## ChemComm

## COMMUNICATION



View Article Online View Journal | View Issue

Cite this: Chem. Commun., 2014, 50, 7488

Received 10th April 2014, Accepted 19th May 2014

DOI: 10.1039/c4cc02658f

www.rsc.org/chemcomm

A versatile electrochemical sensing receptor based on a molecularly imprinted polymer<sup>†</sup>

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Electrochemical molecularly imprinted polymers (e-MIPs) are reported for the first time. Their elaboration is based on the introduction of a redox tracer (vinylferrocene) inside the binding cavities of a crosslinked MIP. Determination of the analyte (benzo[a]pyrene) can be simply performed by measuring the redox tracer signal.

Molecularly imprinted polymers (MIPs) can advantageously replace natural receptors in biosensor devices thanks to their high selective recognition properties, their easy synthesis and high stability.<sup>1-3</sup> MIPs are commonly prepared by formation of a three-dimensional polymer network around a template (such as a molecule or an ion) via a cross-linking step. The removal of this template creates binding cavities responsible for the recognition process. Such polymers are frequently described as "plastic antibodies" due to their high selectivity.<sup>4</sup> Combined with various transduction mechanisms, MIPs are the key stones of a large panel of optical, electrochemical, acoustic, piezoelectric and calorimetric sensors.<sup>5–10</sup> The main challenge remains the incorporation of MIPs as sensing elements in such devices.<sup>11</sup> The MIP-based electrochemical sensors can be schematically divided into two categories depending on the nature of the template, electroactive or non-electroactive.<sup>12-15</sup> In the first case, the template itself can generate the signal and classical MIP materials can be used in the form of nanoparticles mixed with a conductive matrix or as thin layer membranes, for instance. When non-electroactive targets are involved, the signal should be produced either by an external probe<sup>16,17</sup> or by the transducer upon template rebinding. Electrosynthesized MIPs based on conducting polymers are then commonly used and their implementation in the form of thin layer films of controlled thickness is quite easy. Nevertheless, the crosslinking of such a phase is hard to process.

Herein, we report a new generation of the electrochemical sensing receptor based on the introduction of a redox tracer inside the binding cavities of a cross-linked MIP. Such an electrochemical MIP (e-MIP) can serve both as the recognition and the measuring element for the electrochemical determination of a non-electroactive target. e-MIPs can be prepared in an easy and conventional way by copolymerization of a functional monomer presenting electroactive properties with a crosslinker (Fig. 1). To the best of our knowledge, such an approach has never been reported so far.

To validate our concept of e-MIPs, benzo[a]pyrene (BaP), a polycyclic aromatic hydrocarbon (PAH) known for its high toxicity and carcinogenic effects,<sup>18</sup> was chosen as a model system. Its high oxidation potential ( $E_{1/2}$ Ox = 0.94 V vs. Saturated Calomel Electrode (SCE)<sup>19</sup>) prevents its direct detection in aqueous media by an electrochemical method. The absence of any functional group in the chemical structure of BaP limits its possible interaction with functional monomers via H-bonding, ionic or dipolar interactions,<sup>20</sup> thus restraining those interactions with hydrophobic and  $\pi$ - $\pi$  ones.<sup>21</sup> For this reason, we decided to take advantage of its aromatic structure by using ferrocene as a redox tracer. The two cyclopentadienyl rings in its structure could generate aromatic stacking interactions with BaP.<sup>22</sup> Such interactions might promote the recognition of BaP by e-MIPs and cause detectable modifications of the ferrocene redox properties. Ferrocene constitutes an appropriate redox tracer since it can be easily oxidized in a stable ferricenium ion. Ferrocene and its derivatives have been widely used to develop electrochemical sensors due to their reversible oxidation process that is sensitive to the environment.<sup>23</sup> In order to covalently bind this tracer to the polymer network, it was functionalized by a vinyl group to prepare vinylferrocene (VFc, Fig. 1) according to a previously described procedure.<sup>24</sup> In this work, ethylene glycol dimethacrylate (EDMA) was chosen as a crosslinker because its chemical structure prevents the generation of  $\pi$ - $\pi$  interactions with BaP and avoids interferences with the action of the ferrocene moiety.

