Detection of L-nicotine with dissipation mode quartz crystal microbalance using molecular imprinted polymers

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Synthetic materials with imprinted nanocavities can act as highly selective tailor-made artificial receptors. Implementing these materials in a piezoelectric sensing device can offer fast and straightforward detection together with high sensitivity. L-nicotine, a major addictive substance in cigarettes is used as target molecule. The synthetic receptors for L-nicotine are made via the molecular imprinting technique. The target molecule is added to a monomer mixture containing initiator and this mixture is polymerized with heat. Subsequently, microparticles are obtained by crushing the bulk molecular imprinted

polymers (MIPs), which are then immobilized on thin films of polyvinyl chloride. Using Quartz crystal microbalance, L-nicotine could be detected in the submicromolar range and the selectivity of the sensors was verified by reference measurements with L-cotinine. The effectiveness of the sensor was also tested for different aqueous fluids at different pH. It was found that MIPs bind 4.03 times more L-nicotine than non-imprinted polymer in water and 1.99 times more in $0.1 \times$ phosphate buffer saline at pH 9.

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1 Introduction Molecularly imprinted polymers (MIPs) are an important class of synthetic materials mimicking the molecular recognition of low-molecular weight molecules by natural receptors [1–3]. MIPs are mostly used in separation technology but their use in sensing technology is becoming increasingly popular [4]. The technology provides a wide pallet of sensing devices for different kinds of target templates, such as low molecular weight molecules [5, 6] and proteins [7]. The detection of binding events can be achieved by electrochemical transducers with MIP membranes [8–11] and piezo-electric sensing [12–14]. The imprinting technique is based on the development of either non-covalent or reversible covalent complexes formed between the target molecule and suitable functional monomers. Polymerization of preformed mixture of functional monomer and template molecule leads to a matrix in which the complexes are fixed. After extraction of the template molecule from this matrix, a tailor-made, highly selective synthetic receptor is created. These synthetic

receptors can be used for the detection of the respective target molecule via a suitable read out technique. Previously, impedance spectroscopy was employed for the detection of L-nicotine using L-nicotine imprinted MIPs. In this work, a quartz crystal microbalance (QCM) set-up was used for the detection of L-nicotine to see its performance in comparison with L-nicotine MIPs measured with impedance spectroscopy by Thoelen et al. [15]. The advantage of the QCM sensing platform is that it can be used in a variety of ways, be it fast sensing or a way to test the efficiency of the MIP. QCM applies the piezoelectric ability of quartz to sense an increase or decrease of mass on the surface of the quartz crystal. This mass change will cause the resonance frequency to change in a proportional way. This relationship is described in the Sauerbrey equation [16]. With the QCM-D set-up, it is also possible to measure the dissipation [17–20]. Changes in dissipation relate to the loss of energy of quartz crystal after addition of mass. The MIP particles are immobilized on the crystal via a transducer layer, which consists of polyvinyl

chloride (PVC). Measurements are performed in demi-water and phosphate buffer saline (PBS). The fluid environment in which the measurements are performed will influence the ability of the MIP to capture the molecule and finally influence the effectiveness of the sensor. This is important when using real life samples, because an optimal medium to measure in is required. In this work, the effect of pH and ionic strength on molecular recognition is investigated. The measuring condition was optimized using batch-rebinding experiments employing UV–VIS spectroscopy and a QCM.

2 Materials and methods

2.1 Reagents The imprinted polymer was synthesized using ethylene glycol dimethacrylate (EGDM) as the cross-linker, methacrylic acid (MAA) as functional monomer and hexane as the porogen. Prior to polymerization, the stabilizers in the MAA and EGDM were removed. For the initiator, azobisisobutyronile (AIBN) was used. All chemicals used in the polymerization were of analytical grade and were obtained from various commercial sources. The target molecule L-nicotine, $C_{10}H_{14}N_2$ (MW: 162.23 Da) (Fig. 1) was obtained from Acros. A similar molecule, L-cotinine, $C_{10}H_{12}N_2O$ (MW: 172.22 Da) (Fig. 1), which was obtained from Sigma–Aldrich, was used to test the specificity. Cotinine is a by-product of the human metabolism of L-nicotine. PVC was purchased from Sigma–Aldrich.

