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## Studying the Drug Delivery Kinetics of Nanosponges Using a MIP-Based Thermal Sensing Platform

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1 Article

## 2 Studying the drug delivery kinetics of nanosponges 3 using a MIP-based thermal sensing platform

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14 **Abstract:** The implementation of Molecularly Imprinted Polymers (MIPs) into sensing systems has  
15 been demonstrated abundantly over the past decades. In this article, a novel application for a MIP-  
16 based thermal sensing platform is introduced by using the sensor to characterize the drug release  
17 kinetics of a nano-sized silver-organic framework. This so-called Ag-nanosponge was loaded with  
18 acetylsalicylic acid (aspirin) which was used as a model drug compound in this study. The drug  
19 elution properties were studied by placing the nanosponge in phosphate buffered saline solution  
20 for two days and measuring the drug concentration at regular time intervals. To this extent, an  
21 acrylamide-based MIP was synthesized that was able to detect aspirin in a specific and selective  
22 manner. Rebinding of the template to the MIP was analyzed using a thermal sensor platform. The  
23 results illustrate that addition of aspirin into the sensing chamber leads to a concentration-  
24 dependent increase in the phase shift of a thermal wave that propagates through the MIP-coated  
25 sensor chip. After constructing a dose-response curve, this system was used to study the drug  
26 release kinetics of the nanosponge, clearly demonstrating that the metalorganic framework releases  
27 the drug steadily over the course of the first hour after which the concentration reaches a plateau.  
28 These findings were further confirmed by UV-VIS spectroscopy, illustrating a similar time-  
29 dependent release in the same concentration range, which demonstrates that the MIP-based  
30 platform can be used as a low-cost, straightforward tool to assess the efficacy of drug delivery  
31 systems in a lab environment.

32 **Keywords:** Molecularly imprinted polymers, thermal detection, nanosponge, drug delivery.  
33

### 34 1. Introduction

35 Molecular imprinting technology originally focused on the development of imprinted particles that  
36 could be packed into columns for affinity separation, exploiting the affinity and selectivity to extract  
37 a molecule of interest from complex matrices [1-3]. The concept was soon extended by using  
38 molecularly imprinted polymers (MIPs) as antibody or enzyme mimics [4, 5]. One of the most  
39 interesting applications for MIPs is their incorporation into biomimetic sensing devices as they mimic  
40 the affinity of a natural receptor for its target, but are superior in terms of their chemical, thermal and  
41 long term stability [6-8] and can be made through a straightforward and relatively low-cost synthesis  
42 process [9]. Although MIPs have been combined with optical [10, 11], electrochemical [12-15],  
43 microgravimetric [16, 17], and thermal transducers [18-22] in devices with great potential for *e.g.*  
44 diagnostic applications, the translation of these lab-based devices into commercially available sensors  
45 is still challenging due to difficulties with reproducibility, integrated sampling and automated signal

46 processing [23]. Therefore, this paper illustrates a new potential application for MIP-based sensing  
47 systems, *i.e.* the study of the elution kinetics of drug delivery systems.

48

49 Nanosponges are virus-sized particles that can be used as smart drug delivery systems due to their  
50 biodegradable polymer framework [24]. Cross-linking of the polymer leads to the formation of  
51 spherical nanoparticles with a large amount of binding cavities that could be used to store drugs for  
52 steady release [25]. The solubility of drugs that poorly dissolve in water can be regulated by carefully  
53 tuning the composition of the polymer and balancing the ratio of lipophilic and hydrophilic segments  
54 [26]. Over the past ten years, nano-sized metal-organic frameworks have emerged as an interesting  
55 class of nanosponges due to their non-toxic nature and unusually large loadings of a wide variety of  
56 drugs [27]. In addition, they can be made without using organic solvents and have shown to release  
57 drugs in a gradual and sustaining manner which can be tuned by modifying the organic linkers  
58 within their binding cavities [28]. Due to their size they can reach tightly controlled areas of the body,  
59 allowing to selectively target a desired area.

