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PII: S0039-9140(18)30790-2
DOI: https://doi.org/10.1016/j.talanta.2018.07.084
Reference: TAL18913

To appear in: *Talanta*

Received date: 29 March 2018
Revised date: 22 July 2018
Accepted date: 26 July 2018

Cite this article as: Natalia V. Beloglazova, Pieterjan Lenain, Esther De Rycke, Irina Yu. Goryacheva, Dietmar Knopp and Sarah De Saeger, Capacitive Sensor for Detection of Benzo(a)pyrene in Water, *Talanta,* https://doi.org/10.1016/j.talanta.2018.07.084

Capacitive Sensor for Detection of Benzo(a)pyrene in Water

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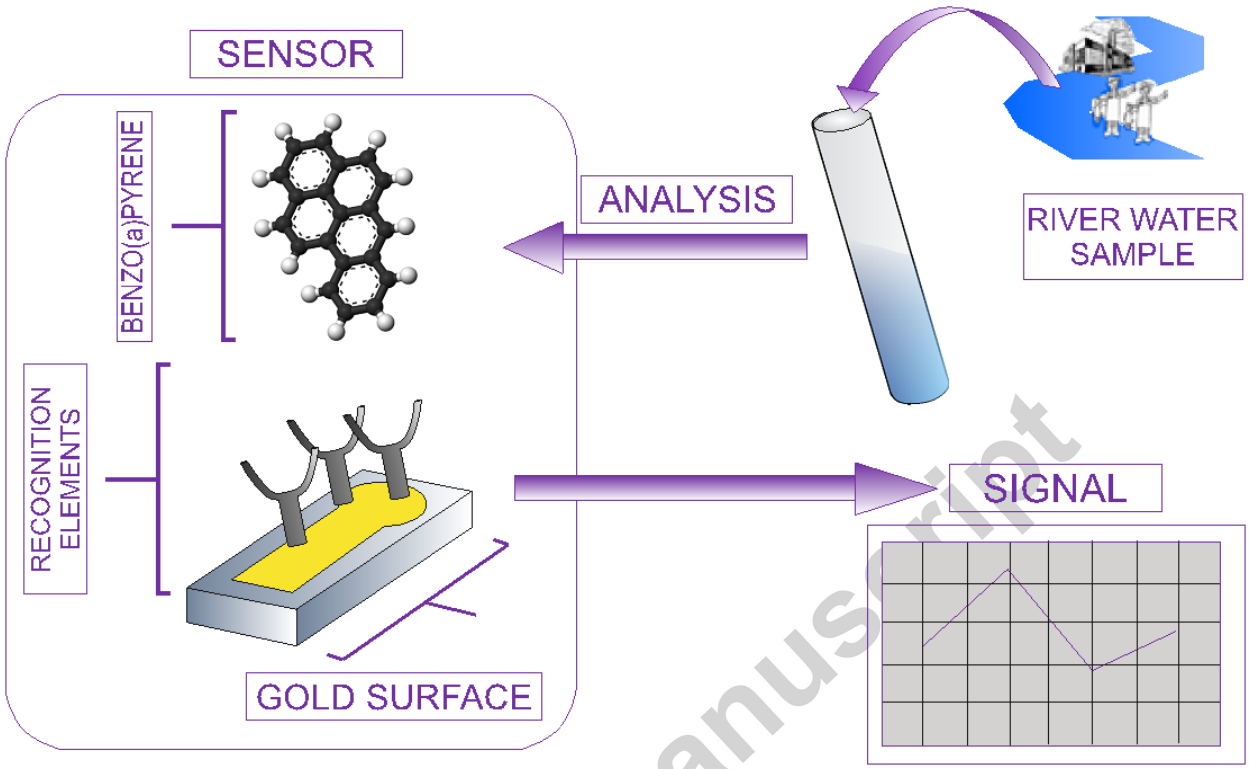
Abstract

An affinity sensor based on capacitive transduction was developed to detect benzo(a)pyrene (BaP) in river water. Two types of recognition elements, the synthetic receptor analogues molecularly imprinted polymers (MIPs) and natural monoclonal antibody (mAb) were tested for this type of biosensor. Different polymerization strategies to obtain MIPs were compared. One approach comprised a preliminary batch synthesis of beads that were subsequently coupled covalently to an electrode surface. Another approach consisted of the in-situ synthesis of MIPs directly onto the electrode surface using electropolymerization. The latter approach provided the best results. To choose the optimal recognition element mAb and MIP-modified electrodes different sets were evaluated with regards to their sensitivity, selectivity, linear range and re-usability. The mAb-modified electrodes were considerably more sensitive toward BaP (ng L^{-1} range *vs* µg L^{-1} range for the MIP-modified one), and showed a broader linear working range than the MIP-modified electrodes. The latter revealed more suitable for group-selective determination of PAHs. The developed capacitive sensor was tested for the detection of BaP in naturally-contaminated water samples collected in different places of Ghent, Belgium. The results obtained with the sensor were coherent and reproducible, and were in a good agreement with the confirmation technique, HPLC-FlD.