2.2 Equipment The binding constants and quality of the MIP were evaluated with UV–VIS spectroscopy using a NanoDrop 2000c UV–VIS NIR spectrophotometer from Thermo scientific. The morphology of the sensor surface was investigated using optical microscopy with a zeis axiovert 40 MAT. Microscope images were processed using the image analysis program ImageJ 1.37v from the national institute of health, USA. Quartz crystals (5 MHz) with Cr/Au electrodes were purchased from Lot-oriel. The QCM (Q-sense E4) was also purchased from Lot-oriel. A flow set-up was connected to a peristaltic pump (IPC-N) from Ismatec.

2.3 MIP synthesis For the preparation of the L-nicotine MIP, a mixture of MAA (12.5 mmol), EGDM (25.5 mmol) and AIBN (0.66 mmol) was dissolved in 7 ml hexane together with the template molecule L-nicotine (6.41 mmol). To exclude oxygen in the mixture, it was degassed for 10 min with N_2 . Subsequently, for polymerization the solution was sealed and kept in a thermostatic water bath at 60 °C (72 h). After polymerization, the solid MIP was grounded with a mechanical mortar and sieved

Figure 1 Structure of L-nicotine (left) and cotinine (right).

through a 25 μ m sieve. Only particles with a size smaller than 25 μ m were used. Next, the L-nicotine was removed from the MIP powder by extensive washing with methanol (48 h), followed by a mixture of acetic acid/acetonitrile (1/1) (48 h) and finally again with methanol (12 h) using a continuous extraction set-up. A non-imprinted polymer (NIP) was synthesized in the same way but without the presence of the target molecule.

2.4 Sensing system The QCM crystals have a resonance frequency of 5 MHz and are modified with Cr/Au electrodes. On the top electrode, a layer of PVC (PVC 0.7 wt% in tetrahydrofuran) was spincoated to obtain a layer of 100 nm thickness. The MIP was then stamped on the surface using a poly(dimethylsiloxane) (PDMS) stamp, which was pressed on the active sensing surface with a small force for a few seconds. This resulted in a thin layer of MIPs on the surface. Subsequently the PVC was heated to 120 °C, which is above the glass transition temperature of PVC, for 10 min causing the MIP particles to sink in the polymer. The samples were then cooled and the excessive MIP powder was rinsed off with deionized water. The surface coverage was assessed with light microscopy and the images were processed with the aid of ImageJ. An average surface coverage of 19.4% for the MIP and 20.2% for the NIP was found (Fig. 2). This is consistent with the surface coverage reported by Thoelen et al. [15]. QCM measurements were performed as follows. First, the system was left to stabilize in the fluid (demi-water or PBS) in which the performance of the MIP was to be tested. This was at a temperature of 22 °C with a flow of 200 μ l/min. After the signal was stable, different concentrations were consecutively connected to the flow system without in between washing. This results in a dose–response curve for the frequency and dissipation of the QCM-D system. The frequency noise was 0.835 Hz on average and the dissipation noise was 0.426×10^{-6} on average.

3 Results and discussion

3.1 Batch rebinding experiments To characterize the binding characteristics for the MIP and NIP in demi water and PBS with varying pH, batch-rebinding experiments were

Figure 2 (online colour at: www.pss-a.com) Optical microscopy image illustrating the surface coverage of MIPs (black areas) on quartz crystal (light area).

Figure 3 UV–VIS spectroscopy of L-nicotine in demi-water at varying pH for MIP and NIP.