60

61 Drug elution kinetics from smart drug delivery systems are traditionally studied by measuring the  
62 concentration of the drug in the medium surrounding the drug carrier. The drug concentration is  
63 usually determined by classic laboratory devices/techniques including radioactive assays [28],  
64 fluorescent resonance energy transfer [29], liquid chromatography [30] and UV-visible spectroscopy  
65 [31]. Although these methods are usually very sensitive and allow for a very accurate determination  
66 of the drug concentration and hence the elution profile, most of them require expensive readout  
67 devices, involve target labeling, or suffer from interference, limiting their performance in complex  
68 media. Therefore selective, label-free techniques have been studied based on *e.g.* electrochemical [32]  
69 and microgravimetric [33] readout methods. These platforms allow for a straightforward and  
70 relatively fast analysis of the eluted medium but still require ultrasensitive, expensive readout  
71 technology, conductive electrodes and involved data processing and interpretation.

72

73 Therefore, we introduce an elegant MIP-based platform for the study of drug elution from a metal-  
74 organic framework in this article. Silver metalorganic frameworks were synthesized crosslinking  
75 AgNO<sub>3</sub> with ethylenediamine in a sodium hydroxide solution leading to a sponge-like structure  
76 containing a wide distribution of nanocavities. These nanosponges were loaded with the model drug,  
77 acetylsalicylic acid (aspirin), one of the drugs most abundantly used for the treatment of pain, fever,  
78 or inflammation that has also demonstrated to possess antithrombotic effects which positively affects  
79 patients at risk for heart failure [34]. The drug elution under physiological conditions was studied in  
80 the lab by applying aspirin-imprinted MIPs to aluminum electrodes and studying the propagation of  
81 a thermal wave through the chip in function of an increasing concentration of acetylsalicylic acid, a  
82 technique that was used successfully for the detection of dopamine in banana juice in previous work  
83 [21]. Next, the constructed dose-response curve from this experiment was used to determine the  
84 aspirin concentration in PBS solution containing drug eluted from the nanosponge. The results  
85 demonstrated that the nanosponge releases the drugs in a burst-like manner, releasing the drug in a  
86 constant fashion during the first hour, after which the concentration in the media surrounding the  
87 sponge remains stable. These results were validated using UV-Visible spectroscopy, which shows a  
88 similar behavior and a similar concentration range, illustrating the potential of the platform for drug  
89 elution studies.

## 90 2. Materials and Methods

### 91 2.1. Chemicals

92 Acrylamide (AA), azobisisobutyronitrile (AIBN), polyvinylchloride (PVC) and silver nitrate  
93 (AgNO<sub>3</sub>) were obtained from Sigma-Aldrich. Methanol (absolute) and acetonitrile were bought at  
94 Biosolve. Acetone (pure), sodium hydroxide, sulfuric acid, ethanol, salicylic acid and phosphate  
95 buffered saline (PBS) tablets were acquired from VWR chemicals. Ethylenediamine (EDA), hydrazine

96 hydrate and ethylene glycol dimethacrylate (EGDM) were procured from Merck Schuchardt OHG.  
97 Polydimethylsiloxane (PDMS) stamps were made with the Sylgard 184 elastomer kit from Mavom  
98 NV. Aluminum chips were purchased at Brico NV and cut to the desired dimensions.

### 99 2.2. Synthesis of the metalorganic framework (silver nanosponge)

100 Nanosponges were synthesized by mixing aqueous AgNO<sub>3</sub> (5 mL, 0.4M, 2 mmol) with aqueous  
101 NaOH (150 mL, 15 M, 2.25 mol). Crosslinking was initiated by the addition of aqueous EDA (1.5 mL,  
102 99% w/v, 0.25 mmol) and aqueous hydrazine hydrate (0.2 mL, 80% w/v, 5.0 mmol) to the solution.  
103 The mixture was then purged with N<sub>2</sub> and refluxed at 80 °C for 90 minutes. After cooling the reaction  
104 flask to room temperature, silver nanosponges were isolated by vacuum filtration and air dried  
105 before being freeze dried for 6 hours.

