Biodiversity restoration and conservation of inland water ecosystems for environmental and human well-being

BioReset

BiodivRestore-406

2020 - 2021 Joint Call
Joint COFUND Call on “Conservation and restoration of degraded ecosystems and their biodiversity, including a focus on aquatic systems”

Deliverable 1.2.1

Electrochemical sensors (MIPs)

Lead Beneficiary	Work package	Delivery month
REQUIMTE	**1**	**18**

1. Executive Summary

BioReset proposes to advance treatment processes (chemical, physical, biological and their combination) to promote ecosystem recovery and conservation and to develop assessment strategies. Diatoms will be used to model ecosystem conservation and restoration since their communities show high levels of biodiversity. The diatoms will provide an expeditious method to compare different recovery strategies and water treatment processes, allowing to address timescale and key conservation/restoration questions. The full environmental, economic, and social viability of the upgraded and innovative treatment technologies will be assessed. Based on this knowledge, scale-up studies in geographically different sites (Portugal and Spain) will be performed to ascertain the technical and economic feasibility at a larger scale and recommended action guidelines will be issued.

BioReset also envisages the creation of a representative space-time picture of the presence of emerging contaminants in inland waters and its correlation to effects on diatom communities. For this, powerful analytical techniques, such as gas- and liquid chromatography, will be used. Besides these methods, and to obtain real-time information, miniaturized analytical platforms that can perform fast and on-site monitoring will also be employed.

Deliverable 1.2.1 is intended to provide information about innovative analytical devices for emerging contaminant (EC) screening. The details of the validation and development of electrochemical sensors based on molecularly imprinted polymers (MIP) is outlined in this report.

2. Task description

WP1 regards analytical methods to analyse EC (pharmaceuticals and microplastics) in inland waters using established and novel methods. Task 1.2 focuses on the production of portable electroanalytical devices, adequate for on-site monitoring of selected EC. It consists of: **i)** development of commercial transducers (screen-printed electrodes, SPE) modified with MIPs, to increase the selectivity of the analysis, and with nanomaterials (e.g., carbon nanotubes and/or gold nanoparticles), to increase the sensitivity; **ii)** transposing these sensing strategies to alternative and much cheaper transducers and electrochemical cells (e.g., pencil leads, transparency sheets, micropipette tips, microcentrifuge tubes, etc.). This will allow the use of these sensors in remote and resource-limited areas since they will be easy to construct and apply. The ability of all the sensors to detect EC will be studied and the sensor's results will be validated by comparison with the results from the UHPLC-MS/MS method developed in Task 1.1.

3. WP1 - Task 1.2 team members

The Team members in WP1, Task 1.2, regarding MIP sensors, are:

Name	Organization	Role	Name	Organization	Role
Cristina Delerue-Matos	REQUIMTE	Task coordinator	Teresa Fernández-Abedul	UNIOVI	Researcher
Hendrikus Nouws	REQUIMTE	Researcher	Estefanía Costa Rama	UNIOVI	Researcher
João Pacheco	REQUIMTE	Researcher	María Cerrato-Álvarez	UNIOVI	Researcher
Patrícia Rebelo	REQUIMTE	Researcher	Javier Menéndez	UNIOVI	Researcher
Elisa González-Romero	UVIGO	Researcher	Carmen Blanco López	UNIOVI	Researcher
Verónica Poza-Nogueiras	UVIGO	Researcher			

4. Developed activities

Validation of an electrochemical MIP sensor for the analysis of atorvastatin

Atorvastatin (ATV) is one of most widely consumed pharmaceuticals worldwide. As a result, previous studies indicate that this statin is among the pharmaceuticals with the highest concentrations in aqueous matrices, such as wastewater (WW), surface water, and groundwater. Wastewater treatment plants (WWTPs) do not fully remove ATV, leading to its persistence in treated wastewaters and, consequently, its release into the environment.

