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Capacitive sensing of N-formylamphetamine based on immobilized molecular imprinted polymers

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Abstract

A highly sensitive, capacitive biosensor was developed to monitor trace amounts of an amphetamine precursor in aqueous samples. The sensing element is a gold electrode with molecular imprinted polymers (MIPs) immobilized on its surface. A continuous-flow system with timed injections was used to simulate flowing waterways, such as sewers, springs, rivers, etc., ensuring wide applicability of the developed product. MIPs, implemented as a recognition element due to their stability under harsh environmental conditions, were synthesized using thermo- and UV-initiated polymerization techniques. The obtained particles were compared against commercially available MIPs according to specificity and selectivity metrics; commercial MIPs were characterized by quite broad cross-reactivity to other structurally related amphetamine-type stimulants. After the best batch of MIPs was chosen, different strategies for immobilizing them on the gold electrode's surface were evaluated, and their stability was also verified. The complete, developed system was validated through analysis of spiked samples. The limit of detection (LOD) for N-formyl amphetamine was determined to be 10 μM in this capacitive biosensor system. The obtained results indicate future possible applications of this MIPs-based capacitive biosensor for environmental and forensic analysis. To the best of our knowledge there are no existing MIPs-based sensors toward amphetamine-type stimulants (ATS).

Keywords: capacitive biosensor, molecular imprinted polymers, N-formyl amphetamine, water analysis.

1. Introduction

Synthetic drugs are one of the most significant current abused substances worldwide. Amphetamine-Type Stimulants (ATS) are globally the second most widely used drugs after cannabis (EMCDDA 2009), exceeding the use of cocaine and heroin. ATS are potent central nervous system (CNS) stimulants, capable of inducing euphoric state similar to cocaine (Sato 1986). ATS production contributes to environmental pollution (EMCDDA 2011), so there is a demand to develop robust and sensitive detection system for ATS in environmental water samples. To perform continuous monitoring, the detecting unit must be submerged directly into the sample or furnish a constant flow-through approach. A possible application is the monitoring of drugs in wastewater which can be used to estimate drug consumption and is called sewage epidemiology (van Nuijs et al., 2011).

Besides the analysis of drugs in wastewater to estimate human consumption one could also look for drug synthesis intermediates to estimate drug production. One of the most common methods to synthesize amphetamine is the Leuckart route(Aalberg et al., 2005). This method consists of two steps with the first step converting benzylmethylketone (BMK) into the intermediate N-formylamphetamine (N-FA) and the second step, which forms amphetamine out of N-FA. Therefore, N-FA is a promising marker substance indicating that an illicit amphetamine synthesis following the Leuckart route took place. It has to be noted in this context that not all of the amphetamine detectable in wastewater originates from illicit production or illegal consumption as amphetamine is also ingested legally as a prescription drug by persons suffering from the attention-deficit hyperactivity disorder (ADHS) or narcolepsy (e.g. dexamphetamine sulphate in Attendin® for the treatment of ADHS).

However, analyzing wastewater means that the sensor has to permanently resist quite harsh environmental conditions like pH changes, biofilm growth, human-made sweepings and temperature influence. All abovementioned obstacles hurdle the use of any kind of natural or engineered naturally based receptors as recognition elements in this kind of detection approach. Amphetamine and methamphetamine have some limited therapeutic use in narcolepsy and ADHD, but most are produced in clandestine laboratories around Europe (King 2009). Amphetamine is the most popular within the group of ATS and its found at the highest concentrations in environmental water samples(Caliman 2013). Amphetamines are frequently found in surface waters across Europe at levels reaching 50 ng/L(Kasprzyk-Hordern et al. 2008).

Illicit drugs continue to be topic of research, since Jones-Lepp reported the first finding of methamphetamine and ecstasy traces in U.S. environmental waters in 2004 (Richardson 2009). Many methods have been reported to assess amphetamine level in aqueous samples. The mass spectrometric analysis of illicit drugs in wastewater and surface water are the most popular and has been recently reviewed by Castiglioni et al. where authors devoted one chapter to amphetamine detection (Castiglioni et al. 2008). To mention only few, Zuccato et al. described presence of several illegal drugs and their