Graphical abstract:



Keywords: Capacitive biosensor; molecularly imprinted polymer; benzo(a)pyrene; monoclonal anti-benzo(a)pyrene antibody; water analysis.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs), which represent a hazard to both humans and the ecosystems, have been receiving an increased interest related to their mutagenic, carcinogenic and endocrine disrupting properties [1–3]. They are formed during incomplete combustion processes of organic matter and, as a consequence, are ubiquitous in the environment [4,5]. PAHs are usually found as a mixture containing two or more structurally related compounds. The U.S. Environmental Protection Agency (U.S. EPA) has prioritized 16 PAHs [4]. Benzo[a]pyrene (BaP) is one of the best known PAHs listed as a group I carcinogen by the International Agency for Research of Cancer. BaP is also considered to act as an endocrine disrupting chemical [2,6,7]. It is frequently used as a marker of PAHs presence [5] . In the European Council Directive 98/83/EC concerning the quality of water intended for human

consumption, a limit value of 10 ng L^{-1} was set for BaP [8]. The development of reliable, sensitive and selective analytical methods for the determination of traces of PAHs in water remains challenging despite active research in this field was carried out over the past years. Numerous publications are devoted to the determination of PAHs in water by means of chromatography [9–11]. Such approach is complex and costly, requiring sophisticated and time-consuming sample preparation steps, that limits its application for screening of a large number of samples. Immunoassay based on antibodies as recognition elements has been presented in various formats, only to mention a few examples [12–16]. Another interesting approach was based on a real-time measuring changes in the oscillation dynamics of DNA probes. It showed that an exceedance of the permissible 10 ng/L limit for BaP was detectable [5].

However, to perform continuous sampling and monitoring of PAHs in rivers, a cheap, robust, and stand-alone, system is necessary. Although antibodies and aptamers are characterized by high sensitivities and selectivity, the natural receptors have some major drawbacks. They have a low stability under pH- and temperature deviations and high prices, the antibody's production process is time consuming and environmentally unfriendly. An alternative to natural antibodies is the use of molecularly imprinted polymers (MIPs), which are tailor-made polymers with cavities that are able to selectively bind a target molecule or a group of structurally related compounds [17–19]. Appealing advantages in chemical recognition offered by MIPs include their robustness and high mechanical and thermal stability in harsh conditions (e.g.in broad pH and t° ranges), especially important in environmental analysis [20]. MIPs are re-usable receptors as the target-receptor complex can be disrupted by a regeneration solution, with preservation binding cavities' affinity. The latter characteristic matches the expectation for their use in a stand-alone equipment. Unfortunately, MIPs often demonstrate lower binding affinity (K_D values in the micromolar range) compared to antibodies [19]. Additionally, they often show a limited selectivity due to unspecific adsorptions on the polymer surface. MIPs have been employed both for solid phase extraction (SPE) of BaP [21,22] and as receptors in biosensing systems. Sensors for PAHs detection in water samples based on group-specific molecularly imprinted polyurethanes coupled with fluorescence and mass-sensitive transducers [23,24] and QCM [25] were published to be operating in ng L^{-1} range. MIP obtained using a mixture of 16 PAHs as a template was employed as a recognition element for group determination of the PAHs in environmental samples by a fluorescence sensor operating in µg L^{-1} range [26]. An optosensor

developed for determination of BaP in water making use of MIPs was reported to reach a detection limit of 10 ng L^{-1} after preconcentration [27].

In this work, for the first time we described the development of a capacitive label-free biosensor using anti-BaP monoclonal antibody [12] or MIPs. Capacitive biosensors belong to a sub-category of impedance biosensors, and the principle of a capacitive biosensor is based on measuring the change in capacitance caused by the change of dielectric properties and/or thickness of the dielectrical layer when a target binds to the recognition element immobilized on the electrode surface. Last years this type of sensors have been gaining a lot of interest due to their superior robustness, simplicity and sensitivity. Detection of various analytes, mainly for medical, biomedical and biological purpose, using capacitive sensors was performed [28–32], with the high sensitivity and selectivity, and with use of the low sample volumes. Measurement could be performed even in pico range [33]. Due to a high stability of baseline during the evaluation of capacitance a measuring unite is nF [34]. To the best of our knowledge this is the first report which compares natural and artificial receptors as recognizing element of a capacitive biosensor for determination of PAHs. A highly sensitive, capacitive sensor was developed by us to monitor trace amounts of amphetamine-type stimulant in aqueous samples [35]. For this MIPs were chosen as recognition elements since antibody and aptamers could lose their functionality in the measuring conditions due to harsh conditions, changing in temperature and pH, potential presence of algae etc. Based on this experience and potentially complicated matrix of river water MIPs towards BaP were designed and synthesized to be used as receptors in the manuscript.

