Development of a Novel Flexible Polymer-Based Biosensor Platform for the Thermal Detection of Noradrenaline in Aqueous Solutions

S. Casadio[1], J.W. Lowdon[2], K. Betlem[2], J. Tadashi[3], C.W. Foster[2],

T.J. Cleij[4], B. van Grinsven[4], O.B. Sutcliffe[2], C.E. Banks[2], M. Peeters[2*]

1) University of Bologna, Department of Industrial Chemistry, Viale Risorgimento 4, Bologna, 40136, Italy.

2) Manchester Metropolitan University, Faculty of Science and Engineering, School of Science and the Environment, Division of Chemistry and Environmental Science, Chester Street, Manchester M1 5GD, United Kingdom.

3) Universidade de São Paulo, Instituto de Química, Avenida Prof. Lineu Prestes, 748, CEP 05508-000 São Paulo, SP, Brazil.

4) Maastricht University, Maastricht Science Programme, P.O. Box 616, 6200 MD Maastricht, The Netherlands.

Abstract:

Molecularly Imprinted Polymers (MIPs) are synthesized for the neurotransmitter noradrenaline with the optimal composition and binding conditions being determined *via* optical batch rebinding experiments. Next, the obtained MIP polymer particles are mixed within screen-printed inks to produce mass-producible bulk modified MIPs screen-printed electrodes (MIP-SPEs). In this contribution, the supporting surface which the MIP-SPEs are screen-printed upon are explored to deviate from conventional polyester, to polyvinylchloride, tracing paper and household-printing paper. The performance of the MIP-SPEs are measured using the Heat-Transfer Method (HTM), a straightforward and low-cost detection technique based on thermal resistance. At first, the noise on the signal is minimized by adjusting the settings of the temperature feedback loop. Second, the response of the MIP-SPEs to noradrenaline is measured and compared for the different substrate materials. Sensors printed onto paper are considered in further experiments as their response to noradrenaline is the highest and

advantageous material properties, including sustainability and flexibility of the material. Subsequently, dose-response curves are determined by simultaneously measuring HTM and Thermal Wave Transport Analysis (TWTA). The latter is a new thermal detection method that relies on the use of thermal waves and has the advantage of a short measurement time (2 min). With these thermal methods, it is possible to specifically detect noradrenaline in aqueous solutions and quantify it at relevant concentrations. In summary, by combining synthetic receptors with thermal measurement techniques it is possible to develop a portable sensor platform that is capable of low-cost and straightforward detection of biomolecules. Through exploring novel SPE substrates, a system is designed that is flexible and holds potential for the use in commercial biomedical devices and complex sensor architectures.

Keywords: Molecularly Imprinted Polymers (MIPs), paper-based biomimetic sensors, heat-transfer method (HTM), thermal wave transport analysis (TWTA), neurotransmitters, screen-printing technology.

Corresponding author: Dr. Marloes Peeters

Manchester Metropolitan University, Chester Street, M1 5GD

Manchester, United Kingdom.

Phone: + 44 – (0)161 – 247 1450

Fax: +44 (0)1612476318

E-mail: m.peeters@mmu.ac.uk

1. Introduction

Noradrenaline is a catechol neurotransmitter that has a crucial role in the function of the renal, hormonal, cardiovascular and central nervous system[1, 2]. It is associated with the fight-or-flight response, as the release of noradrenaline is significantly increased in stressful and dangerous situations, which mobilizes the brain and body for action[3, 4]. Low levels of the neurotransmitter are associated with depression and postural hypotension[5], while high levels indicate stress, thyroid hormone deficiency[6] and congestive heart failure[7]. Noradrenaline is an active component of a variety of

drugs, ranging from treatment of hypertension to organic heart disease, diabetes[8], and anxiety[9]. In recent years, noradrenaline has attracted attention as a tumour biomarker[10]. Urine and plasma tests are used to diagnose pheochromocytoma, an endocrine tumour of the adrenal glands that secretes high amount of catechol amines[11, 12]. If undetected, these tumours present a high risk of mortality[13] since fluctuating levels of noradrenaline can result in organ damage from dangerously high blood pressure, which leads to heart attacks and kidney failure[14]. Recent reports suggest that stress hormones such as noradrenaline are involved in the initiation and progression of tumours, which occurs through the overexpression of enzymes[15, 16]. Work by Choi *et al*.[17] have demonstrated that by stimulating cells with noradrenaline, metastasis of ovarian cancer cells was enhanced. Due to its relevance as a biomarker, various analytical techniques have been employed to determine noradrenaline concentrations in biological samples. The most common methods include chromatographic[18, 19] and electrochemical biosensor techniques[20-22]. Electrochemical methods are inexpensive and fast compared to chromatographic measurements, but applications are limited due to poor selectivity and interference of other metabolites present in biological samples. To enhance the selectivity, Molecularly Imprinted Polymers (MIPs) have been used[23]. MIPs, referred to as plastic antibodies, possess high affinity for their template molecules but have superior stability and are inexpensive compared to natural antibodies[24-26]. The imprinting process takes place by co-polymerizing functional and cross-linking monomers in the presence of a molecular template[27, 28]. After removal of the template, cavities are formed that are complementary in shape, size and functional groups to the template molecule and are able to rebind it with high affinity and selectivity [29, 30]. In certain areas commercial applications are available, and recently, MIPs were used for the first time as an active ingredient in a cosmetic product[31]. Examples of MIPs in literature designed for catecholamines, the class of molecules noradrenaline belongs to, are sparse and focus around chromatographic applications[32, 33]. Huang *et al*.[34] developed a monolithic MIP for the chiral separation of (-)-noradrenaline from buffer solutions. To extract cathecholamines from a biological matrix, respectively human plasma samples, magnetic-carbon nanotubes MIPs were prepared which were combined with ultra-fast liquid chromatography-tandem quadrupole mass spectrometry (UFLC-MS/MS)[35]. Electropolymerization has the advantages over preparation of monoliths that instead of particles an imprinted film is formed on the electrode, but

the main drawback is that a conductive monomer is required[36]. Rosy *et al*.[23] synthesized an imprinted polymer film with o-aminophenol and evaluated noradrenaline binding by determining the increase in peak current. By implementing the MIP into the sensor platform, the selectivity was enhanced compared to traditional noradrenaline electrochemical sensors.