metabolites including amphetamine in several lakes and rivers across the Europe. Cation exchange cartridges were used for drugs extraction, and LC/ MS/MS was used as the detection method were LOD and LOQ for amphetamine were 0.19 ng/L and 0.65 ng/L. Daily loads of the drug residues were measured in the rivers Po, Arno, Lambro and Olona at each sampling site and revealed 30, 1.4, 0.1, 2.4 g respectively (Zuccato et al. 2008). Liquid chromatography / tandem mass spectrometry (LC-MS/MS) was used by Nowicki and coworkers to analyze wastewater samples collected from the main Wastewater Treatment Plant in the city of Poznan. They reposted up to 0.71 ng/L amphetamine coming from drug consumption during their studies (Nowicki et al. 2014). Mentioned chromatographic techniques are accurate but also time consuming, expensive and require highly trained personnel. Biosensor approach is a perfect alternative as cheap, portable machine providing in situ results. The aim of this manuscript was to develop a solution for determination of N-formyl amphetamine, one of ATS stimulants. The detecting system must be able to withstand the harsh working conditions imposed by the water environment. Desirably, system have to be designed so it can regenerate after the binding events; therefore, able to operate over a longer period of time, e.g. several weeks. An important requirement is the desired sensitivity and specificity of the electrochemical sensor: the system must be able to detect specifically target in ppb range (the cut-off was based on the results of preliminary experiments concerning to screening of NFA in water samples). To perform this continuous monitoring, the detecting unit must be submerged in water or furnish a constant flow-through approach, hence permanently stand quite harsh environmental conditions as pH changing, potential presence of algae, human-made sweepings and temperature influence. All abovementioned obstacles hurdle the use of any kind of natural or " engineered naturally-based" receptors as recognition elements in this kind of detecting approach. Contrary to natural antibodies and aptamers (Sellergren 2001; Vlatakis et al. 1993), MIPs, which have no biological origin, are robust and characterized by a high mechanical and thermal stability and show an excellent chemical resistance in a broad pH range (Svenson and Nicholls 2001). This robustness makes MIPs a preferred type of receptors for application in environments where any biological receptors can degrade, denature or lose their affinity. Besides, the opportunity to regenerate MIPs can significantly simplify the use of this sensing unit as sensing elements do not need to be changed after every single measurement, which is an important requirement for the stand-alone equipment. MIPs are polymers synthesized using the molecular imprinting technique which leaves cavities in the polymer matrix which are complementary in size and shape to the target analyte (Suryanarayanan et al. 2010). MIPs are sometimes called artificial or plastic antibodies, these bio-mimetics can react with molecules by covalent or non-covalent binding. This variety of features makes MIPs the perfect sensing layer in a biosensing approach.

Capacitive based label-free sensors are one of the unique platforms that do not require complex sample preparation, it provides a stable signal allowing constant read-out and process monitoring (Erlandsson et al., 2014). A typical capacitive sensor sensitively generates the signal upon binding of the target analyte due to changes in electrical double layer composition on the interface between buffer and electrode surface. Thus, the observed change in the capacitive signal depends mainly on the nature of the targeted molecules and their interaction with sensing element and can thus be used to quantify interactions between ligands immobilized on the metal surface and the target compound.

To the best of our knowledge there are no existing MIPs-based sensors toward amphetamine and N-formylamphetamine (N-FA), which is an intermediate in the production of amphetamine by the Leukart reaction(NATIONS 2006). Also only a few commercial MIPs against amphetamine are available. Therefore, the aim of this study was to compare different techniques to obtain MIPs with high specificity towards ATS and characterize them for following use in a capacitance sensor.

2. Experimental

2.1 Materials and methods

N-formylamphetamine (N-FA), N,N-di(β-phenylisopropyl)amine hydrochloride and benzyl methyl ketone, were kindly provided by BKA/Bundeskriminalamt (Wiesbaden, Germany). Azobisisobutyronitrile (AIBN, 98%), acetonitrile (ACN) methacrylic acid (MAA,99%), ethylene glycol dimethacrylate (EGDMA,98%), hydroxyethyl methacrylate (HEM), acetophenone, methylbenzylamine, hydrogen peroxide (30wt%), tyramine (99%), dipotassium hydrogen phosphate (K_2HPO_4, ≥98%), 1-dodecanethiol (≥98%), itaconic acid, N-Hydroxysuccinimide (NHS), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), 3-Mercaptopropionic acid (MPA, ≥99%), lipoic acid (LA), potassium ferricyanide (K3[Fe(CN)6], Z99.0%), and trimethylamine (≥99%) were purchased from Sigma Aldrich (Bornem, Belgium). Methanol was purchased from Biosolve BV (Valkenswaard, Netherlands). Acetone (99.5%) was obtained from Fiers (Kuurne, Belgium) and ethanol (EtOH absolute, Analar Normapure) from VWR International (Leuven, Belgium). Ciba® IRGACURE® 651 was purchased from Ciba (Basel, Switzerland). Potassium dihydrogen phosphate (KH_2PO_4, p.a.) and potassium chloride (KCl, p.a.) were bought from Merck (Darmstadt, Germany). Ultrapure water was obtained with a MilliQ system from Millipore (Brussels, Belgium). Amphetamine-HCl and methamphetamine-HCl were obtained from Lipomed (Arlesheim, Switzerland). Dimethyl formamide (DMF) was provided by Acros Organics (Geel, Belgium). Development resin (amphetamine MIP) were purchased from MIP Technologies (Lund, Sweden). Sputtered gold electrodes were provided by CapSenze AB (Lund, Sweden).

2.2 Synthesis of N-FA-imprinted polymers

For synthesis of the MIPs towards N-FA (N-formylamphetamine, an intermediate in the production of amphetamine by the Leukart reaction) three approaches, based on the use of different monomers, cross linkers and polymerization techniques were compared (Table 1).

2.2.1 Bulk polymerization

The first technique was based on the modified procedure described by Djozan(Djozan et al. 2012) (MIPs1) and implemented MAA as functional monomer and EGDMA as cross-linker. First, 50 μmol of N-FA were dissolved in 100μL of methanol, then subsequently diluted in 10 mL of ACN. Next 30 mmol of MAA were added, and the mixture was ultrasonically stirred for 5 min prior to the appending of 120 mmol of EGDMA. All compounds were mixed, the solution was blown with nitrogen for 10 min and acted as a pre-polymerization mixture. Thermal polymerization was initiated by addition of AIBN, and the reaction was carried out for 24 h under 60 °C. Non-imprinted polymers (NIPs) were synthesized using the same procedures without the addition of N-FA template. The resultant hard bulk polymers (Fig. 1a) were crushed, ground, and wet sieved in a mixture of methanol/ acetic acid/MilliQ water (4/1/1, v/v/v) on a shaker for 1h and dried.