### 106 2.3. Molecular imprinting procedure

107 Pre-polymerization mixtures composed of aspirin (0.090 g, 0.5 mmol), 3 mmol AA (0.213 g) and 15  
108 mmol EGDM were dissolved in 5 mL acetonitrile. Polymerization was initiated by adding 0.15 mmol  
109 AIBN (0.025 g) to the pre-polymerization mixture. After sonication, the mixture was deoxygenated  
110 by purging it for 5 minutes with N<sub>2</sub> and heated to 60 °C during 24 hours while shielding the mixture  
111 from light to prevent the template from degrading. The resulting monolith was mechanically ground  
112 using a Fritsch Planetary Micro Mill Pulverisette 7 premium line (700 rpm, 5 minutes, 10 mm balls)  
113 and sieved using a using a Fritsch Analysette 3 for 4 hours with a 20 µm mesh. The resulting powder  
114 was extracted during 96 hours at 105 °C, using a Soxhlet apparatus filled with with a mixture of  
115 methanol and ethanoic acid (7:3 v/v). Finally, the MIP particles were dried for three hours in an oven  
116 at 50 °C. Non-imprinted polymers (NIPs), serving as a reference, were synthesized in the same  
117 manner without the presence of a template.

### 118 2.4. Chip preparation

119 Polished aluminium plates were cut to obtain chips with the desired dimensions (10 × 10 mm<sup>2</sup>). To  
120 immobilize MIP particles onto the surface of the measurement chip, a 100 nm PVC adhesive layer  
121 (0.35 wt% PVC dissolved in tetrahydrofuran) was applied onto the chip by spin coating at 3000 rpm  
122 for 60 seconds with an acceleration of 1100 rpm/s. MIP and NIP particles were stamped into this  
123 layer using a PDMS substrate that was covered with a monolayer of polymer particles. The PVC  
124 layer was heated for 2 hours at a temperature of 100 °C way above its glass transition temperature  
125 (80 °C) allowing beads to sink into the polymer layer. The samples were cooled down prior to  
126 thermal measurements and any unbound particles were washed off with distilled water.

### 127 2.5. Selectivity test and dose-response curve

128 The thermal detection platform is described thoroughly in previous work [18-22]. Functionalized  
129 chips (MIP or NIP) were pressed mechanically with their backside onto a copper block serving as a  
130 heat provider. The temperature of the copper underneath the sample, T<sub>1</sub>, was monitored by a K-type  
131 thermocouple (TC Direct). This information was fed into a temperature control unit that stringently  
132 controls T<sub>1</sub> by modifying the voltage over the power resistor (Farnell) that heats the copper, using a  
133 software-based (Labview, National Instruments) proportional-integral-derivative (PID) controller  
134 (P= 10, I= 8, D= 0). The functionalized side of the chip faced a polyether ether ketone (PEEK) flow cell  
135 which was sealed with an O-ring to avoid leakage, defining a contact area of 28 mm<sup>2</sup> and an inner  
136 volume of 110 µL. The flow cell is connected to a tubing system, allowing the administration of  
137 liquids in a controlled and automated fashion by means of a syringe pump. The temperature of the  
138 liquid inside the flow cell, T<sub>2</sub>, is measured by a second thermocouple, placed 1 mm above the chip.  
139 For each rebinding measurement the signal was stabilized in PBS at pH 7.4 which was used as to  
140 mimic physiological conditions.