Small MIP particles were required to introduce the electrochemical MIPs inside a working electrode or a sensing device.

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 $<sup>\</sup>dagger$  Electronic supplementary information (ESI) available. See DOI: 10.1039/ c4cc02658f



Fig. 1 Schematic representations of e-MIPs and e-NIPs syntheses, their integration in the carbon paste electrode and the resulting electrochemical responses.

Performing bulk polymerization followed by grinding and sieving was avoided because of the inherent drawbacks of this method (a tedious procedure with low yields leading to particles of irregular sizes and shapes). Nano- or microsized MIP beads can be easily obtained through precipitation polymerization without addition of any surfactant in a diluted medium using a near- $\theta$  solvent.<sup>25</sup> First attempts to prepare e-MIP particles by this synthetic pathway were unsuccessful. For this reason, distillation-precipitation polymerization was then performed.<sup>26</sup> The basic principle remained unchanged (near- $\theta$  solvent, diluted conditions) but after a short period of polymerization, a partial distillation of the solvent was achieved using a Dean–Stark apparatus. This procedure led to microsized polymer beads in a quite short reaction time (3 h compared to an average of 24 to 48 h for a classical precipitation polymerization).

Various solvent conditions were studied based on the use of the classical solvents, such as acetonitrile and toluene. Pure acetonitrile led to well-formed non-imprinted polymer (NIP) beads but coalescence of the particles was observed for the corresponding MIP (Fig. S1, ESI†). Toluene, a good solvent for BaP, was then introduced in increasing proportions. Spherical independent particles of e-MIPs could only be obtained from a volumetric proportion of 20% of toluene in acetonitrile (e-MIP20), since for lower ratios, the coalescence phenomenon was still observed. Whereas a 30% ratio gave also beads (e-MIP30), no precipitation was observed when 40% of toluene was introduced. The polymerization yields did not exceed 50% as a result of the quenching of the macroradical due to the transfer between the polymer chains and the vinylferrocene monomer. Indeed, the intramolecular electron transfer from the iron center may generate an anionic chain end resulting in a termination reaction.<sup>27</sup> E-MIPs and e-NIPs were analyzed by scanning electron microscopy (SEM) (Fig. S2 and S3, ESI<sup>†</sup>). Microsized beads (from 1.5 to 2.4 µm) were observed and a better homogeneity, in terms of sizes and shapes, was found for e-MIP particles compared to those of e-NIPs (Table S2, ESI<sup>†</sup>). Contrary to e-MIPs, e-NIP particles were also better defined when the toluene percentage was low. These observations showed the impact of the presence of BaP on the polymerization of the vinylferrocene monomer with EDMA. Elemental analysis was performed to assess the correct introduction of the vinylferrocene monomer inside the polymer matrix and the effective leaching of the BaP outside e-MIP particles (Table S3, ESI<sup>†</sup>).

The binding properties were investigated in an aqueous rich medium (water/acetonitrile (99:1, v/v)) in batch mode. Both imprinted polymers exhibited much higher affinity for BaP than the corresponding non-imprinted materials (Fig. 2) leading to imprinting factors of 1.3 and 1.7 for e-MIP20 and e-MIP30, respectively. Binding capacity values of 3.3 and 3.7 mg g<sup>-1</sup> were measured under those conditions. These relatively high values could be the result of the occurrence of specific  $\pi$ – $\pi$  interactions between BaP and the ferrocene moiety. The enhanced values of the imprinting factor and the binding capacity of e-MIP30 compared to e-MIP20 are in agreement with a beneficial impact of toluene on the imprinting effect.

