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Affinity sensor based on immobilized molecular imprinted synthetic recognition elements

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ABSTRACT

An affinity sensor based on capacitive transduction was developed to detect a model compound, metergoline, in a continuous flow system. This system simulates the monitoring of low-molecular weight organic compounds in natural flowing waters, *i.e.* rivers and streams. During operation in such scenarios, control of the experimental parameters is not possible, which poses a true analytical challenge. A two-step approach was used to produce a sensor for metergoline. Submicron spherical molecularly imprinted polymers, used as recognition elements, were obtained through emulsion polymerization and subsequently coupled to the sensor surface by electropolymerization. This way, a robust and reusable sensor was obtained that regenerated spontaneously under the natural conditions in a river. Small organic compounds could be analyzed in water without manipulating the binding or regeneration conditions, thereby offering a viable tool for *on-site* application

1. Introduction

Continuous sampling and monitoring of target compounds in rivers and streams is an extremely challenging analytical task where the assay response time is of crucial importance. Depending on public health risk, quick action might be required. Pollutants in rivers have a great impact on communities and eco-systems both close to the polluted area and downstream. To address this issue, a robust, stand-alone, and preferably cheap solution is needed. Therefore, a sensing unit, which can be submerged in a river to continuously monitor specific target substances, could be of interest. The most important requirement is that the device can operate independently over a longer period of time, *e.g.* several weeks or months. We have in this work developed an affinity sensor based on capacitive transduction, able to perform as the sensing device (detector) in such a unit. Molecularly imprinted polymers (MIPs) were used as recognition elements, where binding of the analyte to the sensor surface caused a capacitance change, which was further assessed by a constant current pulse to the sensor transducer, as described by Erlandsson et al. (2014).

The choice of using MIPs as recognition elements was based on the specific conditions in which the system needs to perform (Ruigrok et al., 2011). Contrary to natural antibodies, MIPs have no biological origin, are robust, and are chemically and thermally stable (Sellergren, 2001). This robustness allows for application in environments where biological recognition elements are unsuitable or will denature. MIPs can also be reused multiple times, which is essential to meet the stand-alone requirement. Metergoline was chosen as a model compound for small organic molecules. Small molecules, such as pesticides, herbicides, antibiotics, are widely discarded and encountered in naturally flowing waters. Imprinted polymers where metergoline was used as the template molecule have been described (Lenain et al., 2012). Metergoline, a drug used to treat disorders associated with hyperprolactinaemia and prophylaxis of migraine, was used as a model compound in this study, representative of small organic structures such as pharmaceutical residues (Reynolds, 1993).

Early attempts comprised the incorporation of MIPs in carbon paste through powder processing. An imprinted bulk-polymerized monolith was crushed, sieved, and mixed with carbon paste to produce a sensor (Blanco-López et al., 2004). A variation on this two-step method was the inclusion of particles in a sol–gel, where the swelling in an aqueous environment intended to facilitate better mass transfer of the analyte to the recognition elements, thus enhancing the sensitivity. However, these attempts suffered from reduced sensitivities, slow kinetics and long response times (Mazzotta et al., 2008). Proper regeneration of the surface was also inhibited, often limiting a sensor to a single use.

Most techniques described in the literature for combining MIPs and electro-chemical sensors utilize *in situ* approaches where the imprinting process and the coupling to the transducer occur in one

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step (Alexander et al., 2006; Blanco-López et al., 2004), and where the most frequently employed technique for producing MIPs to be immobilized onto the transducer surface is direct bulk polymerization (Morita et al., 1997). Similarly, monomers can be physically adsorbed onto a conducting surface in the presence of template, and electro-polymerized, leaving a functionalized layer behind (Suryanarayanan et al., 2010). Other film immobilization techniques include the use of agar gels, silanes or self-assembled monolayer (SAM) formation on the surface (Suryanarayanan et al., 2010). More refined sensor configurations could be obtained by applying grafting procedures, thereby producing functionalized imprinted monolayers (Panasyuk-Delaney et al., 2001). The performance of these sensors relies on the density and proper orientation of the SAM. One disadvantage of these films is the limited surface available for interaction with analytes. Another general drawback is the removal of template after polymerization. In fact, since these are label-free techniques, template leakage can cause false measurements. However, *in situ* prepared sensors cannot be subjected to harsh conditions for removal of template since this affects adsorption-based functionalized surfaces. The authors propose a two-step approach to overcome these drawbacks.

It was opted to produce small, uniformly sized, spherical MIPs, and their non-imprinted counterpart, NIPs, in a separate step by use of emulsion polymerization (Dvorakova et al., 2012; Perez-Moral and Mayes, 2004). NIPs lack specific binding sites and the comparison of their performance with MIPs therefore enables discrimination between specific and non-specific binding. The obtained beads were attached to a gold electrode surface via a thin but well-insulating polytyramine layer. Finally, residual pinholes in the sensing layer were covered with 1-dodecanethiol. This approach offered important advantages. The issue of template leakage was addressed by the off-line bead production since this allowed thorough and complete template removal with organic solvents, bases and increased temperature, which would otherwise damage *in situ* prepared electrodes. Also, employing spherical particles effectively enlarged the surface available for interaction with analyte molecules.