2. Materials and methods

Materials

Benzo[a]pyrene (BaP), pyrene (PYR), anthracene (ANT), fluoranthene (FLA), naphthalene (NAPH), phenanthrene (PHE), sulfuric acid (95–97%), potassium dihydrogenphosphate (KH_2PO4, p.a.), and potassium chloride (KCl, p.a.) were bought from Merck (Darmstadt, Germany). 2-(trifluoromethyl) acrylic acid (TFMAA), sodium dodecylsulfate (≥98.5), divinylbenzene (DVB) (technical grade, 80%, treated with basic alumina before use), 4-vinylpyridine (4-VP), 2-mercaptobenzimidazole (2-MBI), methacrylic acid (MAA, 99%), ethylene glycol dimethacrylate (EDMA, 98%), tyramine (99%), α-lipoic acid (LA), N-(3-

Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), dipotassium hydrogen phosphate (K_2HPO_4, ≥98%), 1-dodecanethiol (>98%), potassium ferricyanide ($K_3[Fe(CN)_6]$, ≥99.0%), glycine, bovine serum albumin (BSA), and trimethylamine (≥99%) were purchased from Sigma-Aldrich (Bornem, Belgium). Other reagents are presented in Supplimentary (SI). IRGACURE® 651 was purchased from Ciba (Basel, Switzerland). Monoclonal anti-BaP antibody 22F12 (100 µg mL-1), BaP-BSA (1 mg), PYR-BSA (1 mg) was prepared at TUM (Munich, Germany). Cyclic voltammetry (CV) scans were performed using an Autolab potentiostat (PGSTAT12, Eco Chemie, The Netherlands). Sputtered gold electrodes were provided by CapSenze AB (Lund, Sweden). The capacitance measurements were performed with an automated flow injection capacitance system based on current pulse capacitive measurements (CapSenze HB, Lund, Sweden). River water samples were collected in different places of Ghent, Belgium.

Modification of electrode surface with monoclonal antibody

Immobilization of mAb on the gold electrode via tyramine

Preliminary cleaned electrodes were modified by applying a polytyramine monolayer via electropolymerization of 0.1 M of tyramine dissolved in methanol containing 0.3 M NaOH. CV (15 potential sweeps) was performed covering a potential range from 0 to 1.5 V with the scan rate of 0.05 V s^{-1}. When the scanning was completed, the electrodes were rinsed thoroughly with MilliQ water and ethanol to remove any non-polymerized tyramine monomers and dried. Glutaraldehyde was used for an activation of amino groups present on the surface: the polytyramine layer was then reacted with 5 % glutaraldehyde in PBS (10 mM, pH~7.2) for 20 min. Excess of tyramine was removed by rinsing the electrode with MilliQ water, and the freshly polytyramine-modified electrodes were then coated with 10 µL of a suspension of AuNPs by allowing the particles to adsorb for 6 h at 4 °C. The modified electrodes were then coated with 20 µL of the mAb (1 mg mL^{-1}) and incubated overnight at 4 °C, followed by the rinsing with phosphate buffer to remove the unbond antibody. The resultant sensing layer was subsequently treated with 10 mM 1-dodecanethiol to block any bare sites on the electrodes surface. Prior to use, the electrodes were treated with ethanolamine to block any possible non-reacted aldehyde groups. The prepared electrodes were kept at 4 °C under nitrogen when not used.

Immobilization of mAb on the gold electrode via lipoic acid

The preliminary pretreated electrode was incubated in the solution of 76 mM LA for 12 h; then rinsed with MilliQ water and ethanol, and dried. This step introduces carboxyl functional groups on the gold surface. Afterwards, the obtained carboxyl groups were activated using a standard carbodiimide technique (EDC/NHS). For this the electrode was submerged for 5 h in the solution containing 0.05 M EDC and 0.03 M NHS in dry acetonitrile, followed by rinsing with PBS buffer and drying. Next, the electrode was incubated with 20 µL of the mAb (1 $mg.mL^{-1}$) overnight at 4 °C. After washing with PBS, the bare spots on the electrode surface were blocked with 10 mM 1-dodecanethiol for 30 min.

Modification of electrode surface with MIPs

Immobilization of MIP beads on the gold surface

The MIP beads toward BaP were prepared using different strategies, i.e, a bulk, precipitation and emulsion polymerization (S1). Five mg of MIP or non-imprinted polymer (NIP) particles were suspended in 1 mL of a 10 mM tyramine solution in a phosphate buffer and ethanol (4/1, v/v) and carefully sonicated. A preliminary cleaned electrode was fixed in a reaction cell with 300 µL of the suspended solution, and the beads were allowed to sediment onto the electrode's surface for 30 min, then the cyclic voltammetry (15 potential sweeps) was performed covering a potential range from 0 to 1.5 V with the scan rate of 0.05 $V\ s^{-1}$ where the gold electrode acted as a working electrode. A polytyramine layer was formed on the electrode's surface. Finally, the electrode's surface was blocked with a 10 mM 1-dodecanethiol in ethanol for 30 min to cap residual pinholes on the gold surface of the electrode.

Direct electropolymerization of benzo(a)pyrene-imprinted polymers

A 50 mM solution of 2-MBI was prepared in the ethanol/water (70/30, v/v, pH~10 adjusted with an addition of NaOH to ensure the conductivity required for electropolymerization) containing 10 mM BaP. The electrodes were submerged in this solution. Four potential sweeps were performed from -0.6 V to 1.5 V using a 0.01 $V\ s^{-1}$ potential step. Afterwards, the template was removed by submerging the electrodes for 5 min subsequently in toluene, ethanol and

MilliQ water. The electrode modified with NIPs was prepared in the same manner without addition of the template (BaP).