2.2.2 Precipitation polymerization

For the second approach, the modified technique described by Piletska et al.(Piletska et al. 2005) (MIPs2) was applied. A mixture consisting of N-FA (50 μmol), HEM (3 mmol), itaconic acid (3 mmol), EDGMA (9 mmol), and Irgacure 651 as an initiator (0,03 mmol) in 2,5 mL of DMF was prepared and blown with nitrogen for 10 min. Polymerization was carried out for 1 hour under UV lamps (wave length range: 300-400nm).

The reactions resulted in a small amount, of aggregates (Fig. 1b), which were sieved in a mixture of methanol/ acetic acid/ MilliQ water (4/1/1, v/v/v) for 1h and then dried. Non-imprinted polymers (NIPs) were synthesized using the same procedure without the addition of N-FA template.

2.2.3 *In situ* polymerization

Due to bulky shape of MIPs1 obtained using the first approach and an insufficient reaction yield of the second approach (MIPs2) an *in situ* MIPs polymerization, directly on the electrode surface was investigated. *In situ* polymerization was prepared with the same pre-polymerization mixture as used in the second approach, implementing two kinds of initiators, AIBN and Ciba® IRGACURE® 651 in order to choose the best method. Thermal polymerization (MIPs3) was initiated by addition of 2,2-azobis-2-

isobutyronitrile (AIBN), and the reaction was carried out for 24 h under 60 °C. UV polymerization (MIPs4) was initiated by Ciba® IRGACURE® 651 (2,2-Dimethoxy-1,2-diphenylethan-1-one), and performed under UV lamps with UV wave length range between 300-400 nm, for 1 hour.

The morphologies of microspherical MIPs particles prepared by bulk, precipitation and *in situ* polymerization were compared using Scanning Electron Microscopy (SEM, FEI Company, Eindhoven The Netherlands, (Fig.1). For reference, these MIPs were compared with commercial MIPs towards amphetamine (Fig. 1d). The obtained functionalized electrodes were eventually tested using an electro-chemical detection of N-FA performed using an automated flow injection system, developed by CapSenze AB (Lund, Sweden).

2.3 Preliminary electrodes pretreatment

Before coupling, the electrode surface was cleaned to remove the protective coatings. Electrode were submerged and sonicated for 10 min in MilliQ water, ethanol, acetone and piranha solution (H_2SO_4/H_2O_2; 3/1, v/v) successively, and subsequently dried under a stream of nitrogen. Finally, microbiological contaminants were removed before modification by 20 minutes' sterilization in a plasma cleaner.

2.4 Immobilization of polymer beads on the gold electrode

Apart from *in situ* approach (see 2.2.3), an alternative method for immobilization of MIPs or NIPs on the gold electrode surface was investigated. The first approach was based on the formation of a polytyramine monolayer on the preliminary pretreated electrode surface via electro polymerization of 0.1 M of tyramine dissolved in methanol. MIPs beads were integrated in the polytyramine layer through mechanism of matrix entrapment(Tenreiro et al. 2007).

Commercial amphetamine MIPs were used as model compound for immobilization tests since they were easily available. MIPs were suspended by sonication in conductive tyramine solution as described before by Lenain and coworkers (Lenain et al. 2015b). Clean electrodes were placed in a reaction cell and filled with the MIPs suspension. MIPs were allowed to sediment for 30 minutes. A wafer golden electrode was employed as working electrode, a platinum wire was inserted in the reaction cell acting as reference electrode and as auxiliary glassy carbon electrode (Metrohm, Herisau, Switzerland) was used. Described composition allowed the electro-oxidation of tyramine by variation of the potential. All electrodes were connected to the potentiostat (Autolab, Utrecht, Netherlands) and electro-oxidation of tyramine was performed using Nova software. Cyclic voltammetry (15 potential sweeps) was performed covering a potential range from 0 V to 1.5 V with scan rate of 0.05 V/s. When scanning was completed,

the electrodes were rinsed thoroughly with MilliQ water and ethanol to remove any non-polymerized tyramine monomers and dried subsequently. To insulate all remaining pinholes, electrodes were kept in 10 mM 1-dodecanethiol for 30 min.

2.5 Electrode surface characterization by cyclic voltammetry (CV)

The insulation of gold electrodes after MIPs immobilization was verified with cyclic voltammetry (CV). CV was carried out in 0.1 M KCl containing 50 mM K3[Fe(CN)6] with potential range of 0.25-0.70 V at 0.05 V/s. The electrochemical measurements were performed in a three-electrode configured batch cell comprising Ag/AgCl as reference electrode, platinum wire and modified gold as counter and working electrode, respectively.

