MIP sensors on the way to biotech applications: Targeting selectivity

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ABSTRACT

Molecular imprinting among others leads to recognition layers toward pharmaceutically or physiologically active compounds that can be applied for rugged mass-sensitive sensing. In the case of ephedrine, quartz crystal microbalance (QCM) measurements in aqueous solution result in a detection limit of ~5 ppm. When assessing two folic acid metabolites, leucovorin yields LoD of 20 ppm and anhydroleucovorin even 1 ppm. Systematic selectivity studies reveal that acrylate-based molecularly imprinted polymers (MIP) for ephedrine detection most of all address the secondary amino group of the compound, followed by the aromatic ring. In contrast to this, the aliphatic part and the hydroxyl group do not substantially contribute to sensing. In the case of the two folic acid metabolites, the present study to the best of our knowledge represents the first successful imprinting approach. Besides very appreciable selectivity between the two metabolites and folic acid, respectively, it also strongly indicates that applying solvent mixtures substantially increases the sensitivity of the resulting sensors.

1. Introduction

Chemical sensing and biochemical sensing have been in the focus of substantial scientific interest for three decades because of their potential to be applied online and in real-time measurements [1]. Synthesizing suitable selective recognition materials is a key aspect during sensor development and often poses a challenge for materials scientists [2]. Molecular imprinting [3] promises a remarkably fast and straightforward possibility to design such artificial receptors. Here, the analyte acts as a template and is mixed with monomers forming a highly cross-linked polymeric matrix based, e.g., on polyurethane, polystyrene or polymethacrylate [4,5]. Such systems have proven an exciting alternative to applying biological receptors in chemical sensing [6–8]. Hence, recent years have seen a substantial amount of MIP-based sensors published, including electrochemical, mass-sensitive, optical and surface plasmon resonance (SPR) measurements [9–16]. However, for actually implementing MIP into technology and hence bridge the gap between fundamental research and application, further studies are required especially focusing on binding mechanisms between MIP and analyte, selectivity, and assessment of ruggedness. Pharmaceutical industry with its quality control requirements is potentially a very interesting customer for MIP-based sensors. Our present study thus takes into consideration key analytes for this application scenario, namely ephedrine and two folic acid metabolites (leucovorin and anhydroleucovorin). Ephedrine, a well-known

alkaloid, is usually used as a stimulant, appetite suppressant, concentration aid, decongestant and for treating hypotension [17]. Folic acid together with its metabolites is analytically interesting both for food supplement and pharma industry as well as for diagnostics. Furthermore, both ephedrine and folic acid derivatives contain a range of functionalities in their structures. This makes the especially suitable candidates for systematic selectivity tests.

2. Experimental

2.1. Chemicals

Tetrahydrofuran (THF), diphenyl methane (DMF), methacrylic acid, ethylene glycol dimethacrylate (EGDMA), methyl methacrylate (MMA), azobisisobutyronitrile (AIBN), ephedrine, N,N′ (1,2-dihydorxyethylene) bisacrylamide, sodium peroxidisulphate and N-vinyl 2-pyrrolidone were purchased from Merck and Fluka, respectively, with maximum available purity and used as received. Leucovorin and anhydroleucovorin were provided by the Department of Nutrition Science, University of Vienna.

2.2. Synthesis of ephedrine MIP

5 mg ephedrine was dissolved in a solvent mixture containing 50 μL tetrahydrofurane (THF) and 10 mg of diphenylmethane (DMF) as a porogen in an Eppendorf reaction tube. Then 20 mg methacrylic acid, 70 mg ethylene glycol dimethacrylate (EGDMA) and 10 mg methyl methacrylate (MMA) were added to the solution and dissolved by sonication. Finally 5 mg azobisisobutyronitrile (AIBN) was added as a polymerization initiator. This was

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followed by UV polymerization (λ_{max} 360 nm and 210 W) for 20 min just prior to reaching the gel point and then stirred overnight to obtain nanoparticles of MIP via a precipitation polymerization approach. The respective non-imprinted polymer (NIP) was prepared in exactly the same way but for template addition. Both approaches resulted in globular particles of 150–200 nm diameter, as determined by AFM. For generating recognition layers, 7 μL of the respective stock solution was spin coated at 3500 rpm onto the measuring electrode of a 10 MHz-QCM prepared according to the procedures shown in [18], while the second electrode was spin coated with NIP. Finally, the devices were kept overnight at room temperature for drying and hardening and then washed with water overnight to extract the template. Layer heights were determined by the frequency shifts they cause on a QCM (1 kHz corresponds to roughly 40 nm). The abovementioned procedures resulted in roughly 160 nm layer height. The apparent contradiction with the particle diameters can be explained by the interstitial place between individual particles, as the frequency change is of course caused by mass, which in this case cannot be translated directly into layer heights.