### 141 2.6. Loading of nanosponges with aspirin

142 Aspirin was absorbed into the nanosponges by solvent evaporation. To this extent 1.85 mmol (0.100  
143 g) of the nanosponge was incubated with a 1.85 mmol aspirin in 6.2 mL of ethanol. The mixture was  
144 shaken for 48 hours at 750 rpm. The solvent was removed under vacuum (30 °C, 300 mbar, 90 rpm)  
145 and dried at 65 °C for 3 hours.

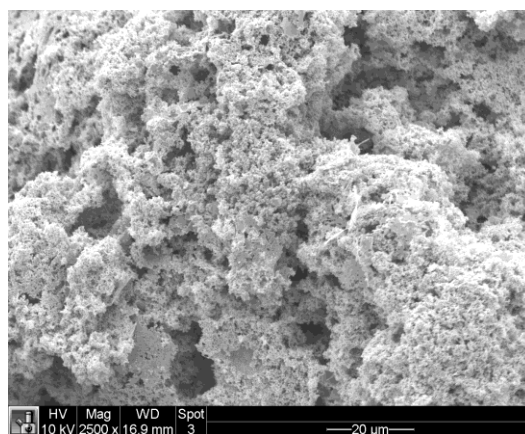
#### 146 2.7. Drug elution analysis

147 Loaded nanosponges (0.1 g) were incubated in 300 mL PBS (pH 7.4 at 37 °C) while gently stirring at  
148 100 rpm to mimic physiological conditions. Over the course of two days, 3 mL samples of the PBS  
149 were taken at regular time intervals and the aspirin concentration was analyzed by both TWTA and  
150 UV-visible spectroscopy to create an elution profile.

### 151 3. Results and Discussion

#### 152 3.1. Surface characterization of Ag nanosponges

153 Upon synthesis the Ag nanosponges were analyzed using scanning electron microscopy (SEM). This  
154 analysis reveals that the metalorganic framework indeeds has a nanosponge structure containing a  
155 large set of nano-sized cavities, providing a large a surface area that can be loaded with drug  
156 molecules.