2.6 Automated flow injection system

Capacitive measurements are based on the electrical double layer theory(Devanathan and Tilak 1965), experiments were performed in triplicates. Electrochemical detection of N-FA was implemented using an automated flow injection system, developed by CapSenze Biosystems AB (Lund, Sweden) and was based on processing the capacitance changes. This approach is based on current pulse capacitive measurements, and was described for the first time by Erlandson et al.(Erlandsson et al. 2014). Ten mM KH_2PO_4/K_2HPO_4 buffer was implemented as a running buffer. Before (to clean the surface and remove weakly bound compounds) and after each injection regeneration of the working electrode was performed using a mixture of MeOH and running buffer with 5% triethylamine (47.5/47.5/5). The capacitance measurement was performed via the current step method where a constant current of + µ10 mA and -µ10 mA were alternately supplied to the electrode surface. The capacitance was calculated from the resulting registered potential profile and plotted as function of time. The binding event between N-FA and the immobilized MIPs or NIPs (flow rate of 1.67 µL/sec, sample volume of 250 µL, regeneration buffer volume 250 µL) on the electrode surface resulted in a decrease in the registered capacitance. The capacitive responses for both the MIP and NIP functionalized electrodes were sampled with 1 min intervals.

3. Results and discussion

3.1. Evaluation of MIPs synthesis

The first approach implementing traditional bulk polymerization and MAA as functional monomer, resulted in rough particles of dimensions exceeding 100 µm. MAA was applied based on literature review

as one of the most commonly used monomers. It was reported as appropriate for MIPs toward amphetamine, which is a structural analog of N-FA (Djozan et al. 2012). After investigation of monomer-template interactions the decision to change functional monomer was taken. MAA was replaced by a mixture of HEM and itaconic acid. Also due to very big size of MIPs obtained by bulk polymerization, precipitation polymerization was implemented. This new method led to vast, uneven clusters of round beads with low yield (Fig.1b). Therefore, precipitation technique was replaced by the *in situ* methodology. This technique resulted in round, uniform beads of around 1μm, attached to the electrode surface through chemical bounding (Fig. 1c). A comparison with commercial MIPs for amphetamine was performed. SEM analysis of the commercial MIPs revealed presented acicular shape crystals approximately 5 μm long (Fig. 1d).

N-FA (Fig. 2a) is a lipophilic compound that contains a phenyl and amide group in its structure to be used in imprinting. This excludes any kind of electrostatic bonding. Therefore, MIPs syntheses were based on non-covalent interaction and attempts to design a highly affine spherical cavity. The hydrophobic cross-linker (EGDMA) and monomers (HEM and IA) containing methylene and carbonyl groups in their structure (Fig. 2b, c), were chosen for the imprinting. The traditional way of first obtaining particles followed by their immobilization on the electrode surface was performed. Two kinds of particles (MIPs3 and MIPs4) were synthesized and integrated with capacitance sensor.

3.2 Immobilization of MIPs particles on the transducer

The function of a transducer is to translate the signal generated upon binding of the target analyte with recognition element, into a quantifiable electrical output(Tothill 2011). It is important to mention here that MIPs, must be in close proximity to the transducer surface. This can be achieved either if polymer beads are generated *in situ* by monomer polymerization directly on the transducer surface, or if the prior-synthesized particles are then attached to the transducer surface via a linker. In this research the gold surface of a wafer electrode was implemented as transducer. Two approaches of MIPs immobilization, *in situ* polymerization and MIPs coupled with linkers were evaluated to choose the best electrode immobilization technique using commercially available MIPs as a model system (to exclude any kind of doubts in quality of the in-house made MIPs).

To elaborate and compare immobilization events, cyclic voltammetry was applied. As expected, all cyclic voltammograms of MIPs-functionalized electrodes gave a lower redox peak current in comparison with bare gold electrode surface (Fig. 3). This was due to the fact that MIPs attached to the Au-electrode decreased the electron transfer mechanism between the redox species and electrode surface. Anodic peaks

were significantly decreased during analysis of tyramine monolayer and *in situ* polymerized MIPs analysis (Fig. 3). Finally, the surface of transducer was completely insulated after immersion in 10 mM 1-dodecanthiol. The electrochemical results demonstrate that the modified electrode was highly insulated and could be further employed for the detection of N-FA in a capacitive flow injection system. Obtained result proved successful functionalization of golden electrodes.

3.3 Selecitivity and impact of initiator

Capacitance measurements for MIPs3, MIPs4 and corresponding NIPs3, NIPs4 were performed in triplicates, the standard deviations between the measurement were < 5%. Drop of capacitance considered as sensor signal was proportional to increasing standard concentration (Fig. 4). In order to compare the results obtained by MIPs and NIPs, the imprinted efficiency (*IE*) was proposed. The *IE* is defined as the amount of template bound to MIPs particles divided by amount of template bound to NIPs. Nevertheless, it is hard to evaluate the quantity of analyte attached to MIPs in electrochemical systems, like the one presented in the current study. To face this challenge, the sensitivity enhancement (*SE*) factor was introduced. SE is defined as the imprinting efficiency of electrochemical based MIPs sensing systems(Suryanarayanan et al. 2010). SE has been modified below as the ratio of the sensitivity of the MIP electrode to sensitivity of the NIP electrode:

SE of MIPs3 and MIPs4 was equal to 2.4, although very different result was obtained for MIPs4 equivalent to 0.27, giving conclusion that MIPs3 were very specific to N-FA (Fig. 4). Although the produced MIPs show adequate specificity for the target compound, it is also necessary to test them with similar structurally-related compounds under experimental conditions. Therefore, several compounds were tested using the same MIPs3 electrode (cross-reactivity section, Fig. 5).