UV-initiated imprinting directly on an electrode

A stock solution of BaP/DVB at a molar ratio of 1/20 was prepared by mixing of BaP (5.0 mg) and Irgacure 651 (2.7 mg) in 56.5 µL of DVB in an Eppendorf tube. Different cross-linker/functional monomer ratios were compared. For this 3.8 µL of 4-VP was added to the first tube at a molar ratio of BaP/4-VP/DVB of 1/4/20) and 7.6 µL of 4-VP - to the second tube at a molar ratio of BaP/4-VP/DVB of 1/8/20). 0.5 µL of each solution was placed on different electrodes and the polymerization was initiated by UV irradiation (365 nm, 0.5 mW cm^2, 2 h). After polymerization, the electrodes were placed in 10 mM 1-dodecanethiol solution for 30 min.

Automated flow injection system

As a working buffer a 10 mM phosphate buffer (without addition of chloride, pH~7.2) was used. Using a multi-port valve up to six samples could be measured in one run. Regeneration of the working electrode was performed by injecting 250 µL of either a solution of 25 mM glycine-HCl (pH~2.4) for the mAb-modified electrode or a regeneration buffer consisting of methanol/running buffer/trimethylamine (47.5/47.5/5, v/v/v). The modified electrode was inserted in the electrochemical flow-cell equipped with two platinum wires performing as an auxiliary and a reference electrode. The capacitance measurement was done via the current step method with a constant current of ± 10 µA. A pulse was given every 60 seconds .The capacitance was calculated from the resulting registered potential profile and plotted as function of time. A flow rate of 1.67 µL s^{-1} was used, and a sample volume of 250 µL was injected. The binding between BaP and the receptor on the electrode surface resulted in a measurable decrease of the registered capacitance (ΔC).

3. Results and discussion

Electrode surface modification with monoclonal antibody

At the beginning, the performance of mAb was verified by ELISA (SI). Two different strategies for antibody immobilization on the electrode surface were compared. The first approach was

based on the electropolymerization of tyramine and subsequent antibody attachment through glutaraldehyde-activated amino groups present on the electrode surface. The second technique was based on the formation of a Self-Assembled Lipoic Acid Monolayer (SALAM) with subsequent covalent immobilization of the mAb via activated carboxyl groups. The formation of a self-assembled monolayer on the electrode surface was monitored by cyclic voltammetry measured in a 10 mM $K_3[Fe(CN)_6]$ in 0.1 M KCl solution (Fig. 1). The cyclic voltammetry of SALAM-modified electrodes only showed minor coverage as the redox peaks decreased significantly less in comparison with the redox peaks after functionalization with polytyramine. Therefore, all following experiments were performed with the electrodes prepared using tyramine as a linker.

After immobilization of the mAb on the surface of a gold electrodes, the electrode was placed in the flow cell of the capacitive sensor. The capacitive sensor from CapSenze HB (Lund, Sweden) was employed to simulate a flowing water body (e.g. river or stream) in a laboratory environment. The principle of the measurements is based on the theory of the electrical double layer (EDL). Prior to the start of the measurements a regeneration buffer (25 mM glycine-HCl, pH~2.4, 250 µL) was injected to the system for cleaning the binding sites of the receptor. Injection of this regeneration buffer causes an increase of capacitance due to the changes in EDL (Fig. 2A). For the analysis, 250 µL of BaP samples in the concentration range of 1-200 ng L^{-1} were injected in the system. The aliquots of the regeneration buffer, the standard, or a sample, were carried through the flow system by a running buffer (PBS, without chloride, at pH 7.2) from the pump to the flow cell. Diluting the sample with the working buffer minimized any possible matrix influences on the immobilized receptor. The regeneration buffer was applied again to disrupt the mAb/BaP complex after each measurement. The total capacitance at a working electrode/solution interface was continuously monitored via the current step method with a constant current of ± 10 µA. A pulse was given every 60 seconds. A binding event between BaP and the immobilized receptor causes the EDL to be displaced, and results in a decrease in the registered capacitance. Therefore, the analytical signal was calculated as the difference in capacitance value of the baseline before and after binding (Figure SI 2). A detection limit for the capacitance measurements was calculated as a signal-to-noise ratio equal to 3. A linear relationship between the capacitance change and BaP dilutions in MilliQ water is

presented in Fig. 2B with a detection limit (LOD) of 1 ng L^{-1} and a linear range of 2.5-100 ng L^{-1}. The sensor's output is presented on Fig. 2A.

To verify the cross-reactivity, several other PAHs, structurally related to BaP, with a relatively good solubility in water like PYR, NAPH, FLA, PHE, and ANT were tested at a concentration level of 50 ng L^{-1}. Except PYR (CR of 23%) and fluoranthene (CR of 18%) other tested PAHs did not show any significant interference with the detection of BaP in the outlined concentration range (CR<10%). The antibody used in this manuscript were tested by an ELISA [12] and a column gel-based immunoassay [15] as well, and demonstrated the comparable specificity, although sensitivity of the capacitive biosensor was much higher (the LOD of 1 ng L^{-1} *vs* 20 ng L^{-1} for the ELISA). Such increase of sensitivity could be explained by a higher sensitivity of capacitive detector in comparison with spectrophotometric one. Comparing the developed MAb-based sensor with commercially available immunoassays for BaP detection in water samples, its high sensitivity must be highlighted: The ELISA kits for BaP detection in water samples supplied by Abraxis BioScience (USA) and Creative Diagnostics (USA) both have the sensitivity of 0.3 ng mL^{-1}; actual quantitation of PAH by ELISA-VIDITEST-PAH (Abnova GmbH, Taiwan) is not due to variability in the relative amounts of the individual PAHs in environmental samples.