Cross-selectivity of e-MIPs towards other PAHs was studied by putting the imprinted and non-imprinted polymers in contact with solutions of BaP with one interfering PAH (chosen between fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene or dibenzo(a,h)anthracene). The selectivity coefficients (k) ranged from 0.40 to 2.86 (Table S5, ESI†). This highest value corresponded to the ratio of the distribution coefficient of BaP vs. phenanthrene for e-MIP20. The low range of k values was in agreement with the very similar aromatic structures of BaP and other PAHs, and the lack of specific chemical functionalities for



Fig. 2 Equilibrium binding isotherms of different polymers (Q = binding capacity in mg of BaP per g of e-MIP/e-NIP particles) in water/acetonitrile (99:1, v/v).

such compounds. Except in the case of fluorene for e-MIP20, and pyrene and chrysene for e-MIP30, the relative selectivity coefficients k', defined as the ratio of k(MIP)/k(NIP), were in favor of the imprinted polymers. This still showed the imprinting effect and spoke in favor of specific interactions between BaP and the vinylferrocene monomer.

Carbon paste was chosen to immobilize e-NIPs and e-MIPs for electrochemical characterization. Carbon paste electrodes (CPEs) are very simple and easy to fabricate and present other advantages such as stability in various solvents, relatively low-background characteristics and a low residual current.<sup>28</sup> For each e-MIP or e-NIP, a mixture of carbon paste/e-MIP or e-NIP (4:1, w/w) was prepared and put in a 5 mm diameter empty working electrode (Fig. S4, ESI†). After drying under vacuum, the carbon paste custom working electrode was polished and used in a classical three electrode cell equipped with platinum as a counter electrode and the Saturated Calomel Electrode (SCE) as a reference electrode.

Cyclic voltammograms (CVs) of e-MIP20CPE and e-MIP30CPE and their corresponding e-NIPCPE were recorded in acetonitrile with *n*-Bu<sub>4</sub>NPF<sub>6</sub> (0.1 M) as a supporting electrolyte. All CVs exhibited anodic and cathodic shoulders related to the oxidation and the reduction of the redox system ferrocene/ferricenium whereas the CV of the bare CPE in the same solution did not show any redox system (Fig. S5 and S6, ESI†). In all cases, anodic  $(E_{pa})$  and cathodic  $(E_{pc})$  potential values were constant with the scan rate. Anodic  $(I_a)$  and cathodic  $(I_c)$  current values were linearly dependent on the square root of the scan rate (5 to 500 mV s<sup>-1</sup>) with correlation coefficients higher than 0.95. These data were in good agreement with a system under diffusion control which could be due to electron hopping between neighbouring redox sites and through counter-ion motion. This might explain the peak separation potential  $(E_{pa} - E_{pc})$  greater than (59/*n*) mV indicating that ferrocene did not act as a reversible system.

Determination of the amount of ferrocene oxidized during the anodic potential sweep was carried out by evaluation of the electrical charge (Q) associated with the process. Q was determined from the area of the oxidation peak and was related to the number of moles by the equation: Q = nFN, where F is the Faraday constant, n is the number of electrons involved in the oxidation process (n = 1 for the ferricenium/ferrocene couple) and N is the number of moles of the transformed electroactive species. In all cases the charge Q decreased with the scan rate and this might be attributed to the carbon paste used to prepare custom electrodes.<sup>29</sup>

To evaluate the BaP effect on the redox signal of ferrocene, electrochemical behaviors of e-NIP20CPE and e-MIP20CPE, on one hand, and of e-NIP30CPE and e-MIP30CPE on the other hand, were investigated by Differential Pulse Voltammetry (DPV) and Square Wave Voltammetry (SWV), respectively (Fig. 3). As expected anodic DPV scans for e-NIP20CPE and e-MIP20CPE showed one electron oxidation peaks of ferrocene at  $E_{1/2} = 0.42$  and 0.43 V vs. SCE, respectively (Fig. 3(a) and (b)). After addition of 100 µL of a 10 mg L<sup>-1</sup> BaP solution in acetonitrile directly into the electrolyte solution (final concentration of BaP = 0.2 mg L<sup>-1</sup>), a very small variation of the current intensity with an amplitude of 1–2 µA was observed for e-NIP20CPE with an oxidation peak