MIPs3 and MIPs4 differed by the applied initiator, therefore the conclusion was made that the initiator has a significant impact on MIPs selectivity. Fig. 4 indicates that the MIPs3 electrode (initiated by addition of AIBN), has much stronger than affinity to N-FA than corresponding non-imprinted polymers. However, the opposite situation was observed for MIPs4 initiated by Ciba® IRGACURE® 651.

3.4 Working range, limit of detection and cross reactivity

N-FA standards in phosphate buffer were examined with a regeneration step (250 μL) in between each sample. According to Lenain et al. (Lenain et al. 2015a) spontaneous regeneration with water occurs during the flow, although to ensure removal of every bounding event from sensing layer regeneration buffer was implemented (flow rate 1.67 μL/s). An analytical signal was detected by two electrodes simultaneously, functionalized with MIP and NIP respectively. The capacitive response profiles of the MIP and NIP functionalized electrodes were completely different: the response of the MIP – functionalized electrode showed a steep inclination after injection of N-FA, whereas the NIP-functionalized electrode demonstrated a less pronounced deviation. However, the signal of both electrodes quite fast returned to baseline level (spontaneous dissociation of the obtained N-FA-receptor complex in case of MIP-electrode and washing off of non-specifically adsorbed substances in case of NIP-electrode). As the flow rate (1.67 μL/s) and the dimensions of the flow cell are quite small, it was assumed that changes for the MIP- and NIP-functionalized electrodes at any given time point were resulted by the same factors. The signal of the NIP-functionalized electrode was considered to be the accumulation of all non-specific interactions, whereas the MIP-functionalized electrode corresponded to all specific and non-specific interactions. Therefore, to register only the specific N-FA-MIP interaction, the calibration curve was built after subtracting the NIP signal from the MIP signal. The response of the MIPs-functionalized electrode was characterized by a steep slope of N-FA calibration curve (Fig. 4), and a limit of detection (LOD) of the developed system for N-FA detection was 10 μM and the working range was 5- 200 μM.

Cross-reactivity experiments were performed implementing compound with four structural analogs: benzylmethylketone, amphetamine, methylamphetamine and acetophenone. Their concentrations were in the same order as for N-FA to obtain a relevant comparison (Fig. 5). Capacitance drops results for structural analogus of N-FA were 10 times smaller for methamphetamine, amphetamine and acetophenone, and almost three times smaller for benzylmethylketone. Studied polymers after integration with sensor device exhibit good selectivity and affinity towards their templates.

4. **Conclusions**

This work was devoted to development of the MIPs-based capacitive sensor for sensitive and specific detection of the amphetamine intermediate by affinity sensor. A capacitive biosensor with high sensitivity was developed to monitor trace amounts of N-formylamphetamine (N-FA), an intermediate of clandestine amphetamine production, in aqueous samples. A set of MIPs was synthesized for amphetamine intermediate. Molecularly imprinted polymers in the form of bulk polymers and microspheres for the target analyte, were successfully obtained. N-FA MIPs were effectively attached to the surface of a gold transducer (electrode) by chemical bounding and tyramine electro-polymerization. By investigation and

optimization of polymerization conditions MIPs with the best target sensitivity were selected. Synthesized resins possessed good recognition properties and stability, and were successfully integrated in sensor platform, characterized by LOD equal to 10 μM. MIP functionalized electrodes were able to detect more template molecules in comparison with NIP electrodes. A proportional relation between analyte concentration and sensor capacitance was observed in concentrations between 10 and 250 μM. Signals observed after injection of structurally related compounds were at least three times smaller than for the template molecule. Designed polymers demonstrated good recognition properties and stability, and could be recommended for analysis of real samples. To the best of our knowledge this is a first existing MIPs-based sensor toward amphetamine-type stimulants (ATS) or toward specific markers for their illicit production. The development of a new robust chemical receptor for illicit drug detection reported in this paper could be beneficial for analytical and forensic sciences.

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Figure(s)

Table 1. Composition of the polymers.

	MIPs 1	NIPs 1	MIPs 2	NIPs 2
N-FA	50 μM	n.a.	50 μM	n.a.
DMF	10 mL	10 mL	2,5 mL	2,5 mL
MAA	15 mM	15 mM	n.a.	n.a.
EGDMA	60 mM	60 mM	9 mM	9 mM
HEMA	n.a.	n.a.	3 mM	3 mM
IA	n.a.	n.a.	3 mM	3 mM
initiator	50 mg	50 mg	50 mg	50 mg

Acronyms: IA – itaconic acid; HEM – hydroxyethyl methacrylate; MAA – methacrylic acid; EGDMA – ethylene glycol dimethacrylate; DMF – dimethylformamide.