Figure 5 UV–VIS spectroscopy of L-nicotine in PBS water at varying pH for NIP.

performed. To achieve this, 20 mg of MIP or NIP was added to 5 ml of water (or PBS) with different pH and different L-nicotine concentrations. These mixtures were stirred for 3 h on a rocking table at room temperature. Afterwards the mixtures were filtered using syringe filters (1 μM) in order to remove the MIP and NIP particles. The concentration of L-nicotine left over in the fluid (C_f) is measured via UV–VIS spectroscopy. With this information, the amount of L-nicotine bound per gram (S_b) of MIP/NIP can be calculated and binding isotherms for MIP and NIP for demi-water and PBS can be made. From these experiments, it is apparent that in demi-water the MIP binds L-nicotine very well in comparison with the NIP (Fig. 3). Fits in Figs. 3–5 are achieved with $y = ax^b$. The difference in binding capacity proves that this MIP/NIP is a very good candidate for use in sensing applications as the difference at low concentrations between MIP and NIP is significant. It is also clear that a difference in pH does not affect the binding ability of the MIP or NIP.

In PBS, the amount of bound L-nicotine is significantly reduced in comparison to demi-water (Fig. 4). This is probably due to the ions present in the PBS. In PBS, the pH plays a more important role in the binding ability. This is due

to protonation and deprotonation of L-nicotine and the functional monomer MAA, respectively. A more acidic PBS gives the least amount of binding. PBS at pH 5 binds as much L-nicotine as the NIP does at pH 10. The more base the PBS is, the better the amount of bound L-nicotine until it reaches its optimal pH of 9. Similar results with NIP in demi-water were observed where the NIP does not bind much L-nicotine and the effect of pH on the amount of bound L-nicotine is significantly diminished (Fig. 5).

3.2 QCM in water As mentioned before, the actual sensing system is a QCM-D, which works on the principle that an increase of mass on the sensor surface will induce a shift in the resonance frequency, which is proportional to the added mass. Also dissipation shifts or loss of energy can be measured. This dampening of the oscillating crystal can thus also indicate changes at the sensing surface. The prepared MIP and NIP crystals were exposed to different L-nicotine concentrations in demi-water (12.5–150 μM). The pH of the demi-water was unaltered because the UV–VIS spectroscopy showed that changing the pH did not affect the binding capacity of the MIP. In Fig. 6, it is clear from the

Figure 4 UV–VIS spectroscopy of L-nicotine in PBS water at varying pH for MIP.

Figure 6 QCM-based dose response curve of the frequency change for L-nicotine and cotinine in water.

Figure 7 QCM-based dose response curve of dissipation change for L-nicotine and cotinine in water.

frequency change that the MIP binds L-nicotine much more than the NIP does (Fig. 6). On an average, the MIP binds 4.64 times better than the NIP. At higher concentrations, the response of the MIP is lower, meaning that the MIP is getting saturated. This is also apparent from the NIP as the only frequency shift occurs at lower concentrations while at higher ones there is no further response. As a control reference for specificity, cotinine is also measured with same concentrations and conditions (Fig. 6). It is evident that neither MIP nor NIP bind any cotinine. The dissipation changes also indicate that the MIP binds L-nicotine more than the NIP (Fig. 7). The difference is less obvious in comparison to the frequency as the amount of bound L-nicotine on average is only 2.13 times larger for the MIP. From these results, it is again clear that cotinine does not bind to the MIP or NIP (Fig. 7). In Fig. 8, it can be seen that the frequency drops after the first 50 μM addition of L-nicotine and then rises during the washing step with demi-water. During the second addition of 50 μM L-nicotine, the frequency drops once again thereby confirming the reproducibility and reusability of the sensor.

Figure 8 MIP frequency response shifts illustrating the reusability of the sensor by consecutive additions of L-nicotine with an intermediate washing step.

Figure 9 QCM-based dose response curve (MIPs) of the frequency change for L-nicotine in PBS at pH 9 with varying ionic strengths. As a control, affinity for cotinine in 0.1 $\times$ PBS at pH 9 is presented.

3.3 QCM in PBS From the UV-VIS spectroscopy results in PBS, it is clear that binding of L-nicotine to the MIP is optimal at pH 9 and that ions present in the fluid inhibit interactions between the MIP and L-nicotine. To see how much this ionic content influences the binding, L-nicotine concentrations were measured in different PBS concentrations (1 $\times$ -, 0.5 $\times$ - and 0.1 $\times$ PBS). Each PBS buffer at a different concentration was adjusted to the optimal pH 9. For these measurements, a higher concentration range was chosen (100–500 μM) to give a very clear view on the difference of recognition between the buffers.