Electrochemical techniques offer fast and low-cost measurements, but are not compatible with every target molecule and there are limitations to the selectivity. The Heat-Transfer Method (HTM), is a promising and straightforward alternative that relies on thermal detection[37]. It was first discovered for the process of DNA melting; with the transition from well-defined double-stranded DNA to single-stranded DNA where the strands are random coils without a regular structure. As a result, the surface coverages increases by 150% and this leads to a significant increase in the electrical resistance due to the formation of an additional insulating layer on the surface[38]. The electrical resistance is linked to the thermal resistance and this additional insulating layer blocks the heat-flow in a certain direction, which corresponds to a measurable increase in the thermal resistance. This effect has been well-studied for various applications, such as detection of proteins with aptamers[39], screening of cancer cells[40] and studying of DNA mutations[38]. For sensors with MIPs as recognition element, binding of the templates to specific cavities in the porous polymer layer, leads to increase in the thermal and electrical resistance that is described by the "pore-blocking" model[41]. Advantages of this thermal method include that a low-cost home-made set up is used, which requires the use of only two thermometers and an adjustable heat source. In recent work, screen-printed electrodes (SPEs) were used to prepare a biosensing platform by the direct mixing of the MIP particles into the screen-printing ink, which results in mass producible MIP-SPEs[42]. As a first proof-of-concept, the binding of the neurotransmitter dopamine was studied by using HTM and Thermal Wave Transport Analysis (TWTA). The latter is a novel thermal method that relies on applying thermal waves rather than keeping the thermal resistance of samples fixed[43]. It is demonstrated that this improves the detection of dopamine by lowering the noise ratio on the signal and it has the additional benefit of shorter measurement time[42].

In this contribution, a MIP with a high specificity for noradrenaline is developed by evaluating the composition of various charged monomers. Next, MIP particles are mixed with screen-printing ink and screen-printed onto a variety of substrates; polyester, which was used in previous work[42], and an

array of substrates that have not been used before in combination with MIPs namely, polyvinylchloride (PVC), tracing paper, and household printing-paper. The effect of the SPE substrate on thermal detection is studied by exposing the prepared MIP-SPEs to an aqueous solution with a 1 mM noradrenaline concentration. In consideration of all the substrates, the paper-based SPEs ensure a high stability and exhibit the highest analytical response (measured as a phase shift in the thermal signal) towards noradrenaline. Therefore, these MIP-SPEs are evaluated in further thermal measurements (HTM and TWTA) and dose-response curves are constructed to quantify the noradrenaline concentration in aqueous solutions.

The developed polymer-based platform is sustainable due to the use of paper and has potential for the use in pharmaceutical applications because of it its simplicity, low-cost, and portability of the set up[37]. The ability to adapt the MIP layer offers a great versatility[26] as the sensor can be targeted towards other disease markers, additionally this platform could be placed into an array format offering a great opportunity as a point-of-care sensor for drug screening.

2. Experimental

2.1 Reagents and instrumentation

Ethylene glycol dimethacrylate (EGDMA), itaconic acid (IA), methacrylic acid (MAA), acrylic acid (AA), serotonin hydrochloride salt (98%) and dopamine hydrochloride salt (99%) were purchased from Acros (Loughborough, United Kingdom). Prior to polymerization, the stabilizers in MAA, AA and EGDMA were removed by passing the solutions over a column packed with alumina. 4,4'-azobis(4-cyanovaleric acid), (±)-noradrenaline hydrochloride, (±)-adrenaline hydrochloride, tyramine, 3,4-dihydroxy-L-phenylalanine (L-Dopa) (98%), ascorbic acid and solvents were obtained from Sigma Aldrich (Gillingham, United Kingdom). In further text, for simplicity we will refer to (±)-noradrenaline hydrochloride as noradrenaline. For the heat-transfer measurements, home-made phosphate buffered saline (PBS, 1x) solutions were prepared. All solutions were prepared with deionized water of restively

18.2 Ω cm. All solutions, unless stated otherwise, were vigorously degassed with nitrogen to remove oxygen prior to analysis.

2.2 MIP synthesis

MIPs for noradrenaline were synthesized with varying compositions, which are listed in Table 1. The MIP which was able to bind noradrenaline with the highest affinity was prepared in the following manner. First, a mixture of the functional monomer IA (0.77 mmol), crosslinker molecule EGDM (7.7 mmol), and initiator 4,4-azobis(4-cyanovaleric acid) (50 mg) was dissolved in dimethylsulfoxide (DMSO) together with the template molecule (0.38 mmol). The mixture was then sonicated for 5 min and subsequently degassed with N_2 before starting the polymerization. Polymerization was performed by heating the mixture up to 65˚C and keeping it there at 12 h, allowing full completion of the reaction. The obtained bulk polymer was ground and sieved to achieve microparticles with sizes smaller than 20 μm. Finally, noradrenaline was removed from the MIP powders by continuous Soxhlet extraction with a 50/50 mixture of acetic acid and methanol. After 24 h, the template was fully extracted which was verified by AT-IR spectroscopy with a Nicolet 380 FT-IR device from Thermo Scientific (Loughborough, United Kingdom) (**Supporting Information, S-1).** The powders were then dried overnight in an oven at 100˚C. Thermal stability of the MIPs was ensured by running a ThermoGravimetric Analysis (TGA) experiment on a TG4000 from Perkin Elmer (London, United Kingdom), which is enclosed in **Supporting Information, S-2.**

Table 1. The composition of the different MIPs is described in Table 1, listing the amount of template, functional monomer, crosslinker monomers, initiator, and porogen used.

	MIP-23	**MIP-24**	**MIP-31**	**MIP-32**	**MIP-33**
Noradrenaline (mmol)	0.38	0.38	0.38	0.38	0.38
IA (mmol)	-	-	0.77	0.77	0.77
MAA (mmol)	0.77	-	-	-	-

AA (mmol)	-	0.77	-	-	-
EGDM (mmol)	7.7	7.7	7.7	7.7	7.7
Initiator (mg)	50	50	50	50	50
DMSO (ml)	4	4	3	4	5

Non-imprinted polymer (NIP) were synthesized accordingly, but without the presence of the target molecule. These MIP and NIP powders were used in all further batch-rebinding and heat-transfer experiments.

2.3 Batch rebinding experiments

Optical batch rebinding experiments were evaluated with an Agilent 8453 spectrophotometer (Stockport, United Kingdom). For each experiment, 20 mg of MIP or NIP powder was added to 5 ml of aqueous solutions with noradrenaline concentrations between 0-0.3 mM. The pH of the solutions was adjusted in a range from 3-6 by addition of drops of a HCl solution (0.1 mM). The resulting suspensions were stirred for 1h on a rocking table at room temperature. Subsequently, the samples were filtered and the free concentration of noradrenaline (C_f) was determined by UV-vis spectroscopy. This enabled to calculate the bound concentration (S_b) of noradrenaline per gram of MIP and NIP powder and construct the corresponding binding isotherms. To obtain the imprint factor (IF), the binding of noradrenaline to the MIP was divided over that by the NIP at C_f = 0.05 mM. Selectivity experiments were performed with adrenaline, dopamine, L-Dopa and tyramine, molecules that are similar in chemical structure to noradrenaline.

2.4 Preparation of MIP bulk modified Screen-Printed Electrodes (MIP-SPEs)

The procedure to prepare the MIP-SPEs is described in detail in ref[42]. A carbon-graphite ink formulation (Product Code: C2000802P2; Gwent Electronic Materials Ltd, UK) was printed onto different substrates (thickness listed in Table S-3) and cured at 60˚C for 30 mins. Substrates that were used include tracing paper, paper, polyvinylchloride (PVC) and polyester. Details about the supplier and grade of the substrates can be found in ref[44]. MIPs were incorporated into the bulk ink of the

SPEs with a mass percentage of 30% of mass particulate vs mass ink (M_P/M_I). This is the highest amount of particles that can be incorporated before the ink loses its printability. SEM images were recorded on a Supra 40VP Field Emission SEM from Carl Zeiss Ltd (Cambridge, United Kingdom) to demonstrate the presence of the MIP particles onto the SPEs. Prior to analysis, a thin layer of Au/Pd was sputtered onto the MIP-SPEs with a SC7640 from Polaron (Hertfordshire, United Kingdom) to enhance contrast of the images.