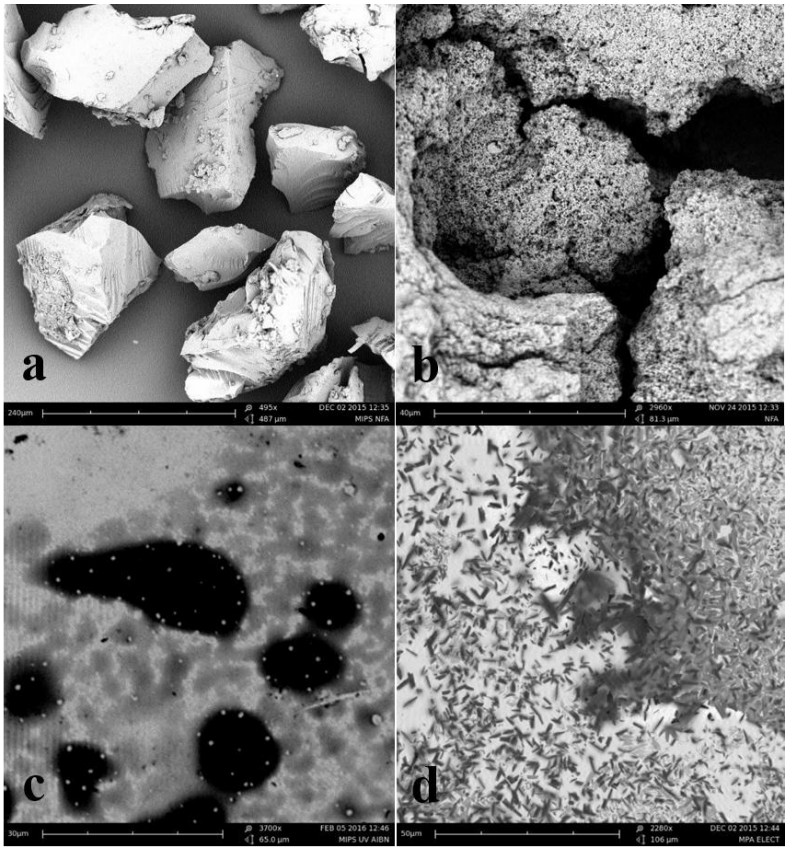


Figure 1. Overview of Scanning Electron Microscopy (SEM) pictures of synthesized molecularly imprinted polymers (MIPs), (a) MIPs for N-formylamphetamine (N-FA) prepared using bulk polymerization; (b) MIPs for N-FA prepared by precipitation polymerization, (c) MIPs for N-FA prepared using *in situ* polymerization, (d) commercial MIPs for amphetamine.

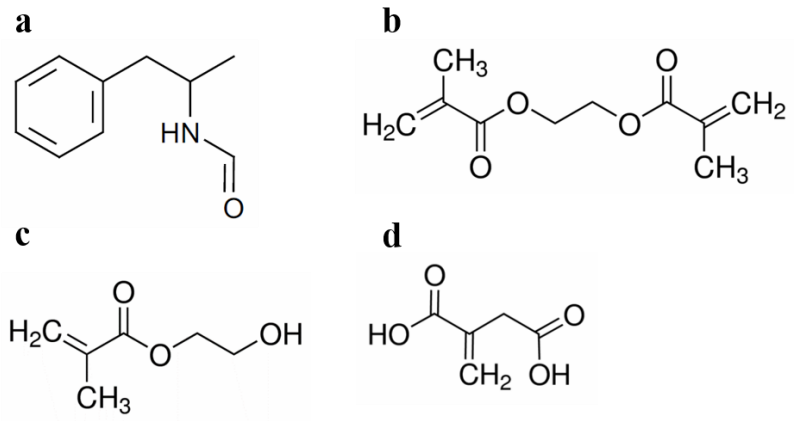


Figure 2. Molecular structure of: (a) template, N-formylamphetamine (N-FA); (b) cross-linker,ethylene glycol dimethacrylate (EGDMA); (c) monomer: 2-hydroxyethyl methacrylate (HEM), (d) functional monomer, itaconic acid (IA).