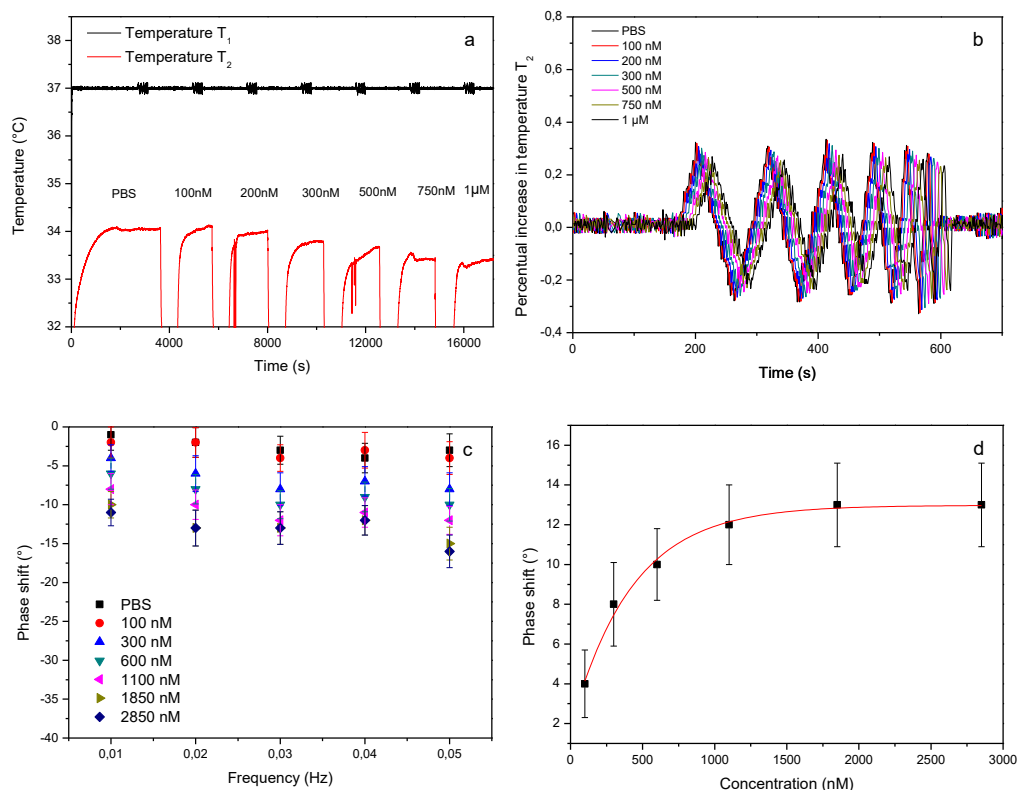
157

158 **Figure 1.** Surface analysis of an Ag-nanosponge using a scanning electron microscope at magnification 2500x.

#### 159 3.2. Quantification of aspirin in PBS

160 To assess whether it was possible to accurately determine the concentration of drug eluted from the  
161 nanosponge, a dose-response curve was constructed by exposing a MIP-coated chip to an increasing  
162 concentration of aspirin in PBS. The thermal analysis clearly indicates that exposing the MIP to  
163 aspirin in increasing concentrations results in a decrease of the liquid temperature inside the flow  
164 chamber (Figure 2a) and a increase in the phase shift observed in the transmitted wave (Figure 2b).  
165 The results in figure 2b were used to construct a thermal bode plot which shows a the phase shift for  
166 each transmitted frequency in function of the cummulative concentration of aspirin present in the  
167 flow cell (Figure 3c). Although the phase shift at every concentration is most pronounced at the  
168 highest input frequency, the resolution appears to be optimal at 0.03 Hz. These findings are in line  
169 with previously obtained results with dopamine MIPs in a similar setup [21]. The time-dependent  
170 TWTA data at 0.03 Hz are used to construct a dose-response curve (figure 3d), which will be used to  
171 assess the concentration of aspirin that has eluted from the Ag-nanosponge.

172



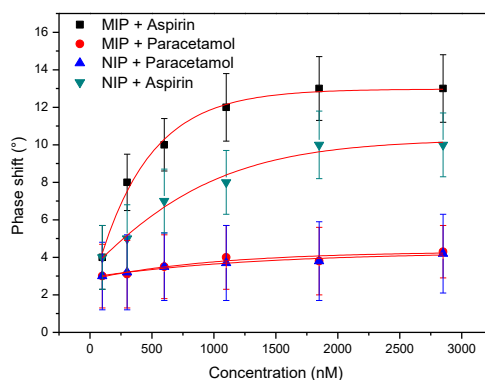
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174

175 **Figure 2.** Aspirin rebinding analysis. The time-dependent temperature data (a) and thermal wave transport  
 176 analysis spectrum (b) are shown in response to adding an increasing concentration of aspirin. The phase shift at  
 177 an optimal resolution at frequency 0,03 Hz (c) and a dose-response curve (d) are constructed from these data  
 178 and are plotted in function of the cummulative concentration present in the flow chamber.

179 *3.3. Selectivity test*

180 In order to assess whether the aspirin recognition was selective and specific, the experiment  
 181 summarized in the previous section was repeated for a NIP-coated electrode. In addition, both MIP  
 182 and NIP were exposed to an increasing concentration of acetaminophen (paracetamol). The resulting  
 183 dose-response curves and the corresponding fits are shown in Figure 3.