2.5 HTM measurements with MIP-SPEs printed on different substrates

All thermal measurements were performed with a Perspex flow cell with an in-house design, as described in detail in ref [38]. The flow cell, with an inner volume of 110 µl, was sealed off with an O-ring and connected to the thermal set up (Figure 1). MIP-SPEs are pressed mechanically onto a copper block, which served as the heat sink. Its temperature, T_1, is actively steered by a proportional-integral-derivative (PID) controller and measured by a thermocouple type K (Onecall, Leeds, United Kingdom). For all HTM measurements with noradrenaline, T_1 was kept at 37 ± 0.04 ˚C to mimic body temperature. A second thermocouple was placed above the sensor surface to measure the temperature in the liquid (T_2).

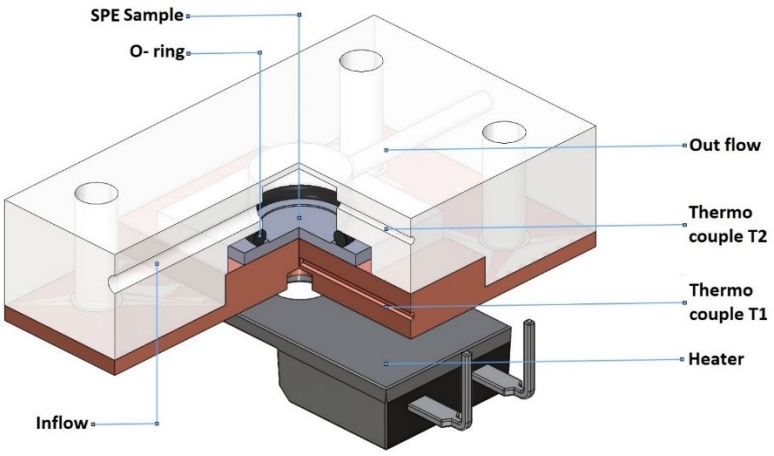


Figure 1: is a schematic representation of the in-house designed flow cell. MIP-SPEs are placed onto copper (T_1) of which the temperature is actively steered. The temperature in the fluid (T_2) is monitored and are correlated to changes at the solid-liquid interface.

MIP-SPEs with different substrates, including paper, tracing paper, PVC and polyester, were mounted into the sensor set up described in Figure 1. The thermal resistance, R_{th} (˚C/W), is defined as the temperature gradient ($T_1 - T_2$) divided over the input power P (W). P is defined as the voltage over the heat source that is required to keep T_1 at a constant temperature. The temperature at T_2 is sampled every second.

$$\text{Equation 1:} \quad R_{th} = \frac{T_1 - T_2}{P}$$

First, the influence of the PID settings on the noise of the signal was evaluated, as previous work showed that the feedback loop is the main origin of the noise on the thermal measurements[45]. The PID parameters were varied from 1-6-0, 1-9-0, 1-10-0, 2-4-0.1, 3-3-0.2 and 10-10-10. The first two configurations were used in past papers and gave the lowest Limit of Detection (LOD) for MIPs deposited onto aluminum[41]. The feedback of the system is material dependent and to study the SPEs the parameters were adapted to look at settings between and at the extreme (10-10-10) of the PID spectrum. In these experiments, the noise on the level was studied when the MIP-SPEs stabilized into a buffered aqueous solution. Subsequently, the configuration for the different substrates with the lowest noise level on the baseline signal was determined. In further experiments, the PID parameters were then fixed and the effect of the SPE substrates was studied. MIP-SPEs printed onto the different substrates were stabilized in water and aqueous solutions with a 1 mM concentration (pH=6) of noradrenaline were added to the set up. After the first addition with noradrenaline, the signal was left to stabilize for 30 min and flushed with an aqueous solution. To test the reusability, the sensor was again exposed to a solution with a 1 mM noradrenaline concentration. The response of the system was then determined by calculating the effect size, which corresponds to the thermal resistance at a 1 mM concentration divided over the baseline level.

2.6 HTM and TWTA experiments with MIP-SPEs printed on paper to determine dose-response curves

MIP-SPEs printed onto paper were used in further dose-response curve measurements due to its high effect size and advantageous material properties of the system. The MIP-SPE were first stabilized for 1

h in distilled water and then solutions of increasing noradrenaline concentrations (0, 0.5, 1, 2.5, 5, 10, 25 mM) in distilled water were added into the flow cell with an automated NE500 programmable syringe pump from ProSense (Oosterhout, The Netherlands). The solutions where injected at an interval of 30 minutes with a flowrate of 200 µL/min with a total volume of 1 mL.

After stabilization of the signal, the R_{th} values at the specified concentrations were determined. The relative response in R_{th}, which will be called the effect size, is defined according to Equation 1. The R_{th} at t=0 refers to the baseline signal, while R_{th} at t=c refers to the thermal resistance that is measured after a certain concentration of the target is added. Equation 2 is used to construct dose-response curves and quantify the concentration of noradrenaline in aqueous solutions.

Equation 1: $$Effect\ size = \frac{R_{th}\ (t=c)}{R_{th}\ (t=0)}\ x\ 100$$

Simultaneously with these HTM measurements, thermal wave transport analysis (TWTA) measurements were performed. A thermal wave (amplitude of 0.1˚C, frequency range from 0.0083 Hz to 0.05 Hz) was applied from the heat-sink to the MIP and the thermal wave output was measured at the position in the liquid of thermocouple T_2. Delays in the thermal wave output were measured because of two effects. First, the solid-liquid interface of the MIP-SPE with the aqueous solution induced a delay in the thermal wave output and, second, a further delay was caused by binding of neurotransmitters to the MIP layer. To solely study the binding effect, the delay in seconds for the MIP-SPEs exposed to known noradrenaline solutions was compared to the output measured in a pure aqueous solution. This was then converted into a phase shift by taking the time of one thermal wave (pure aqueous solution, c=0 of noradrenaline concentration) measured at the output as 360˚. Dose-response curves were obtained by plotting the normalized phase shift versus the noradrenaline concentration. To determine the specificity of the developed sensor platform, identical HTM and TWTA measurements were performed for the reference NIP and by exposing MIP-SPEs to adrenaline and dopamine, molecules that have a similar chemical structure as noradrenaline.

Finally, a TWTA measurement with a MIP-SPE exposed to noradrenaline solutions in PBS of pH =7 was performed to give an outlook in application for clinical samples. Ascorbic acid (1 mM) was added as an anti-oxidant, which ensures stability of noradrenaline in solution[46]. The phase shift, and determined dose-response curve, were obtained in the same manner as for the other TWTA measurements.