2.3. Synthesis of leucovorin MIP

Firstly, 400 μL of water was filled into a reaction tube followed by adding 32 mg methacrylic acid along with 8 mg of N,N' (1,2-dihydorxyethylene) bisacrylamide. After stirring thoroughly and adding 6 mg of sodium peroxidisulphate a radical initiator, 4 mg leucovorin was dissolved in this stock solution, which was then polymerized under UV (same wavelength as power as above) for 40 min till the reach of gel point. The NIP was prepared keeping all the components and parameters except the template addition constant. Then 5 μL MIP oligomer solution was spin coated at 2000 rpm onto the measuring electrode of a QCM while NIP was spin coated onto the second electrode. The QCM were kept overnight for drying as well as hardening and were then heated at 100 °C in the oven for 2 h. Finally the template was removed with warm water at 50 °C for 1.5 h prior to the mass sensitive measurements.

2.4. Synthesis of anhydroleucovorin MIP

This material was synthesized by dissolving 10 mg of methacrylic acid, 10 mg of N-vinyl 2-pyrrolidone and 30 mg EGDMA thoroughly in 200 μL of a mixed solvent consisting of 3 parts (v/v) methanol and 2 parts (v/v) DMF containing 4 mg anhydroleucovorin. The polymerization then was initiated after adding 1 mg of AIBN under UV and pre-polymerized for 3 h. The resulting oligomer solutions were spin-coated onto QCM in the same way as above.

2.5. Sensor measurements

Mass sensitive measurements based both on network analyzer (Agilent Technologies E5062A) and dual-channel frequency counter (Agilent Technologies 53131A), respectively, were carried out following previously published protocols [18]. We used the same custom-made oscillator circuit for operating the quartz and a LabView Routine for signal readout.

3. Results and discussion

Coating ephedrine MIP nanoparticles (MIP-NPs) on QCM electrodes yields appreciable sensor responses depending on the concentration of ephedrine as can be seen in Fig. 1. Obviously, solutions containing down to 20 ppm of the analyte yield significant frequency responses allowing for a limit of detection of ∼5 ppm for the QCM sensor. Furthermore, the response characteristic in
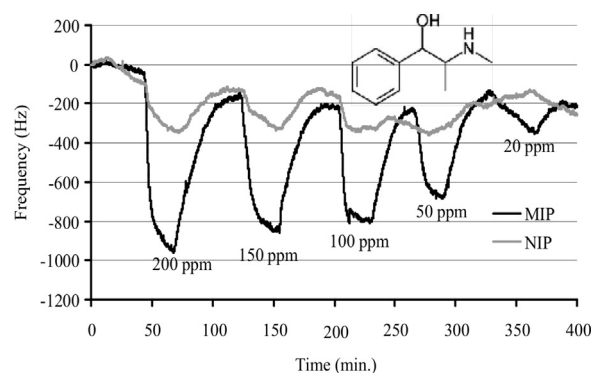

Fig. 1. The sensor signal of molecularly imprinted polymer nanoparticles (MIP-NPs) toward different concentrations of ephedrine.

the concentration range between 200 ppm and 20 ppm indicates fully reversible and linear sensor signals. It is worth to note that ephedrine thin-film MIP – i.e., not based on nanoparticles – do not yield any sensor responses in this concentration range (data not shown). The rationale for selecting acrylic polymer systems for this MIP is determined by the —NH group present in the molecule. So the compound can be expected to interact with the —COOH functionality of the polymer backbone.

Still, sensitivity data alone does not necessarily indicate the main binding group responsible for analyte–MIP interactions. However, even straightforward QCM measurements can yield further evidence via systematic selectivity studies with the sensors. For obtaining such structural information, it is necessary to determine selectivity toward structurally related compounds and/or compounds that basically can be regarded as "substructures" of the ephedrine molecule. Hence it does not make sense to test toward possible (technological) contaminants or competing compounds expected in a real-life matrix. Fig. 2 shows the QCM selectivity pattern resulting from exposing the MIP to 200 ppm of ethyl amine, 2-butanol, 1-propanol and toluene, respectively, all of which contain structural elements that are also present in ephedrine.

Obviously, 1-propanol and 2-butanol yield no significant sensor responses, whereas toluene gives rise to an effect of about 100 Hz. However, from Fig. 1, it is evident that at this concentration ephedrine yields a net sensor signal as high as 600 Hz (900 Hz on the MIP minus 300 Hz effect on the NIP). Obviously, the aliphatic OH group of the compound does not play a significant role in recognition, because otherwise the two primary alcohols would at least give rise to some frequency response on the device. For the same reasons, any major interaction between the polymer backbone and the aliphatic part of the ephedrine molecule can be ruled out. The somewhat higher sensor response of the toluene, however, indicates that the aromatic ring (probably together with the
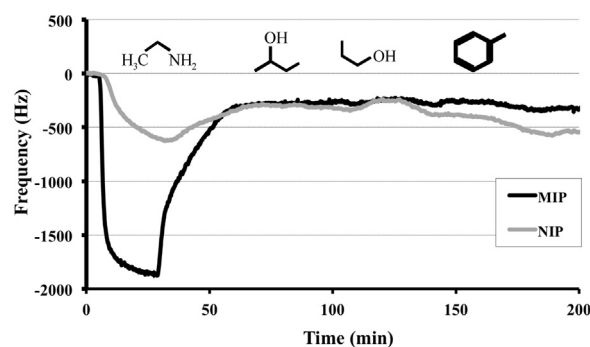

Fig. 2. Selectivity behavior of ephedrine imprinted polymer toward ephedrine and its analogs.