The frequency response between the buffers with the MIP is very distinct (Fig. 9). The 0.1 $\times$ PBS has the best response due to the lowest ionic content followed by 0.5 $\times$ PBS and 1 $\times$ PBS the latter giving the least response. This trend also exists with the NIP samples, however, the response is much lower in comparison with the MIP (Fig. 10). Taking into account both the MIP and NIP, recognition in 0.1 $\times$ PBS is 3.3 times more effective than 1 $\times$ PBS and 1.71 times better than 0.5 $\times$ PBS. Again, cotinine was used as a control

Figure 10 QCM-based dose response curve (NIPs) of the frequency change for L-nicotine in PBS at pH 9 with varying ionic strengths. As a control, affinity for cotinine in 0.1 $\times$ PBS at pH 9 is presented.

Figure 11 QCM-based dose response curve (MIPs) of the dissipation change for L-nicotine in PBS at pH 9 with varying ionic strengths. As a control, affinity for cotinine in 0.1 $\times$ PBS at pH 9 is presented.

Figure 12 QCM-based dose response curve (NIPs) of the dissipation change for L-nicotine in PBS at pH 9 with varying ionic strengths. As a control, affinity for cotinine in 0.1 $\times$ PBS at pH 9 is presented.

reference for specificity with the same conditions and concentrations. As can be seen in both Figs. 9 and 10, neither the MIP nor the NIP show any binding affinity to cotinine.

The dissipation has the same trend as the frequency response except that the difference between 0.1 $\times$ and 0.5 $\times$ PBS is less pronounced with the NIP samples (Figs. 11 and 12).

Looking at the differential signal of MIP and NIP, recognition in 0.1 $\times$ PBS is 4.49 times better than 1 $\times$ PBS and 2.07 times better than 0.5 $\times$ PBS. The dissipation does not change with the addition of cotinine (Figs. 11 and 12) thereby proving the specificity of the MIPs for L-nicotine. From these results, it is evident that the optimal fluid for measuring the recognition of L-nicotine is 0.1 $\times$ PBS at a pH 9. In order to compare the results with recognition in water, the same concentration range was measured in 0.1 $\times$ PBS at pH 9. For the same concentrations of L-nicotine, the frequency response in water (Fig. 6) is 2.08 times bigger than in 0.1 $\times$ PBS at pH 9 (Fig. 13). This lowered response is again

Figure 13 QCM-based dose response curve of the frequency change for L-nicotine in 0.1 $\times$ PBS at pH 9.

Figure 14 QCM-based dose response curve of the dissipation change for L-nicotine in 0.1 $\times$ PBS at pH 9.

due to the presence of ions in the PBS. On the other hand, there is no difference in the dissipation response as water (Fig. 7) has 0.96 times the response than 0.1 $\times$ PBS at pH 9 (Fig. 14).

4 Conclusion MIPs for the detection of L-nicotine were successfully developed via bulk polymerization for integration in sensing systems. UV–VIS spectroscopy proved the effectiveness of the MIP/NIP in demi-water and showed that the pH has little to no effect on recognition. UV–VIS spectroscopy was also utilized to examine the effect of pH in 1 $\times$ PBS buffer. In PBS, the pH plays a much larger role in the binding capacity of the MIP. It was shown that a pH of 9 was optimal for maximum binding in 1 $\times$ PBS. QCM-D proved to be a fast, sensitive, specific and reproducible sensing platform. The best performance was achieved in demi-water. As seen in both the frequency and dissipation change, the sensor was the most sensitive in the 12.5–50 μ M region. At higher concentrations, the sensor began to be saturated. Cotinine was used as a control for the

specificity of the MIP. It did not show any response in the frequency or in the dissipation. QCM-D was also used to test the influence of the ionic strength of PBS. Here, it was found that a higher ionic strength is detrimental for the binding of L-nicotine. The optimal buffer fluid to use as a medium for measuring was $0.1 \times$ PBS at pH 9. The reason for using $0.1 \times$ PBS is the need for a good medium to perform measurements on real life samples without losing the buffering capabilities but maintaining a good sensitivity for L-nicotine.

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