Batch synthesis of MIPs beads towards benzo(a)pyrene

BaP is a highly lipophilic compound that does not contain any functional groups in its molecule to be used to form strong non-covalent interactions in imprinting. This excludes any kind of electrostatic bonding, therefore, the MIPs syntheses were performed using non-covalent (hydrophobic and π-π) interactions and attempts to design a highly affine steric cavity. According to the published research several monomers and cross-linkers have been tested for MIPs toward BaP, of which 4VP/DVB [22,36] and MAA/EGMA [26] demonstrated good binding properties. Therefore, 4-VP, MAA, and TFMAA were compared as monomers together with DVB and EDMA as cross-linkers. Six different bulk polymers were synthesized, and their binding potentials were evaluated. The maximum rebinding capacity was obtained by the MIPs prepared with VP/DVB, probably due to lower polarity of the functional monomer (Fig. 3). It was shown that the traditional bulk polymerization resulted in rough particles with a heterogeneous particle size distribution with a high cross-reactivity (~68%) towards the PAH

smaller being in molecular length (PYR). According to the published research a majority of MIPs towards BaP obtained via a bulk polymerization demonstrated this behavior [21,26]

Therefore the bulk polymerization was changed towards emulsion and precipitation polymerization. For these two approaches the same combination of monomer and cross-linker was used with photoinitiator Irgacure 651. The particles obtained via emulsion and precipitation polymerizations were much smaller in size of around 1 µm (Fig. 4), and therefore could be tested as biosensing layer. The microspheres were suspended in the tyramine solution and attached to the electrode by electropolymerization of polytyramine where the beads were integrated. But still both polymerization techniques resulted in the non-uniform beads, which clearly caused series of false measurement. Besides, to give a reliable signal MIPs must be in close proximity to the transducer surface (gold surface of the electrode), therefore, the batch-synthesized beads were not optimal.

In-situ synthesis of MIPs towards benzo(a)pyrene on the electrode surface

In addition to the more traditional way of bead synthesis and followed immobilization on the electrode surface, the *in-situ* polymerization on the electrode surface was tested. For comparison, two *in-situ* approaches were chosen, photo- and electropolymerization. For the photopolymerization the same bulk polymerization which was described above was performed directly on an electrode surface. Two molar ratios of the template and the functional monomer (1:4 and 1:8) were compared, but with none of the imprinted layers reusable electrodes were obtained. A constant decrease of ΔC over repeated injection/regeneration cycles using 10 µg L^{-1} of BaP solution was observed. We speculate that this can be explained by the partial removing of the MIPs from the electrode surface or insufficient regeneration For the electropolymerization 2-MBI was used as a monomer as it contained a mercapto group which might consequently improve the polymer-gold binding characteristics. The electropolymerization of 2-MBI was carried out using 15-cycles of CV and led to the formation of a nonconductive polymer layer. The oxidation-reduction peaks (the redox peak current) reduced in comparison to a bare electrode surface, indicating that the polymer completely covered the surface and decreased the electron transfer between the redox solvent and electrode surface. Finally, the gold electrode was blocked with 10 mM 1-dodecanethiol. The LOD of BaP in MilliQ water was determined to be 1 µg L^{-1} (Fig. 5A) and a working range from 3 to 20 µg L^{-1}. The sensor output is shown at

Fig. 5B. Calibration curves for MIP-based capacitive biosensor are usually linear only till a certain concentration, and then come to a slope [34]. The possible explanation of this phenomenon could be in a possible unavoidable non-specific absorption of interferences on MIP surface which cannot be completely eliminated even by blocking and regeneration. This effect would be very pronounced for such a highly lipophilic molecules as BaP. This saturation phenomenon was detected in the chosen working range. Cross-reactivity experiments were performed with PYR, NAPH, FLA, PHE, and ANT at a concentrations of 10 µg L^{-1}. The assay was found to be group selective: the tested PAHs competed for the selective binding sites and simulate the presence of BaP in river water. The signal generated by BaP was almost in the same order of magnitude as those of all tested cross-reactants except of fluoranthene (for that the signal was three times lower than that of BaP, Fig. 6). Thus, the obtained MIP could be used only for a group-selective determination of PAH. All together the abovementioned facts indicated a possibility to use the MIP-based capacitive biosensor for a group-analysis of present PAH. To the best of our knowledge no highly specific MIP toward PAH was published.

Almost all earlier described MIPs towards BaP were mainly employed for SPE [17,21,36]. The MIP-based RT phosphorescence optosensor for determination of BaP in water was reported to reach a detection limit of 10 ng L^{-1} using anyhow 20 mL as sample injection volume [27]. The MIP-based capacitive biosensor presented by us detects BaP in a very small volume of pumped water sample (250 mL) that allows to neglect any potential matrix influences and facilitate the measuring procedure and data processing.