**Fig. 3** DPV of e-MIP20CPE (a) and e-NIP20CPE (b) with and without BaP after 4 h. SWV of e-MIP30CPE (c) and e-NIP30CPE (d) with and without BaP after 4 h. Voltammetric conditions for DPV: 50 mV pulse amplitude, 5 mV step potential; for SWV: 150 mV pulse amplitude, 2 mV step potential. (e) Change in the current intensity of e-MIP30CPE and e-NIP30CPE using SWV and as a function of BaP concentration in electrolyte solution. (f) Normalized current curves where *I* is the current obtained in the presence of BaP and *I*<sub>0</sub> the current before BaP addition (formulated in percentage as a function of added BaP).

value rather constant (Fig. S7, ESI<sup>†</sup>). In contrast, for e-MIP20CPE significant modifications of current intensity and oxidation peak values were rapidly observed. The oxidation peak of ferrocene shifted positively and decreased under the initial value before addition of BaP. On the other hand, the oxidation peak value of ferrocene with BaP was unstable (Fig. S7, ESI<sup>†</sup>). SWV of e-NIP30CPE and e-MIP30CPE revealed the anodic peak of ferrocene at  $E_{1/2} = 0.53$  and 0.47 V vs. SCE, respectively (Fig. 3(c) and (d)).

After addition of 100  $\mu$ L of BaP at 10 mg L<sup>-1</sup> in acetonitrile (final concentration of Bap = 0.2 mg L<sup>-1</sup>), only a decrease of the current intensity by 4–5  $\mu$ A was observed with a constant oxidation peak value for e-NIP30CPE. Nevertheless, e-MIP30CPE showed a different behavior compared to e-MIP20CPE. After addition of BaP, an increase of the current intensity was observed first with a constant value of oxidation peak (Fig. S7, ESI†). After 4 h, an important decrease of the current intensity was observed with a positive shift of the oxidation peak from 0.47 to 0.55 V and then the signal stayed relatively constant.

Because SWV showed a much higher current sensitivity, it was used to estimate the limit of detection of BaP (Fig. 3(e) and (f)). The plot of percentage of current intensity variation of e-MIP30CPE and e-NIP30CPE at 0.46 V *vs.* concentration of BaP consisted of two linear segments with slopes of 3.55 and 1.80%/µM, respectively, in the concentration ranges of 0 to 1 mg L<sup>-1</sup> (0.08 to 3.97 µM;  $R^2 > 0.995$  in both cases). The limits of detection calculated for e-MIP30CPE and e-NIP30CPE were found to be 0.09 and 0.24 µM of BaP, respectively. Limits of quantification obtained for e-MIP30CPE and e-NIP30CPE were 0.32 and 0.82 µM of BaP respectively.

In conclusion, original MIPs were synthesized integrating a redox tracer during the polymerization process. This tracer was used as an electrochemical sensing element to recognize a target compound without redox properties (BaP in this study). Therefore, intrinsic electrochemical MIPs were reported for the first time and are called e-MIPs. Electrochemical measurements revealed drastically different behaviors of e-MIPs and their corresponding e-NIPs. This property is very promising for future applications in the field of sensors. A versatile kind of MIP-based receptor for electrochemical sensors has thus been designed and can be applied to a wide range of analytes, with or without redox properties, by only changing the template, with a compatible redox tracer, during the MIP synthesis.

As far as BaP detection is involved, next challenges will be: (i) to increase selectivity and sensitivity by choosing a more aromatic redox probe in order to enhance  $\pi$ -stacking interactions with BaP during polymerization, and (ii) to prepare a screen printed carbon electrode with two working electrodes designed for e-MIPs and e-NIPs, respectively, in order to conduct relative electrochemical measurements.

The authors acknowledge financial support from the "Société du Canal de Provence" and from the Regional Council of Provence Alpes Côte d'Azur (France) and Bernard Fache from MAPIEM Laboratory for SEM images.