2. Experimental

2.1. Materials

Chloroform (98%) was purchased from Novolab (Geraardsbergen, Belgium). Methanol (MeOH, LC-MS grade) was obtained from Biosolve BV (Valkenswaard, Netherlands), acetone (> 99.5%) from Fiers (Kuurne, Belgium), and ethanol (EtOH absolute, Analar Normapure) from VWR International (Leuven, Belgium). Sulfuric acid (95–97%), potassium dihydrogen phosphate (KH_2PO_4, p.a.), and potassium chloride (KCl, p.a.) were bought from Merck (Darmstadt, Germany). Azobisisobutyronitrile (98%), sodium dodecyl sulfate (≥ 98.5), metergoline (p.a.), methacrylic acid (MAA, 99%), ethylene glycol dimethacrylate (EGDMA, 98%), hexadecane (98%), hydrogen peroxide (30 wt%), tyramine (99%), dipotassium hydrogen phosphate (K_2HPO_4, ≥ 98%), 1-dodecanethiol (> 98%), potassium ferricyanide ($K_3[Fe(CN)_6]$, ≥ 99.0%), and triethylamine (≥ 99%) were purchased from Sigma Aldrich (Bornem, Belgium). Ultrapure water was obtained with a MilliQ system from Millipore (Brussels, Belgium).

2.2. Emulsion polymerization of metergoline-imprinted polymers

First, stock solutions of the disperse and continuous phases, respectively organic and aqueous phases, were prepared in order to minimize weighing errors. The disperse phase consisted of 5 ml chloroform and 113 mg azobisisobutyronitrile, dissolved in a 10 ml round-bottomed flask. The continuous phase comprised/(consisted of?) 25 ml ultrapure water and 720 mg sodium dodecyl sulfate mixed in a 50 ml flask, thus obtaining a 0.1 M surfactant solution. Template (metergoline), functional monomer (MAA) and crosslinker (EGDMA) were added in a 1:6:24 M ratio. In a long, small 20 ml glass flask, 50 mg metergoline (0.124 mmol), 63 μl MAA (0.743 mmol), 19.33 μl hexadecane, 0.561 ml EGDMA (2.97 mmol) and 0.95 ml disperse phase were added and stirred at 500 rpm with a magnetic stirrer. Hexadecane served as a co-stabilizer for the surfactant micelles. Next, 5 ml of the continuous phase was added and the content homogenized at 24,000 rpm for 60 s using an IKA-mixer (IKA-Werke, Yellow line DI 25 Basic, 12 mm diameter mixer bar). The obtained mixture was transferred to a 25 ml round-bottomed flask together with a small magnetic stirrer bar and a tap was placed on the flask. This was submerged in an ice bath (0 °C) and allowed to cool down for a few minutes. Cooling is necessary to reduce the evaporation of chloroform in the next step. Vacuum was applied to the flask by use of a vacuum pump after which the tap was sealed. Nitrogen was introduced into the flask which then was placed in a thermostatic cooling chamber (Thermotron, set at 10 °C) above a magnetic stirrer plate and stirred at 250 rpm during the entire reaction. The flask was located 20–22 cm away from the UV light (75 mW cm^{-2}; $\lambda = 365$ nm) and the polymerization reaction was initiated and allowed to react for 1 h.

After polymerization, the mixture was collected in aliquots and centrifuged at 12,000 g (Awel MF48-R, NuAire, Chateau Gontier, France). The supernatant was discarded and MeOH was added to remove the template and unreacted monomers. The aliquots were placed in a shaker for 30 min, then centrifuged (12,000 g) and the supernatant was discarded. After the centrifugation step, a sample of the supernatant was injected in a time-of-flight-mass spectrometer to verify the absence of template. This process was repeated several times until all template was removed. Subsequently, the aliquots were placed in an oven, overnight, at 40 °C. Non-imprinted polymers were prepared in exactly the same manner, however, without addition of metergoline. The size of the beads was measured with a Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK) and the size distribution of the MIP obtained by dynamic light scattering (DLS) is displayed in Supplementary Fig. 1. Ninety percent of the MIP beads were distributed between 200 and 800 nm, with the median at 350 nm.

2.3. Coupling of MIP beads to an electrode

Disposable electrodes were prepared by use of thermal evaporation to coat a silicon wafer with chromium (50 nm) and gold (200 nm) as described by Teeparuksapun et al. (2009, 2012). Before coupling, the electrode surface was cleaned to remove protective coatings on the surface. The electrode was submerged and sonicated for 10 min in acetone, ethanol, and piranha solution (H_2SO_4:H_2O_2; 3:1), successively, and subsequently dried under a stream of nitrogen.

MIP or NIP particles were suspended by sonication in a 10 mM conductive tyramine solution. This solution was prepared by dissolving 0.0137 g of tyramine (0.100 mmol) in 2.5 ml ethanol. Once all tyramine was dissolved, 7.5 ml of phosphate buffer (10 mM KH_2PO_4/K_2HPO_4 in ultrapure water, pH = 7.2) was added. An electrode was fixed in a reaction cell and the suspended solution was transferred to this cell. The beads were allowed to sediment onto the electrode's surface for 30 min. The electrode acted as a working electrode, and the reaction cell was further equipped with a platinum reference and auxiliary electrode that allowed the electro-oxidation of tyramine by variation of the potential. After 15 potential sweeps between 0 V and 1.5 V with a 0.05 V s^{-1}