Detection of benzo[a]pyrene in naturally-contaminated water samples

Prior to start screening of naturally-contaminated samples a calibration curve for BaP determination was set up in river water in order to bring the analysis conditions closer to real samples. As it is shown in Fig. 2 and 5, no significant difference in the assay performance for MilliQ and river water was observed. The electrode with the immobilized antibody could be reused for at least 13 times with buffer samples and 9 times with surface water samples keeping the signal intensity >90%. The reduced operating life with river water should be caused by matrix interferences, e.g., humic acids. In comparison, MIP-functionalized electrodes prepared via direct electropolymerization could be regenerated for at least 25 times using MilliQ water as sample matrix and mixture of methanol/phosphate buffer at pH 7.2/triethylamine, (47.5/47.5/5,

v/v/v) as a regeneration buffer. With river water, operating life dropped to 17 times with the same regeneration buffer. Repeatability of the developed systems was estimated by injecting the same concentration of the target analyte, BaP, in water for 20 times spread over 5 days. The concentration of BaP in a case of mAb-modified sensor was chosen as 10 ng L^{-1}, which correspond to the its established normative limit in drinking water, and therefore, can be considered as screening target concentration (STC). As a LOD for the MIP-based system is higher than the established normative, the concentration chosen for validation was equal to the LOD, i.g. 1 µg L^{-1}. RSD_r for the mAb based system was 3.6%, for the MIP-based system – 5.8 %. Precision of the systems were checked using the same amounts of replicants in the chosen concentrations, and characterized by RSD of 4.2% and 6.3% for mAb- and MIP-based systems, respectively.

For testing the developed capacitive sensor for the analysis of naturally-contaminated water samples, the random river water samples were collected in different places of Ghent, Belgium: a port, an industrial area, close to a park widely used for barbeque on summers, the center of the city, a suburb. Tap water samples were obtained from local water works. Bottled water samples used as a negative standard were purchased in a local market. Only the mAb-modified electrodes were used for screening of naturally-contaminated samples. The water samples were directly used without any filtration. Because BaP molecule has its own fluorescence, an HPLC-FlD was used as a confirmation technique (SI). The results of sensing were coherent and reproducible, and were in a good agreement with the confirmation technique (Table 1). Some conclusion could be drawn on a relationship between the determined BaP concentration and the geographical location of the sampling, but this work still needs a careful validation, and could be a possible continuation of the presented work.

4. Conclusions

Immobilization of either natural (monoclonal antibody) or synthetic antibodies (molecularly imprinted polymer, MIP) on a gold electrode surface for its subsequent use in a capacitive sensing system was successfully performed. For modification of the electrode with the monoclonal BaP antibody tyramine was used as a linker. Different polymerization strategies were compared to synthesize a robust and reusable BaP-specific MIP. The first strategy comprised a preliminary batch synthesis of beads that were subsequently covalently coupled to

an electrode surface. Another approach consisted of the synthesis of MIPs directly onto the electrode surface. The latter approach provided the best results. While the mAb-modified electrodes were considerably more sensitive (ng L^{-1} *vs* µg L^{-1}) and showed a broader linear range, the MIP-coated electrodes were exhibited some higher re-usability with fresh water samples.

Acknowledgements

CapSenze Biosystems (Lund, Sweden) is highly acknowledged for their help and support in this work.

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Figure 1. Optical microscopy and cyclic voltammograms of a gold electrode in a 5 mM K3[Fe(CN)6] solution containing 0.1 M KCl, at scan rate of 0.1V s−1. All potentials are given w.r.t. Ag/AgCl reference electrode: (blue) bare gold electrode; (green) self-assembled lipoic acid-antibody electrode; (orange) self-assembled polytyramine monolayer-antibody electrode; (red) 1-dodecanethiol end-capped electrode.

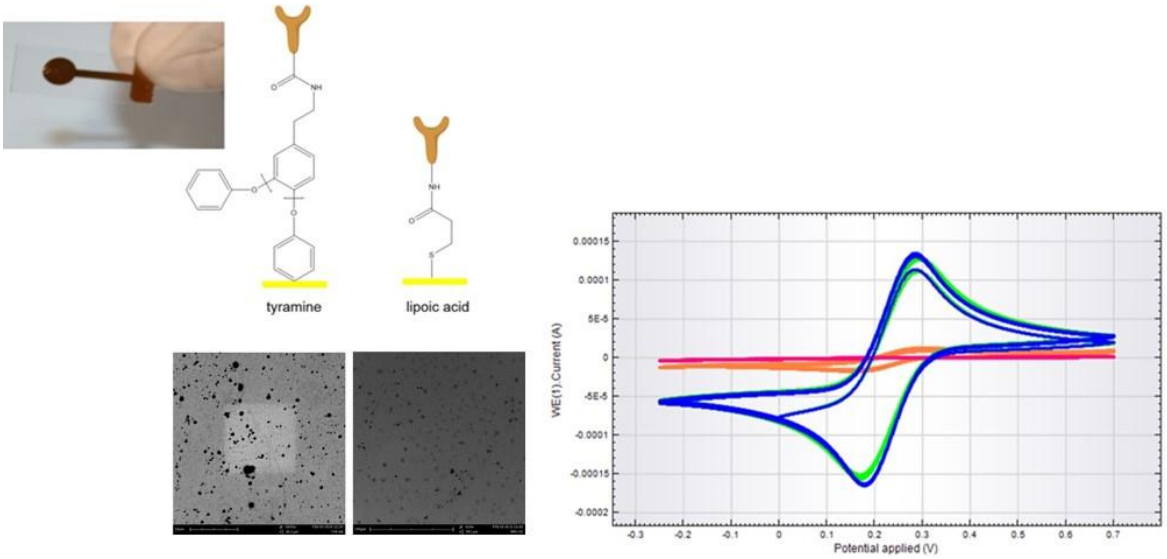
Figure 2. (A.) Difference between capacitance changes (nF) of the antibody-functionalized electrodes in function of BaP concentration. The measurements (n=3) with use of regeneration buffer between each injection was performed in triplicate, average from the measurements was implemented to draw graph. (B.) Capacitance changes after the mAb-modified electrode regeneration with 25 mM glycine HCl, pH 2.4 and injection of different concentrations of BaP.