184

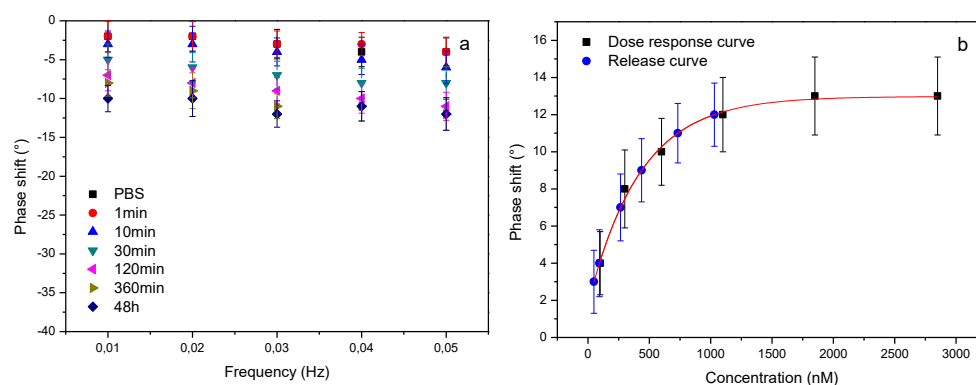
185 **Figure 3.** MIP selectivity test. The data show that exposing both the aspirin MIP and the NIP do not respond to  
 186 an increasing concentration of the analogue molecule. However, the imprinting factor is limited as the difference  
 187 between MIP and NIP is small.

188 The results in figure 3, illustrate that although the MIP is suprisingly selective in discriminating  
 189 between paracetamol and aspirin, the imprinting factor is small. This in line with previous findings  
 190 with similar AA-based MIPs and can be explained by the fact that at a neutral pH, not all binding

191 sites and functional groups on the target will be protonated ( $pK_a$  of aspirin is 3.49) [35]. The fact that  
192 paracetamol only slightly increases the phase shift is surprising as the non-specific binding to the MIP  
193 was expected to be similar. Although previous work has indicated that the MIP would be more  
194 specific and therefore, selective at acidic pHs, the authors decided to continue measuring at a pH of  
195 7.4 to simulate drug elution. The results in Figure 2 indicate that the dose-response curve is highly  
196 usable and as the release pattern will be studied in PBS no interference from other molecules is to be  
197 expected. However, if the concept would be extend to complex matrices in the future the MIP  
198 synthesis route should be revised.

### 199 3.4. Thermal analysis of drug elution

200 The elution of aspirin from the nanosponges was studied by incubating them in PBS and retrieving  
201 a sample from the surrounding medium after 1, 10, 30, 120 and 360 minutes and after 48 hours. The  
202 elutions were diluted 5000 times with PBS to fit the linear range of the sensor. MIP-coated electrodes  
203 were exposed to these diluted elutions and their response was summarized in a temperature bode  
204 plot (figure 4a). The resulting phase shifts at 0.03 Hz were used to construct an elution profile that  
205 was compared to the previously obtained dose response curve (figure 4b) to determine the aspirin  
206 concentration in each of the eluted samples.



207

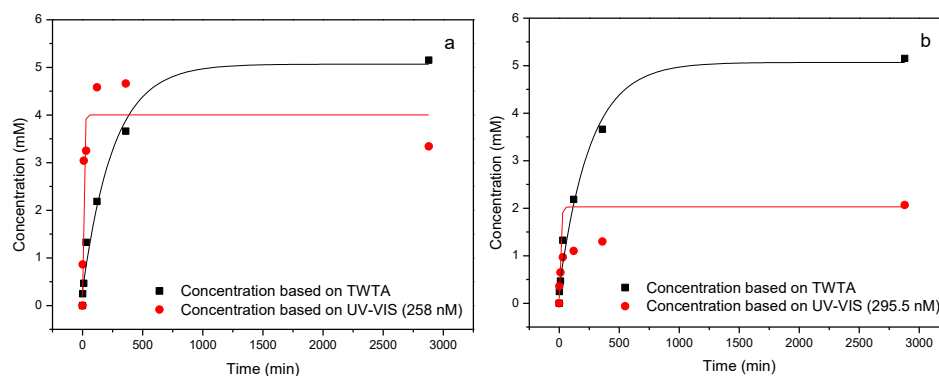
208 **Figure 4.** Drug elution analysis. The drug elution was studied using TWTA and the resulting Bode plot shows a  
209 concentration-dependent phase shift indicating that the concentration of aspirin gradually increases with time  
210 (a). The dose-response curve was used to determine the concentration in the eluted solutions (b).