3. Results

3.1 Batch rebinding results

First, batch rebinding experiments with MIP023, MIP024 and MIP032 were performed. These MIPs have a similar composition but differ in the functional monomer that was used. MAA and AA (one charged functional group) and IA (two charged functional groups) were studied. The time of the experiments was fixed at 1h since after 30 min no significant increase in specific binding of the noradrenaline to the MIP particles was observed. In the experiment, 5 ml of noradrenaline solutions (Ci =0.05, 0.1 and 0.2 mM) were added to 20 mg of MIP particles. Table 2 shows the amount of template bound per gram of polymer and allows to compare the three different MIPs.

Table 2. The amount of template bound (S_b) per gram of polymer for MIP023, MIP024 and MIP032.

MIP	Functional monomer	S_b (µmol/g) C_i = 0.05 mM	S_b (µmol/g) C_i = 0.1 mM	S_b (µmol/g) C_i = 0.2 mM
MIP023	MAA	3.6	6.7	8.9
MIP024	AA	2.2	7.1	10.2
MIP032	IA	9.6	12.9	16.8

The results from Table 2 are in accordance with literature[34], Huang *et al*., determined that IA has a higher recognition ability for noradrenaline compared to MAA and AA because of its higher charged functionality. Therefore, IA was used as monomer in the MIP synthesis in all further experiments.

In the next step, batch rebinding experiments with MIP31,32,33 were performed in aqueous noradrenaline solutions in pH range 3-6. Binding isotherms were constructed by plotting the free concentration C_f vs the amount of noradrenaline bound, S_b. The imprint factor (IF), which is defined as

the binding of the MIP divided over that of the NIP at a certain concentration, was used as a measure for the specificity. To determine the IF, the data was fitted with a two-parameter allometric fit (Equation 2).

$$\text{Equation 2:} \quad S_b = A \cdot C_f^v$$

This equation is referred to as the Freundlich isotherm and is often used for MIP binding isotherms due to the heterogeneity of the binding sites[47]. Table 3 shows the IF values for MIP31,32, and 33 at C_f=0.05 mM for different pH values. Noradrenaline is only soluble in water under acidic conditions and therefore neutral pH and alkaline solutions were not considered. All fits had a R^2 value of above 0.92, indicating the Freundlich isotherm gives an accurate representation of the data. Table 3 enables to compare two parameters: the pH of the noradrenaline solutions and the amount of porogen that was used in MIP synthesis.

Table 3. The IF for MIP31, MIP32 and MIP33 is provided for batch rebinding experiments performed at pH 3, 4, 5 and 6. Experiments were performed in duplicate.

pH	IF MIP31	IF MIP32	IF MIP33
3*	1.01	0.97	1.20
4	1.16	1.35	1.35
5	1.10	1.28	1.31
6	1.19	**1.61**	1.52

*at pH 3, binding was beneath 10 μmol/g and a linear fit was used to determine IF instead of the Freundlich isotherm.

The protonation behavior of the monomer and template is dependent on the pH. IA has two carboxylate groups that have pKa values of 3.85 and 5.44[48]. This means that at pH 3, the molecules do not possess a negative charge and this makes binding to the positively charged nitrogen of noradrenaline very unlikely. From pH 5 onwards, the majority of the acid will be in its ionized form and there is a higher

occurrence of binding, which is reflected in a higher IF for all the polymers as seen in Table 3. For all polymers, the most specific binding and highest IF was at pH 6.

With Table 3, it is possible to compare MIP31, 32, 33, which were prepared with a varying amount of the porogen DMSO. It is crucial to determine the right amount of porogen; it needs to be sufficient to create a porous network but too much can result in a loss of binding sites. MIP 32, with a moderate amount of porogen, possessed the highest IF and was capable of binding the highest amount of noradrenaline per gram of polymer.

The full binding isotherm of the MIP and NIP at optimal conditions (pH =6, MIP 32) is provided in Figure 2. Selectivity experiments were performed by evaluating the amount of adrenaline, dopamine, L-Dopa and tyramine bound to the MIP. Error bars were calculated by taking the average of three independent measurements.

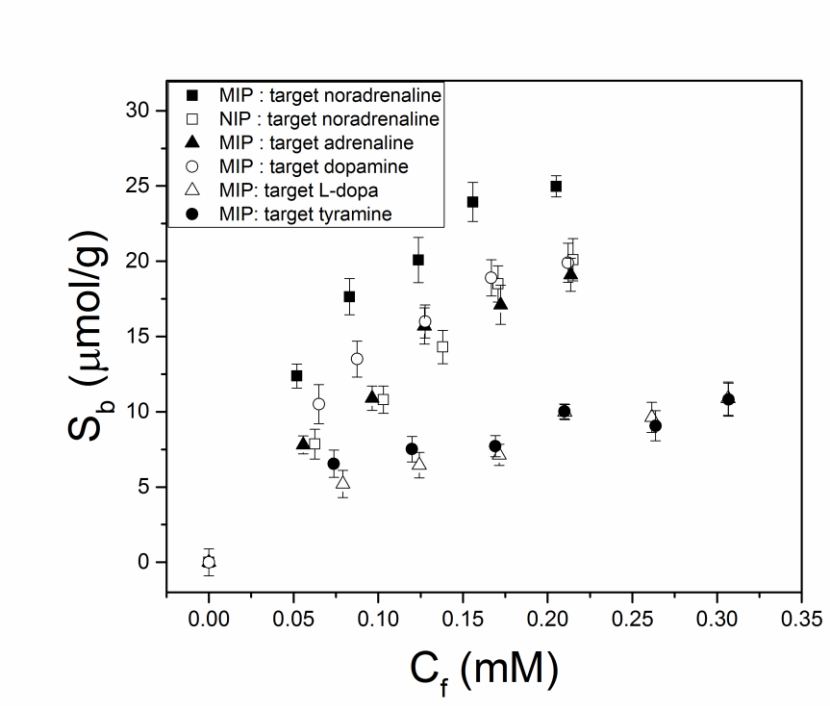


Figure 2: Binding isotherms of MIP (solid squares) and the corresponding NIP (open squares) upon exposure to aqueous noradrenaline solutions (pH = 6) for 12h on a rocking table. To test the selectivity, the response of the MIP to adrenaline (solid triangles), dopamine (open circles), L-dopa (open triangles) and tyramine (solid circles) was studied. The imprint factor at Cf = 0.05 mM for the optimized blend is 1.6 ± 0.05.

Figure 2 shows that the developed MIP is specific; binding of noradrenaline to the MIP is significantly higher compared to that of the reference NIP. In addition, binding to the MIP by a variety of noradrenaline metabolites, including adrenaline, dopamine, tyramine and L-dopa, was significantly lower compared to the original template noradrenaline. After determining the optimal conditions for MIP synthesis and noradrenaline binding, detection was performed with thermal measurements.