Figure 3. Binding capacities of the polymers synthesized by bulk polymerization using different monomer/cross-linker pairs.

Figure 4. Scanning Electron Microscopy (SEM) picture of the MIP synthesized by the emulsion polymerization.

Figure 5. (A.) Difference between capacitance changes (nF) of the MIP-functionalized electrodes in function of BaP concentration. The measurements with use of regeneration buffer between each injection was performed in triplicate, average from the measurements was implemented to draw graph. (B.) Capacitance changes after the MIP-modified electrode regeneration with a mixture of methanol/water/ triethylamine (47.5/47.5/5, v/v) and injection of different concentrations of BaP.

Figure 6. Cross-reactivity study.



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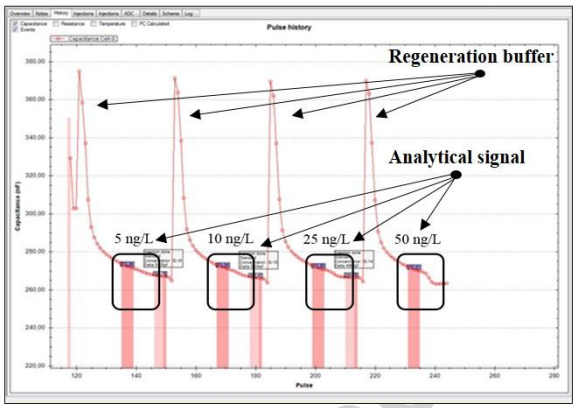

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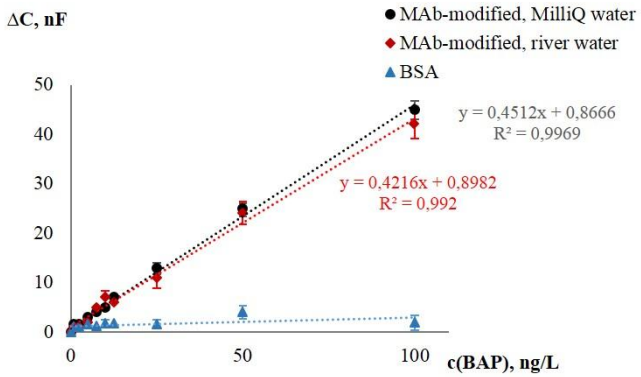

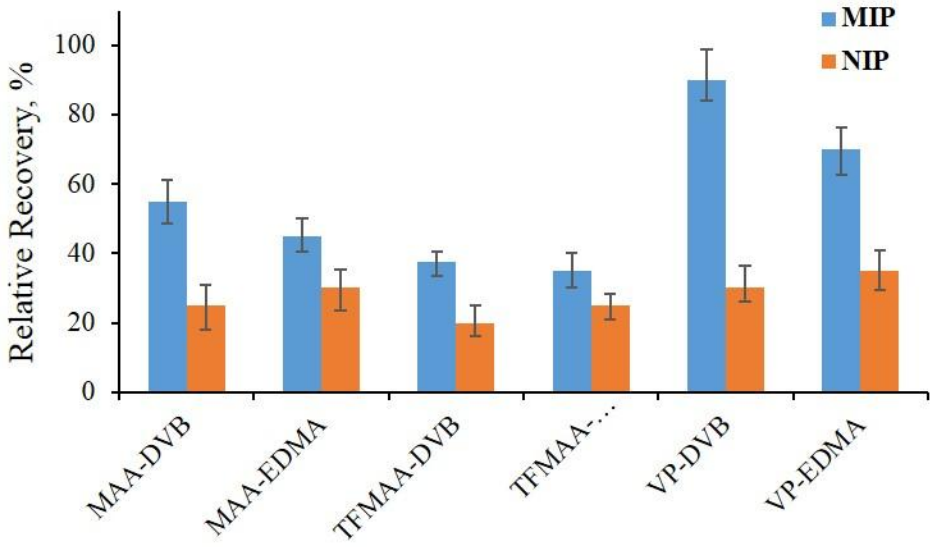
MIP
NIP
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40
20
0
MAA-DVB
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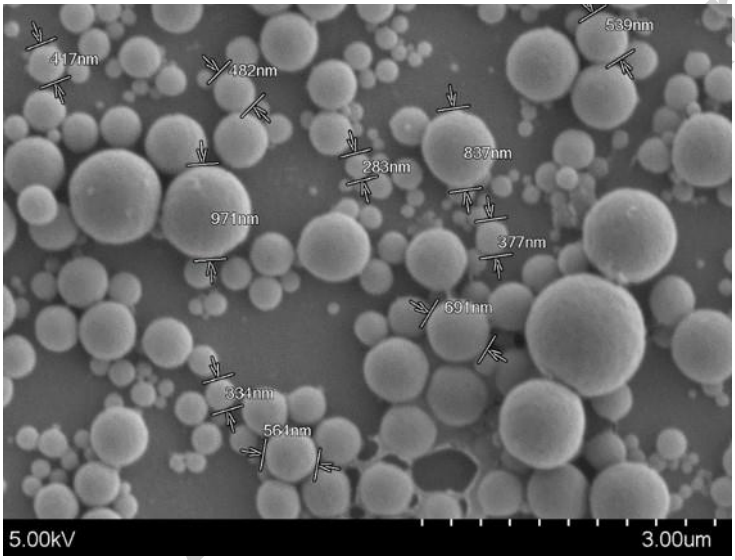
VP-DVB
VP-EDMA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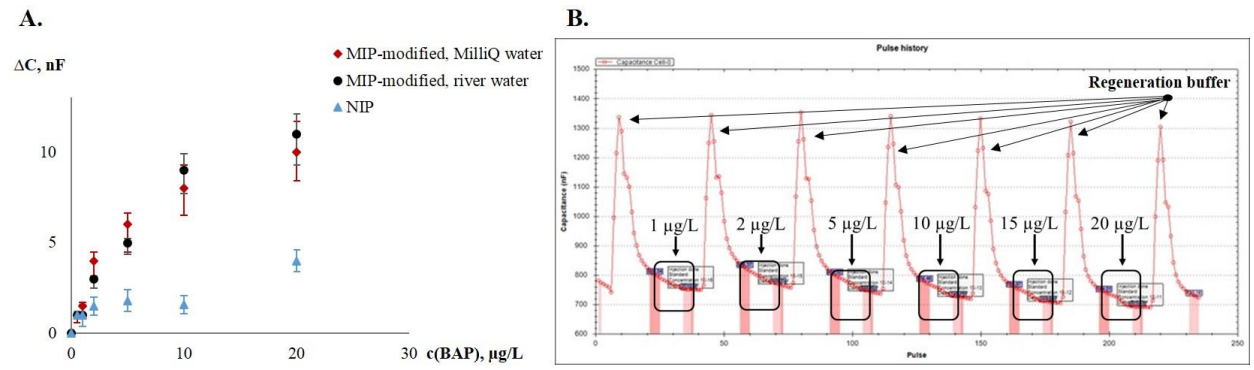