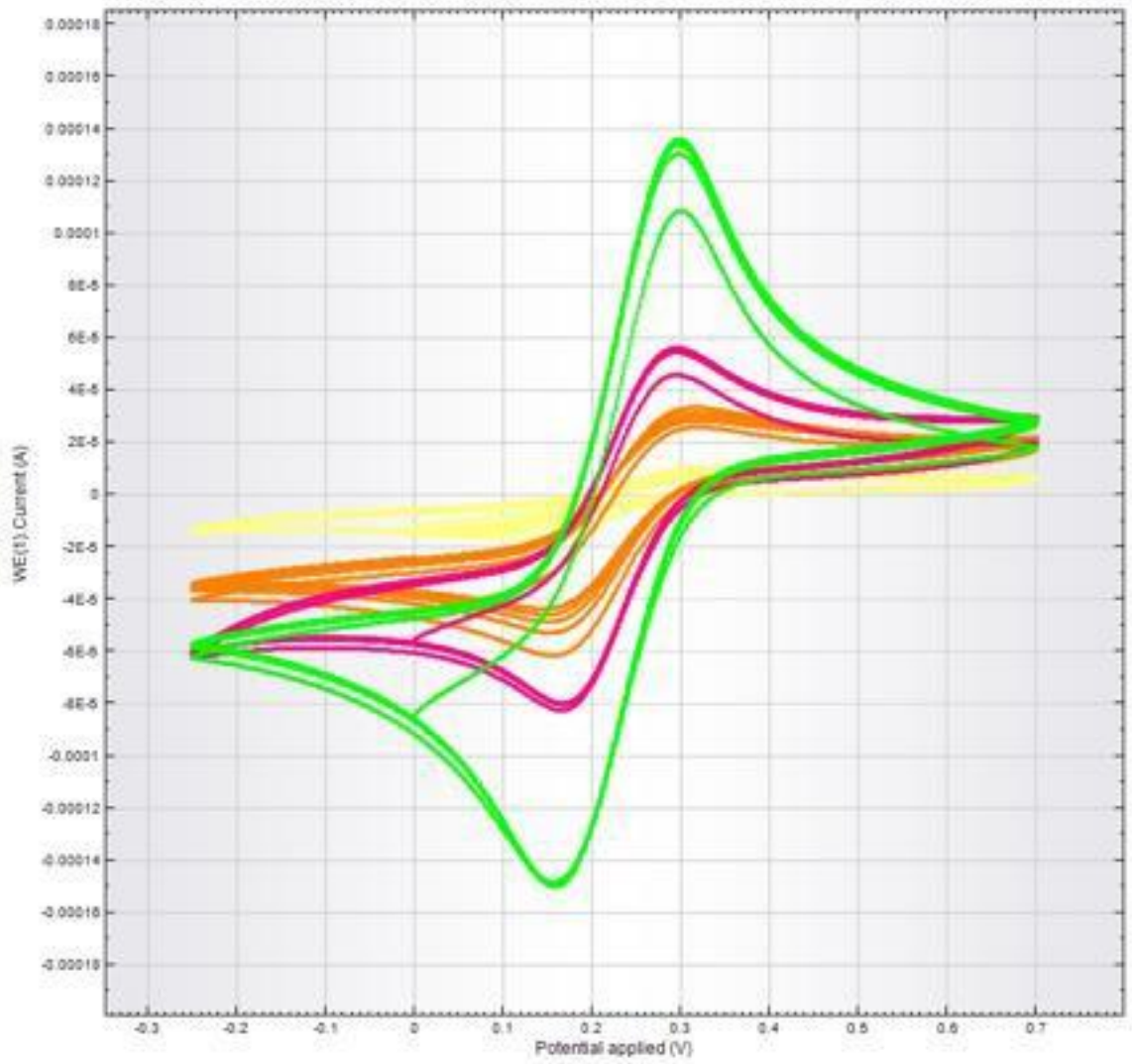
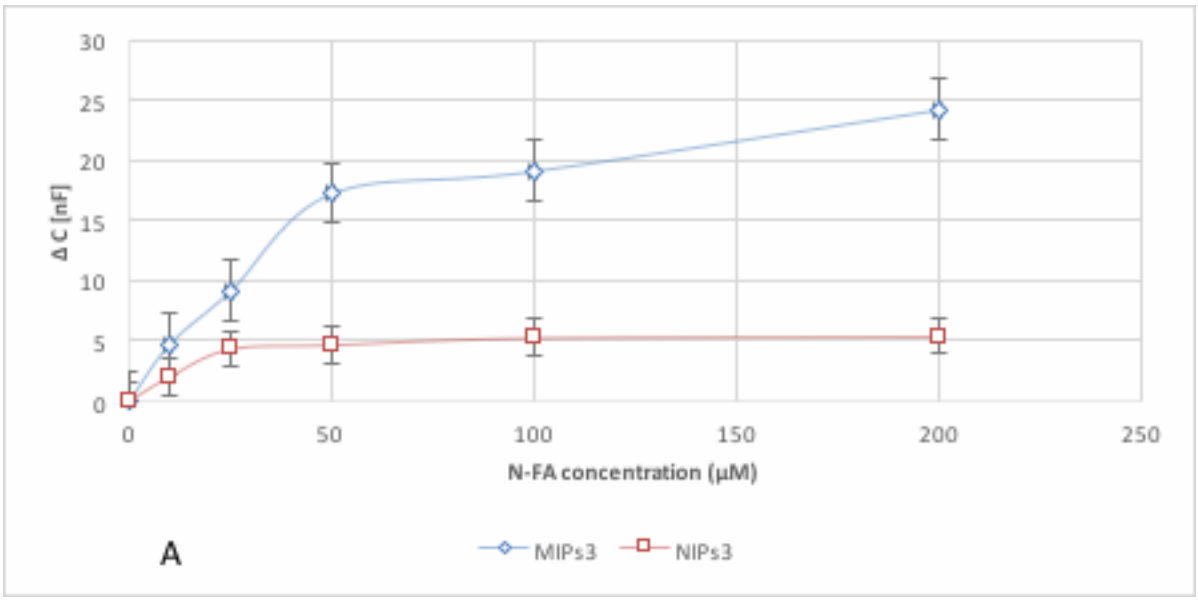


Figure 3. Comparison of electrodes insulation with the use of cyclic voltammetry recorded in 10 mM K3[Fe(CN)6] in 0.1 M KCl. The potential was swept in the range between -300 and 800mV (vs Ag/AgCl) with a sweep rate of 100 mVs-1; electrodes (a) bare; (b) modified with MPA and MIPs; (c) modified with LA and MIPs; (d) MIPs electropolymerization with tyramine; (e) after treatment with 1-dodecanethiol.