## Notes and references

- 1 K. Haupt and K. Mosbach, Chem. Rev., 2000, 100, 2495-2504.
- 2 L. Ye and K. Haupt, Anal. Bioanal. Chem., 2004, 378, 1887-1897.
- 3 A. A. Volkert and A. J. Haes, Analyst, 2014, 139, 21-31.
- 4 G. Vlatakis, L. I. Andersson, R. Müller and K. Mosbach, *Nature*, 1993, **361**, 645–647.
- 5 M. J. Whitcombe, N. Kirsch and I. A. Nicholls, *J. Mol. Recognit.*, 2014, 27, 297–401.
- 6 C. Alexander, H. S. Andersson, L. I. Andersson, R. J. Ansell, N. Kirsch, I. A. Nicholls, J. O'Mahony and M. J. Whitcombe, *J. Mol. Recognit.*, 2006, **19**, 106–180.
- 7 Y. Fuchs, O. Soppera and K. Haupt, *Anal. Chim. Acta*, 2012, **717**, 7–20. 8 M. J. Whitcombe, I. Chianella, L. Larcombe, S. A. Piletsky, J. Noble,
- R. Porter and A. Horgan, *Chem. Soc. Rev.*, 2011, 40, 1547–1571.
  9 M. C. Moreno-Bondi, F. Navarro-Villoslada, E. Benito-Pena and
- J. L. Urraca, *Curr. Anal. Chem.*, 2008, 4, 316–340.
- F. L. Dickert, O. Hayden, R. Bindeus, K. J. Mann, D. Blaas and E. Waigmann, *Anal. Bioanal. Chem.*, 2004, 378, 1929–1934.
- 11 Y. Zhang and K. D. Shimizu, in *Chemosensors: Principles, Strategies*, ed. B. Wang and E. V. Anslyn, John Wiley & Sons, 2011, pp. 107–120.
- 12 S. A. Piletsky and A. P. F. Turner, *Electroanalysis*, 2002, 14, 317–323.
- 13 V. Suryanarayanan, C.-T. Wu and K.-C. Ho, *Electroanalysis*, 2010, 22, 1795–1811.
- 14 C. Malitesta, E. Mazzotta, R. A. Picca, A. Poma, I. Chianella and S. A. Piletsky, Anal. Bioanal. Chem., 2011, 402, 1827–1846.
- 15 M. C. Blanco-López, M. J. Lobo-Castañón, A. J. Miranda-Ordieres and P. Tuñón-Blanco, *TrAC, Trends Anal. Chem.*, 2004, 23, 36–48.
- 16 R.-N. Liang, D.-A. Song, R.-M. Zhang and W. Qin, Angew. Chem., Int. Ed., 2010, 49, 2556–2559.
- 17 N. Luo, D. W. Hatchett and K. R. Rogers, *Electroanalysis*, 2006, 18, 2180–2187.
- 18 G. Mastrangelo, E. Fadda and V. Marzia, Environ. Health Perspect., 1996, 104, 1166–1170.
- 19 R. Dabestani and I. N. Ivanov, Photochem. Photobiol., 1999, 70, 10-34.
- 20 J.-P. Lai, R. Niessner and D. Knopp, Anal. Chim. Acta, 2004, 522, 137-144.
- 21 X. Song, J. Li, S. Xu, R. Ying, J. Ma, C. Liao, D. Liu, J. Yu and L. Chen, *Talanta*, 2012, **99**, 75–82.
- 22 J. Burdeniuc, R. H. Crabtree, A. L. Rheingold and G. P. A. Yap, Bull. Soc. Chim. Fr., 1997, 134, 955–958.
- 23 P. Molina, A. Tárraga and A. Caballero, Eur. J. Inorg. Chem., 2008, 3401–3417.
- 24 K. M. Joly, R. M. Gleixner, S. M. E. Simpkins, D. M. Coe and L. R. Cox, *Tetrahedron*, 2007, 63, 761–767.
- 25 J. Wang, P. A. G. Cormack, D. C. Sherrington and E. Khoshdel, *Pure Appl. Chem.*, 2007, **79**, 1505–1519.
- 26 F. Bai, X. Yang and W. Huang, Macromolecules, 2004, 37, 9746–9752.
- 27 C. U. Pittman, J. Inorg. Organomet. Polym. Mater., 2005, 15, 33-55.
- 28 I. Švancara, K. Vytřas, K. Kalcher, A. Walcarius and J. Wang, *Electroanalysis*, 2009, 21, 7–28.
- 29 R. Martínez, M. T. Ramírez and I. González, *Electroanalysis*, 1998, 10, 336–342.