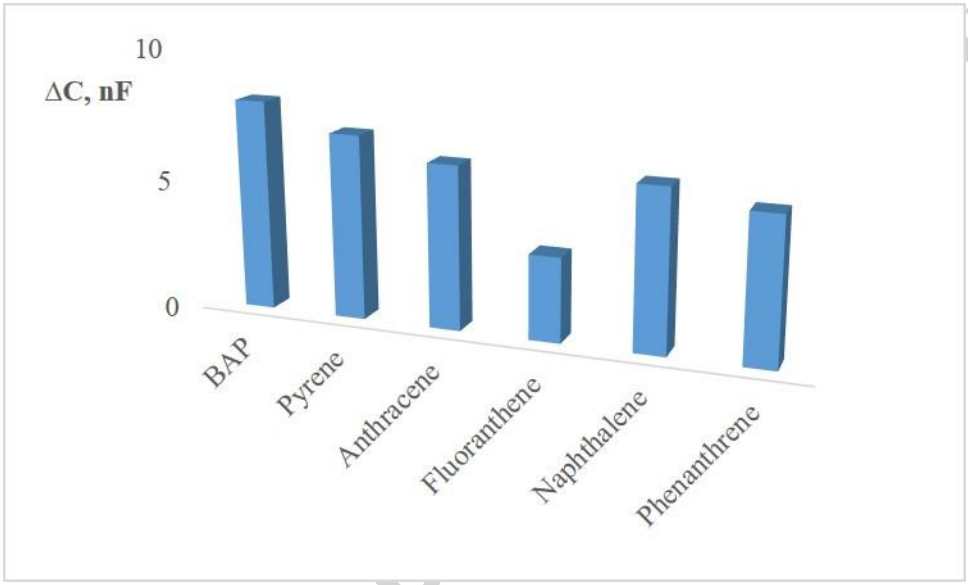

Table 1. Benzo(a)pyrene (BaP) determination of different water samples by the antibody-covered capacitive biosensor.

Water type	**BaP concentration, ng.L^{-1}**	
	MAb-based capacitive biosensor	**HPLC-Fld**
river water - port	523±31	516±14
river water – industrial area 1	414±27	405±17
river water – industrial area 2	298±18	305±11
river water – park area 1	99±6	108±12

river water – park area 2	110±13	119±13
river water – centrum 1	87±11	91±9
river water – centrum 2	94±15	88±7
river water – suburb 1	23±8	19±5
river water – suburb 2	51±6	47±5
tap water	8±2	6±3
bottled water 1	nd	nd
bottled water 2	nd	nd

nd- not detected

Highlights

- An affinity capacitive sensor was developed to determine benzo(a)pyrene in water.
- As a receptor the antibody was compared to molecularly imprinted polymers.
- Two strategies for antibody immobilization on the electrode surface were compared.
- To obtain MIPs, different polymerization strategies were tested.
- The receptors were evaluated regard their sensitivity and re-usability.