3.2 Thermal resistance measurements MIP-SPEs in phosphate buffered saline solutions

MIPs were incorporated into the bulk ink of the SPEs with a mass percentage of 30% of mass particulate *vs.* mass ink (M_P/M_I). This was done for SPEs printed onto different substrates, including tracing paper, paper, PVC and polyester. Figure 3 shows the typical structure of the MIP particles on the surface of a SPE printed onto polyester as determined by SEM image analysis.

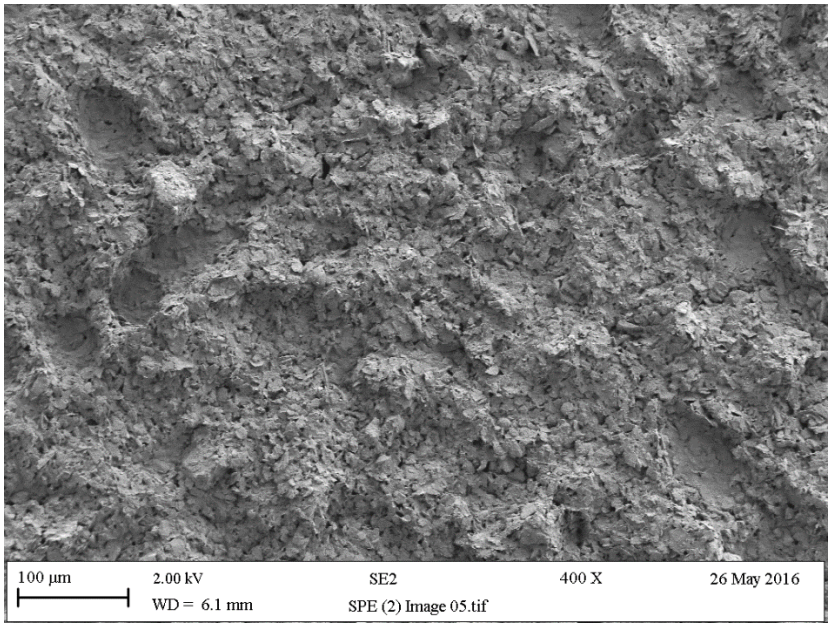


Figure 3. Scanning Electron Microscopy (SEM) image of the MIP-SPEs with a 30% mass percentage of MIP particles.

The bulk of the ink consists of graphite, which has a layered planar structure. A SEM image of the plane SPE electrode is enclosed as a figure in **Supporting Information S-3**. Figure 3 shows the presence of heterogeneous MIP particles on the surface of the SPE, confirming the samples can be used for thermal

measurements. As a first step, the influence of PID settings to the noise level on the baseline signal was studied, as optimizing this will result in lower detection limits. The samples, printed on the substrates polyester, PVC, paper, and tracing paper were mounted into the set up and stabilized in a phosphate buffered saline (PBS) solution for 1h. In all experiments, the solutions were kept at pH 6 since this was determined to be optimal for noradrenaline binding. To determine the noise ratio, the average percentage error on the R_{th} signal was calculated for at least 250 measurement points. These results are summed up in Table 4.

Table 4. Effect of the PID settings on the noise ratio for MIP-SPEs printed onto substrates of polyester, paper, PVC, and tracing paper. Noise levels are determined by averaging out R_{th} values of at least 500 measurement points.

PID parameters	Polyester Noise ratio (%)	Paper Noise ratio (%)	PVC Noise ratio (%)	Tracing paper Noise ratio (%)
1-6-0	1.01	1.42	1.06	1.34
1-9-0	0.97	1.08	1.02	1.11
1-10-0	**0.85**	**1.05**	**0.95**	**0.90**
2-4-0.1	1.01	11.36	1.45	1.08
3-3-0.2	1.91	6.2	1.30	1.30
10-10-10	3.35	3.84	1.25	1.04

The PID settings need to be adapted based on the material that is used; while for tracing paper and PVC varying the PID settings had little effect on the overall noise ratio, fluctuations were observed in the case of polyester and paper. The optimal PID settings for all substrates were 1-10-0, which is similar to what has been reported in literature before[45]. These parameters were implemented for the next set of experiments, in which the response of the MIP-SPEs to a 1 mM aqueous solution of noradrenaline (pH=6) was measured. Table 5 provides an overview of the thermal resistance measured after stabilization in PBS (pH = 6), the thermal resistance after addition of a 1 mM noradrenaline solution in

PBS, and the effect size. Error bars are calculated by taking the averaging of 50 measurement points. The response (%) is defined as the percentage increase in thermal resistance after the addition of the noradrenaline solution.

Table 5. Data showing the increase in thermal resistance after addition of an aqueous noradrenaline solution (c=1 mM) to MIP-SPEs printed onto different substrates.

SPE substrate	R_{th} value Stabilization (˚C/W)	R_{th} value Addition 1 mM noradrenaline solution (˚C/W)	Response (%)
Polyester	3.83 ± 0.02	4.83 ± 0.03	26
Paper	3.61 ± 0.04	4.62 ± 0.03	28
Tracing paper	3.8 ± 0.1	4.3 ± 0.1	13
PVC	4.12 ± 0.03	4.93 ± 0.03	20

Table 5 shows that the SPEs substrates stabilized into aqueous solutions have different thermal resistance values. This is because this property depends on the thickness (listed in Table S-4) and thermal conductivity of the substrates. There is a difference in chemical nature between the paper and polymer substrates: paper tends to absorb water while polyester and PVC are hydrophobic, meaning no significant water uptake will be observed. In **Table S-4**, the effect on the thickness and weight of the substrates after soaking them in distilled water solutions for 30 min is listed. The weight of the tracing paper electrodes increased with a factor of eight, which resulted in a measurable increase of the thickness and deformation of the SPEs. This was reflected in the noise on the signal that was significantly higher for tracing paper compared to the other substrates, and therefore they were determined unsuitable for measurements with aqueous solutions. In contrast, paper electrodes showed good stability, are water-compatible and had the highest percentage effect size. The results of the full measurement, including stabilization, addition of noradrenaline (1 mM in aqueous solution), washing and a second addition of noradrenaline (1 mM in aqueous solution), are shown in Figure 4.

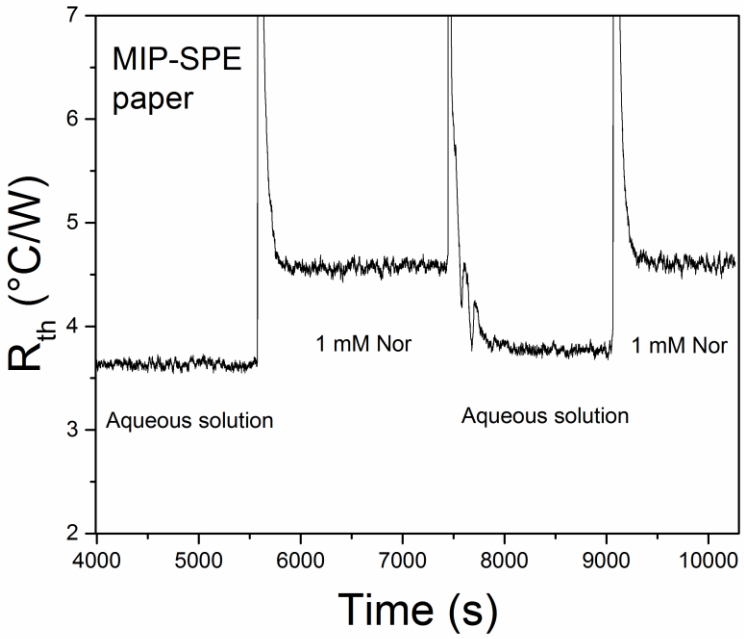


Figure 4. Thermal resistance of the MIP-SPE printed onto paper plotted vs the time after stabilization in an aqueous solution followed by additions of 1 mM aqueous noradrenaline solutions with a washing step between the additions.