211 The results obtained in Figure 4 were corrected for the dilution factor to create an elution profile that  
212 validated using a gold standard reference technique, *i.e.* UV-Visible spectroscopy. The elution  
213 profiles of both techniques demonstrate a similar behavior (Figure 5a): a sharp increase within the  
214 first two hours after which a stable plateau is reached that does not significantly change over the next  
215 two days. This indicates that the nanosponge releases the drugs in a relatively quick burst which is  
216 suitable for some applications requiring immediate effect. However, to actually achieve sustained,  
217 prolonged delivery of drugs the nanosponge should be functionalized with molecules that bind the  
218 drug and actually release it slowly over time.

219 When analyzing the elution profile obtained by UV-Visible spectroscopy, a small decrease in the  
220 concentration of aspirin can be observed over the course of two days. This can be explained by the  
221 fact that some of the acetylsalicylic acid will be converted into salicylic acid in PBS. This is confirmed  
222 by analyzing UV absorbance at 295.5 nm, which shows that salicylic acid is indeed present in the  
223 elution and its concentration will increase slightly over the course of two days (Figure 5b). The fact  
224 that this is not shown in the TWTA data is due to the fact that both compounds will bind to the MIP  
225 in a similar manner [35].

226





227

228 **Figure 5.** Validation of TWTA data by UV-Visible spectroscopy. The drug elution profile derived from the TWTA  
 229 data in figure 4 were compared to the drug elution profile obtained with UV-Vis and both show a initial burst  
 230 of aspirin release in the first two hours in the millimolar regime (a). The decrease in aspirin concentration for the  
 231 UV-vis data shown in figure 5a can be explained by conversion of aspirin into salicylic acid which is confirmed  
 232 by analyzing absorbance at 295.5 nm.

#### 233 4. Conclusions

234 The data shown in this article illustrate the potential use of a MIP-based thermal detection platform,  
 235 which has previously been used for diagnostic purposes, for analyzing the drug release kinetics of  
 236 drug delivery matrices. A proof-of-principle was demonstrated by validating the results obtained  
 237 with the biomimetic sensor using UV-Visible spectroscopy, demonstrating a similar profile in the  
 238 same concentration range. The metalorganic framework synthesized in this work appears to release  
 239 the model drug, aspirin, within the first two hours. Future research should be aimed at  
 240 functionalizing the framework to get to a more gradual release of the drug. In addition, loading and  
 241 elution of other, potentially more relevant drugs should also be studied. As the MIP platform is  
 242 generic, it can be used to study a wide variety of targets in a wide variety of matrices, by changing  
 243 the MIP receptors or optimizing their selectivity or performance in more challenging media and  
 244 chemical conditions (pH, temperature,...). Additionally, the platform has been recently tested for  
 245 detecting molecules directly on a thermocouple wire [36], this opens up perspectives in terms of  
 246 analyzing drug release *in vivo* using the proposed platform.

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249 **Author Contributions:** The thermal wave transport device was designed by B.v.G. and T.J.C. TWTA  
 250 measurements, data processing and interpretation were performed by B.v.G. and K.E. All nanosponges and  
 251 MIPs were synthesized by C.J.P. and K.F. while input on the nanosponge synthesis and loading was provided  
 252 by A.P.G. Insights on the MIP synthesis and template removal protocol was provided by E.S.R. and H.D. Input  
 253 on possible experimental design were provided by G.T., C.J.P. and A.P.G. The manuscript was jointly written  
 254 by P.J.W., K.E and A.P.G. All authors have given approval to the final version of the manuscript.

255 **Conflicts of Interest:** The authors declare no conflict of interest.

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