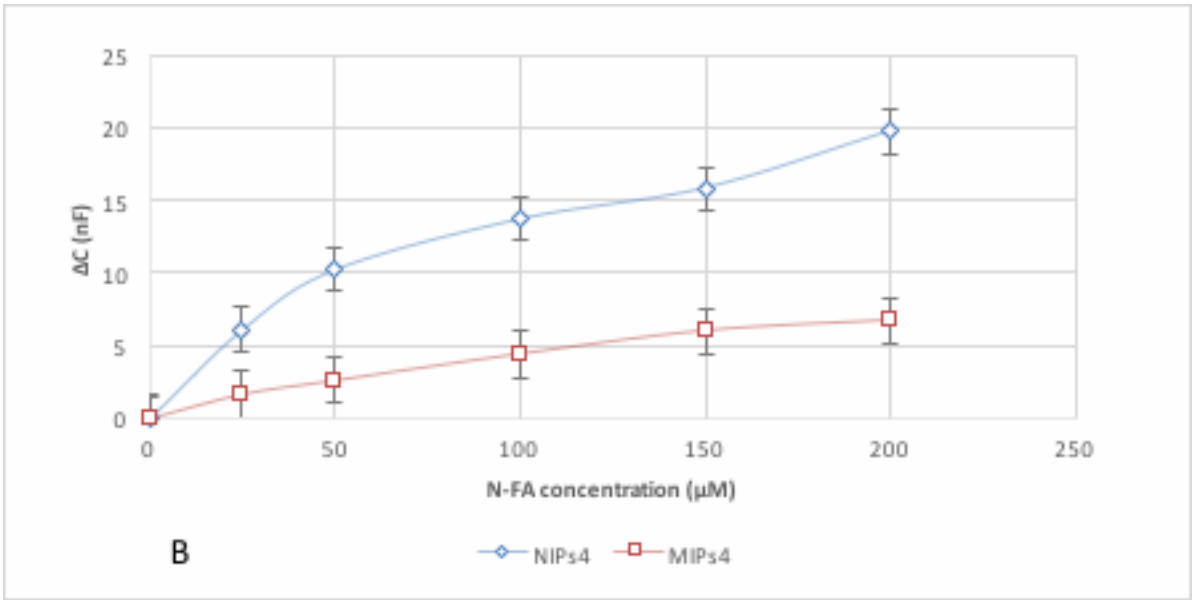


Fig.4 Difference between capacitance changes (nF) of the MIP and NIP functionalized electrodes in function of N-FA concentration (µM), differences in sensitivity according to implemented initiator (A) AIBN MIPs3, (B) Irgacure 651 MIPs4. The measurements (n=3) with use of regeneration buffer between each injection was performed in triplicate, average from the measurements was implemented to draw graph.

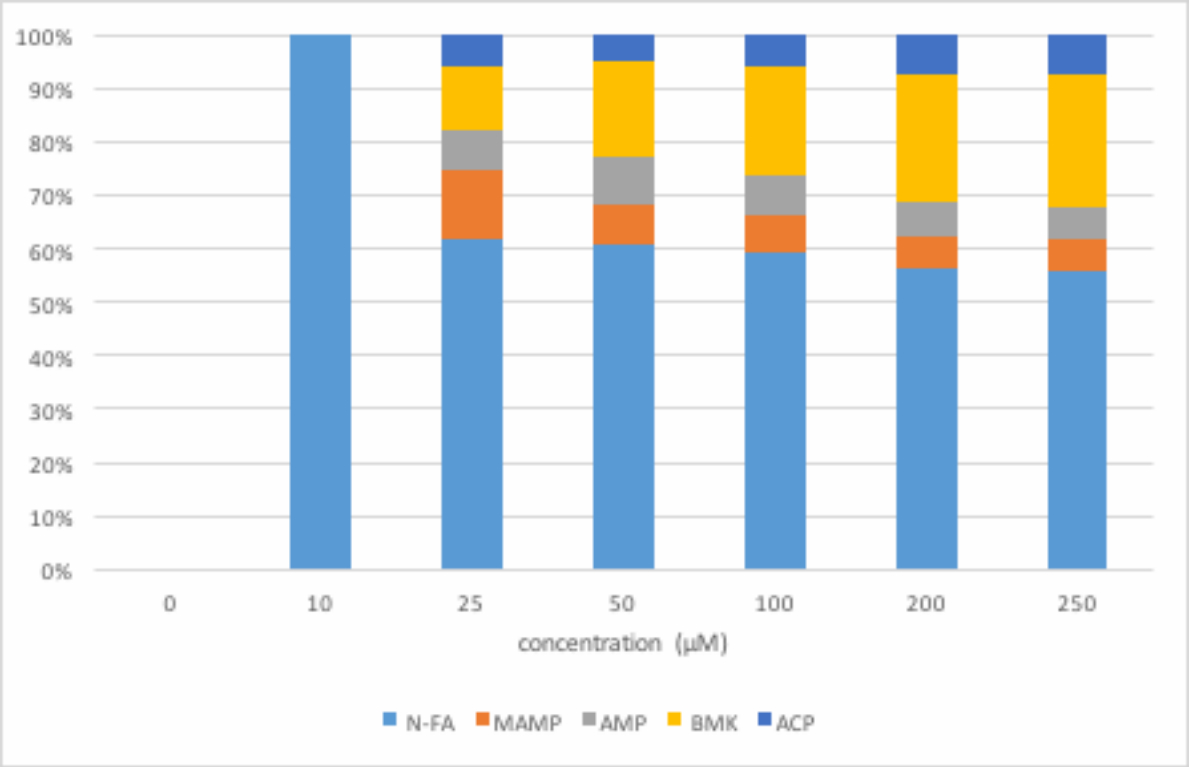


Fig. 5 Cross-reactivity test, graph presenting capacitance changes (nF) of the MIP functionalized electrode in function of concentration (µM) for separate injections of N-formylamphetamine (N-FA), methamphetamine (MAMP), amphetamine (AMP), benzylmethylketone (BMK), acetophenone (ACP).