The MIP-SPE printed onto paper was stabilized in an aqueous solution for 1h, which resulted in a thermal resistance of 3.61 ± 0.04 ˚C/W. After addition of an aqueous noradrenaline solution, the thermal resistance increased to 4.62 ± 0.03 ˚C/W. This corresponds to a percentage increase of 28%, which is well above the noise level on the baseline (1%). In the next step, a washing step was performed with a PBS solution, which lowered the R_{th} to 3.83 ± 0.4 ˚C/W. The signal did not go back to the original baseline value as to extract the template from MIPs layer either a prolonged washing period is required or the use of an organic solvent. After exposing for the second time with a solution of a noradrenaline concentration of 1 mM, the thermal resistance rose back to 4.65 ± 0.03 ˚C/W. This indicated the reusability of the sensor if the surface was washed with an appropriate solvent.

Besides the high effect size, additional advantages of printing onto paper include sustainability of the sensor platform and flexibility of the paper SPEs, which enables wrapping of the sensors around coiled

electrodes and making them suitable for detection in medical devices such as catheters. Therefore, measurements to obtain dose-response curves were performed with MIP-SPEs printed onto paper.

3.3 Thermal resistance measurements and TWTA measurements on MIP-SPEs for determination of dose-response curves in aqueous solutions

To construct the dose-response curves, the thermal resistance with MIP-SPEs printed onto paper was measured after exposing them to solutions of distilled water with varying noradrenaline concentrations. For the first time, distilled water samples are measured by HTM and TWTA. The stability of the system has been improved and therefore it is possible to conduct experiments in distilled water, which guarantees less stability than buffered solutions but is a more accurate representation of pharmaceutical samples. At first, the sensors were soaked into distilled water, mounted into the set up and then stabilized in distilled water for 1h. Subsequently, at fixed times solutions with increasing noradrenaline concentrations (0, 0.5, 1, 2.5, 5, 10, 25 mM) were added to the flow cell with an automated programmable syringe pump.

During the stabilization and after each addition, a thermal wave with an amplitude of 0.1˚C was applied from the heat sink to the MIP-SPE. The specificity of the system was determined by performing identical experiments with reference NIP-SPEs. The response of the MIP-SPE to the competitor molecule adrenaline was evaluated to demonstrate selectivity of the sensor platform.

In Figure 5a, the time-dependent R_{th} of the MIP-SPE is provided after exposing the sensor to solutions with increasing noradrenaline concentrations. The spikes in the graph correspond to additions of the noradrenaline solutions that are injected at room temperature, while the flow cell has an internal temperature of 37.00˚C. After each addition, a distinct pattern of the thermal wave was observed. The thermal wave at the baseline was conducted at 1800 s and is not represented in the graph; this was done in order to show the stability of the signal prior to the addition of noradrenaline solutions. The thermal waves that were applied to the MIP-SPE are, respectively, at 4800 s, 6000 s, 9000 s, 10200 s, 13200 s, and 14400 s.

Figure 5b shows the dose-response curves for the MIP-SPE, a reference NIP-SPE and a MIP-SPE to which the competitor adrenaline was added instead of noradrenaline (Figure 5b). Calculations were done according to Equation 1.

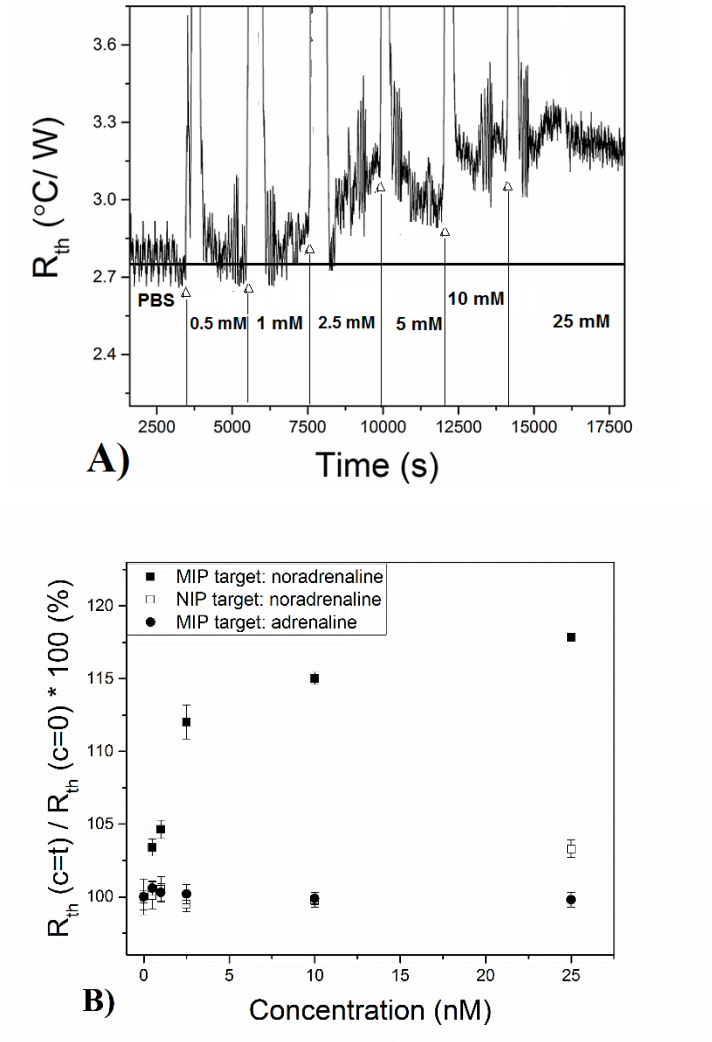


Figure 5 a) demonstrates the thermal resistance in time when the MIP-SPE printed onto paper was exposed to aqueous noradrenaline solutions with increasing concentrations (0, 0.5, 1, 2.5, 10 and 25 mM). The line in the graph gives an indication of the average baseline signal. Arrows indicate when solutions were added to the system, which was at respectively 3450, 5630, 7580, 9910, 1195, 14090 s. b) shows the HTM dose-response curves for the MIP and NIP-SPE exposed to solutions of noradrenaline and adrenaline.

