polymer. The polymers were heated for 120 s to a temperature above their glass transition temperature to allow the MIPs to sink into the adhesive layer. Following, a polydimethylsiloxane (PDMS) cylindrical stamp was used to gently push the imprinted powder in the heated polymer layer. The plates were left at room temperature for 5 min for cooling, after which excess MIP particles were removed by rinsing the chips with MQ water and blow drying them using a steady nitrogen flow.

**Coverage Analysis:** After MIP/NIP particle deposition, optical microscope images were acquired in order to evaluate the area covered by the polymer powder. 10× magnification was used in order to evaluate an area of 0.54 mm<sup>2</sup> for each image. Three different images were taken for each sample. The free software ImageJ was used to compute the covered area. First, the images were translated to 8-bit format, then a threshold analysis was done in order to differentiate the darker substrate from the particles, matching the original image (**Figure 2**). Differences in surface coverage were analyzed using one way ANOVA to establish statistical relevance.

**2-MXP Detection Experiment:** After preparing the coated samples and assembling the experimental setup as shown in **Figure 6**, a solution of 2-MXP in MQ water at a concentration of 200 nM was prepared. After stabilization of the signal in MQ water, injections of 3 mL of the spiked solution were performed at a rate of 0.250 mL per minute to gradually increase the concentration of 2-MXP in the measurement chamber. After each injection the system was left to stabilize for 20 min. The thermal

resistance was calculated using following formula:

$$R_{th} = \frac{T(\text{heater}) - T(\text{chamber})}{PowerOutput}$$

The data were normalized by dividing the response at a certain target concentration by the baseline response when no target is present in the measuring chamber. The effect size at a certain concentration was calculated as the normalized response of the MIP minus the normalized response of the NIP.

## Supporting Information

Supporting Information, consisting of all data sets used for constructing dose-response curves, is available from the author.

## Acknowledgements

The authors are grateful for the financial support received from the Province of Limburg (LiMe) and for funding from Taskforce for Applied Research of the Dutch Research Foundation (SIA-NWO) through the RAAK-PRO project "SURFSCAN."

## Conflict of Interest

The authors declare no conflict of interest.

## Keywords

adhesive layer composition analysis, designer drug detection, heat-transfer method, molecularly imprinted polymers

Received: November 28, 2018

Revised: February 13, 2019

Published online: April 23, 2019

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