After stabilizing the MIP-SPE into distilled water for 1h, a thermal resistance of 2.75 ± 0.04 ˚C/W was reached. A higher thermal resistance value (3 -4 ˚C/W) was measured when screening the different substrates, which was because a phosphate buffered solution has a different conductivity than distilled water. After addition of aqueous noradrenaline solutions, spikes in the signal were observed due to the temperature difference between the flow cell and the sample (37.00˚C *vs.* ambient temperature). The cell was then left for at least 10 min to stabilize after which the R_{th} was determined by averaging out at least 50 points measurement points. The thermal resistance steadily went up with increasing concentrations, which is attributed to the binding of noradrenaline to the MIP layer. This leads to blocking of the pores on the surface and thereby the heat-flow through the polymer particles is decreased[41]. The NIP-SPE was measured with an identical temperature programme and identical noradrenaline solutions, but no significant increase in the thermal resistance was observed. As a further proof of the specificity of the sensor platform, the MIP-SPEs were exposed to solutions of adrenaline. From **Figure S-6**, it can be concluded that there is no significant response of the MIP-SPE towards the competitor molecule of noradrenaline.

The response of the thermal resistance vs the noradrenaline concentration for the MIP-SPE was linear up to 2.5 mM after which a saturation of the signal was observed. At the highest concentration of 25 mM the thermal resistance went up to 3.22 ± 0.02 ˚C/W, which corresponds to a percentage increase of 17%. The limit of detection, defined as the concentration at which the signal equals three times the standard deviation, was estimated by assuming that at low concentrations there is a linear relationship between the increase in thermal resistance and the concentration. This was determined to be approximately ~270 μM, which is similar to concentrations of noradrenaline when it is used as a drug (40 mg/L) for treatment of low blood pressure in emergency situations.

During the HTM measurements, at certain points in time (at 4800 s, 6000 s, 9000 s, 10200 s, 13200 s, and 14400 s) a thermal wave was applied with an amplitude of 0.1˚C. As can be seen in **Figure S-6,** the peaks in the temperature shifted and there was a slower response in the system to temperature changes when MIP-SPEs were exposed to noradrenaline solutions. In Figure 6a, the delay in seconds is plotted at different frequencies of the thermal wave. This was converted into a phase shift, which is

subsequently normalized to the baseline level. Dose-response curves for the MIP, NIP and MIP measurements with the competitor adrenaline are shown in Figure 6b.

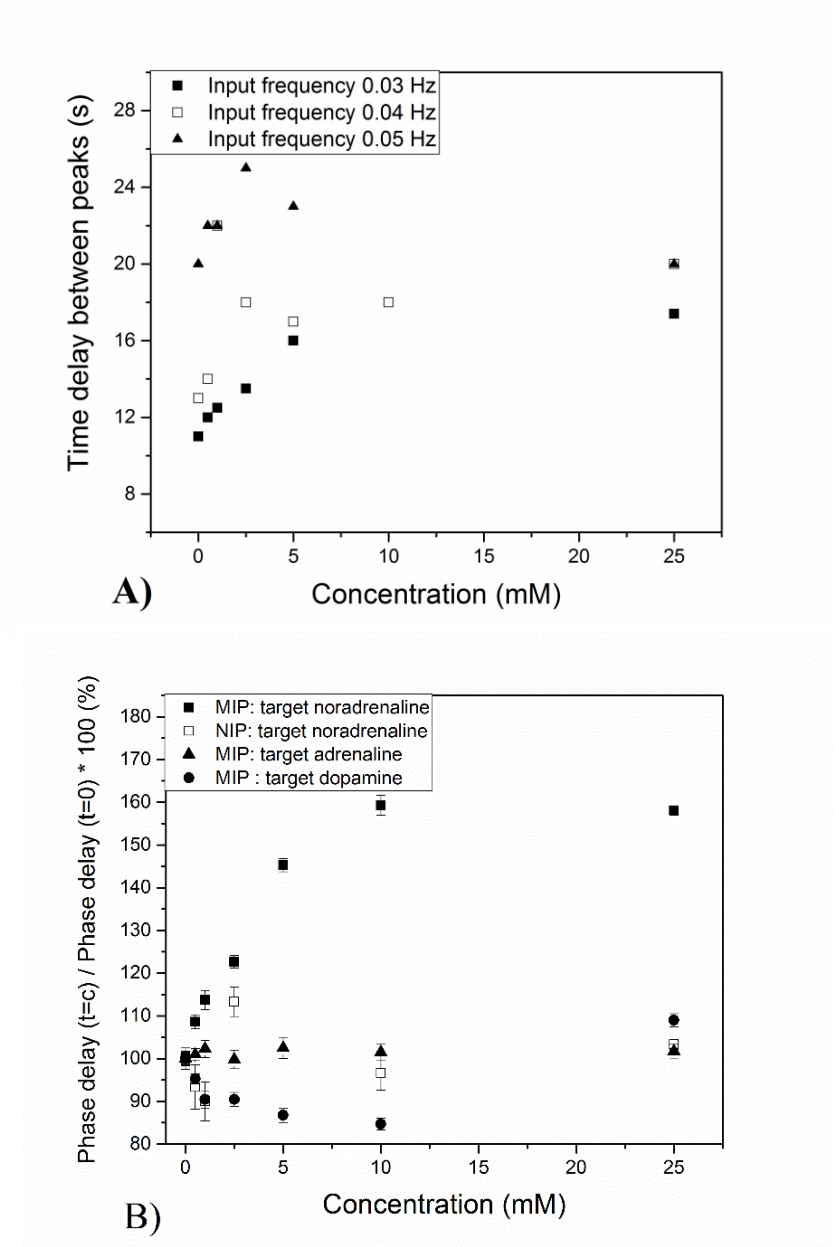
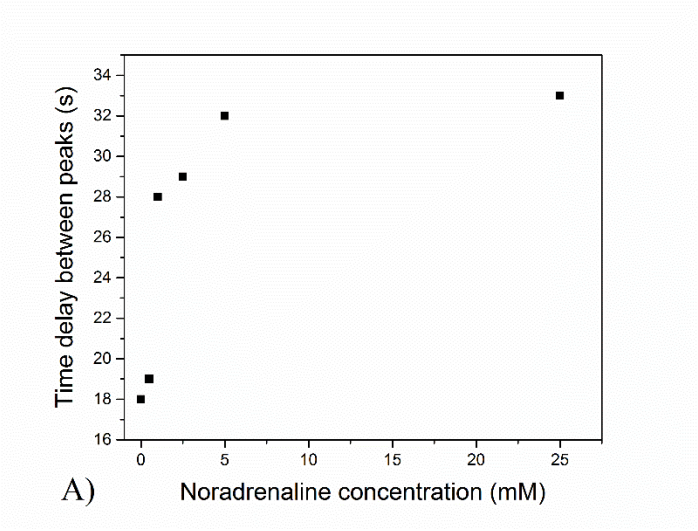


Figure 6 a) demonstrates the delay in seconds (input frequency 0.03, 0.04 and 0.05 Hz) between the input and output thermal wave when MIP-SPEs printed onto paper were exposed to aqueous noradrenaline solutions with increasing concentrations (0, 0.5, 1, 2.5, 10 and 25 mM). b) shows the TWTA dose-response curves with the normalized phase shift (at input frequency 0.03 Hz) for MIP and NIP-SPEs exposed to solutions of noradrenaline, adrenaline and dopamine.

The copper block with MIP-SPEs attached to it has a known thermal mass and this means a certain time is required to heat up and subsequently cool down the sample by 0.1˚C. In previous work[42], it was empirically determined that frequencies of the thermal waves for this system are possible between 0.008 and 0.05 Hz. At an input frequency of 0.03 Hz, a temperature delay of 18s was measured between the MIP-SPE at a 10 mM noradrenaline concentration compared to the baseline signal. A similar response was observed at an input frequency of 0.04 Hz, while at 0.05 Hz no significant effect of the noradrenaline concentration was observed. An input frequency of 0.03 Hz was used to construct dose-response curves as it ensured a superior stability of the signal and a better fit in the linear regime (R^2= 0.98) of the sensor platform. The delay in seconds was then converted into a phase shift by using the whole length of the signal as one wave. With a delay of 18 s, this correspondeded to 72 ±2˚ as the output thermal wave at the baseline was equal to 85s. The results were then normalized to the baseline signal and represented as a dose-response curve (Figure 6b). The dose-response curve showed that the linear regime of the sensor was in the range of 0-10 mM, after which saturation effects were observed. With a linear fit the limit of detection was estimated to be ~230 μM, which is a slight improvement compared to HTM measurements. The sensor was proven to be selective towards noradrenaline, as no significant effect in thermal resistance was observed when solutions of adrenaline and dopamine were added to the set-up.

To determine whether the developed sensor platform has potential for clinical applications, it is necessary to increase to scope to neutral and buffered solutions. Therefore, the samples were treated with ascorbic acid, which acts an anti-oxidant and ensures the stability of catecholamines in solution.

In a first attempt, the response of the MIP-SPE to noradrenaline solutions (c = 0, 0.5, 1, 2.5, 5, 10, 25 mM) in PBS of pH =7 was studied. The protocol, including stabilization time and when solutions were added to the set-up, was kept identical as for the experiments in water. Figure 7a shows the delay (s) between the peaks in the TWTA signal at an input frequency of 0.02 Hz, which is then converted into a dose-response curve (Figure 7b).



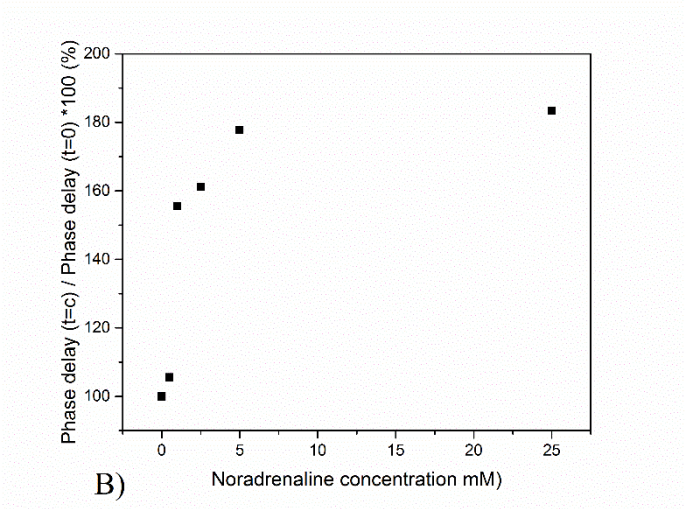


Figure 7 a) demonstrates the delay in seconds (input frequency 0.02 Hz) between the input and output thermal wave when MIP-SPEs printed onto paper were exposed to noradrenaline solutions in PBS pH =7 with increasing concentrations (0, 0.5, 1, 2.5, 10 and 25 mM). b) shows the TWTA dose-response curves with the normalized phase shift (at input frequency 0.02 Hz) for the MIP-SPEs exposed to solutions of noradrenaline.

The addition of ascorbic acid guaranteed that stability of the noradrenaline was maintained in the buffered solution. A linear increase in the phase delay was observed with additions of noradrenaline in the 0-5 mM range, after which saturation occurred and the signal went to a stable plateau level. By fitting the signal of the sensor in the dynamic range and taking into account a standard deviation of ±1

s on the baseline signal, a limit of detection was estimated of ~350 μM. This is comparable to what was previously achieved with measurements in aqueous solutions and is a first indication of the use of the sensor in buffered (biological) samples.

The LODs that are obtained with TWTA are comparable to what has been obtained with HTM meaurements. However, the main advantage of the TWTA measurements is that no additional stabilization period of 15 min is required and measurement time is equal to the length of the output thermal wave (85s for input frequency of 0.03 Hz). This makes the MIP-based sensor with TWTA read-out a fast and low-cost alternative compared to chromatographic techniques, with the additional advantages that the developed set-up is portable and can be brought on-site for point-of-care applications.

Conclusions

MIPs for the detection of noradrenaline were synthesized with the monomers IA, AA and MAA, who are similar in chemical structure. Batch rebinding experiments determined that MIPs with IA as monomer had a higher recognition capability for noradrenaline due to a higher charge density of the functional groups. Next, MIPs with IA were synthesized with varying amount of the porogen DMSO. A moderate amount of porogen resulted in the highest amount of noradrenaline binding as sufficient is required to form the typical porous MIP structure, but an excess will dilute the number of binding sites. Finally, the optimal conditions for noradrenaline binding in aqueous solutions were evaluated. Batch rebinding experiments were performed with the optimized MIP with IA as monomer in aqueous solutions with a pH range 3-6. It was concluded that pH =6 was the best for noradrenaline binding, which is because at this pH the monomers are fully deprotonated and there is a higher chance of binding to the positively charged nitrogen of the noradrenaline. The prepared MIPs were ground to obtain micron-sized particles and functionalized within SPEs by direct mixing of the particles with the screen-printing ink with a ratio of particles vs. ink of 30%. As substrates, polyester, PVC, tracing paper and paper were used, of which only polyester had been applied before for the use in MIP-SPEs. PID settings were adjusted in order to minimize the noise and the signal and then the response of the MIP-SPEs to a solution with a known noradrenaline concentration was studied. Paper electrodes showed the highest

response (28% increase in signal), which can be attributed to the low thickness of the substrate and water-compatibility of the system that promotes heat-flow from the electrode to the liquid. Therefore, further dose-response curves were measured with paper substrates. HTM and TWTA, a novel thermal measurement technique with the benefit of a shorter measurement time (~2 min), were performed simultaneously. The detection limits were in the micromolar regime, which indicated the potential use of MIP-SPEs for the screening of noradrenaline drug concentrations in aqueous solutions. In addition, a first test indicated it is possible to measure in buffered solutions, which is a first step towards medical applications. In this area, there is a great need for cost-effective and flexible electrodes such as the paper-printed MIP-SPEs. The whole set up is portable and can be brought on-site, a TWTA measurement on a sample would not require more than 2 min. Molecular imprinting is versatile and by adapting the MIP, other neurotransmitters, proteins or bacteria can be targeted. The developed sensor platform is generic and for future measurements, this can be transformed into a suitable array format.

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