The first electrochemical MIP sensor for the analysis of ATV in water samples was previously developed by some members of the REQUIMTE team [1]. The applicability of this sensor was tested for ATV analysis in surface waters and wastewaters from different

WWTPs, and the results were compared with the ones obtained by UHPLC-MS/MS (Task 1.1). Considering the LOD of the sensor, several optimization studies were carried out, specially aimed to optimize the preparation and pre-concentration of the water samples. In brief, 10,00 mL of the water samples were evaporated under a gentle stream of nitrogen and were reconstituted with 500 µL of ultra-pure water. These solutions were then filtered through 0.22 µm nylon syringe filters and analysed using the MIP sensor.

Development of a MIP sensor for the analysis of atenolol

Atenolol (ATL) is a beta blocker drug used to treat cardiovascular diseases, including hypertension and arrhythmias. Its effectiveness and relatively low cost have contributed to its widespread use. After testing on commercial SPE, a disposable electrochemical MIP sensor for the selective determination of ATL is being developed. Pencil leads were chosen as electrodes, because of their price and availability. A transparency sheet was used to construct the electrochemical cell. The working area (a circle with a diameter of 4 mm) was prepared by depositing 4 µL of carbon ink on the transparency sheet that was previously cut in rectangles (2.5 cm × 1.5 cm). The MIP was prepared by electropolymerization (through cyclic voltammetry (CV)), using 4-aminobenzoic acid (ABA) as functional monomer in the presence of ATL. To evaluate the efficiency of MIP sensor, the same procedure was employed to prepare a non-imprinted polymer (NIP) sensor, i.e. without ATL in the polymerization solution.

Development of a MIP sensor for the analysis of venlafaxine

Venlafaxine (VFX) was chosen for the development of another low-cost analytical device. This drug appears on the 2022 update of “Watch List of substances for European Union-wide monitoring in the field of water policy”. First a selective MIP sensor was constructed via direct imprinting of VFX onto the surface of a commercial SPE through electropolymerization (by CV) of a solution containing the functional monomer 3,4-ethylenedioxythiophene (EDOT) and VFX. This MIP-based sensor would serve for selective assessment of VFX in case a current is observed in the continuous monitoring of water deposits (current-time curves). The electrochemical cell employed for recording these curves is based on the use of pencil leads as electrodes, in this case not only because of their price and availability, but also because their cylindric shape allows radial diffusion of analytes and a tunable electrodic area. First it was evaluated voltammetrically employing a well-known redox probe. Thus, it consists of a disposable three-electrode assembly, with pencil leads used as working electrode (WE), pseudo reference electrode (RE) and counter electrode (CE), as well as a microcentrifuge tube (electrolyte container). The RE and CE were used without any pretreatment procedure or surface renewal during the measurements. However, the pencil lead used as WE was modified with functionalized multiwalled carbon nanotubes. The electrical contact with the pencil leads was achieved by using a hook clip. The final design the electrochemical cell is shown in Figure 1.

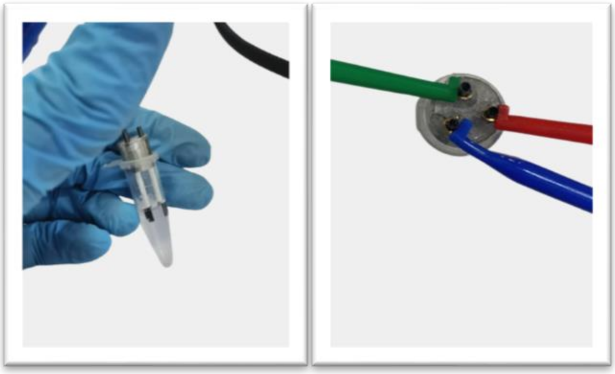

Figure 1. Design of the electrochemical cell.

To develop an adequate analytical methodology, the voltammetric response obtained for potassium ferrocyanide with different commercial pencil leads was studied, selecting 1.4 mm B pencil leads (commercial Faber-Castell leads).

All the experimental parameters involved in the preparation of the transducer (modification with nanomaterials and geometrical surface area) as well as instrumental variables (accumulation potential and accumulation time) were extensively investigated. The electrochemical cell was tested for the determination of VFX in spiked wastewater collected at the inlet and outlet of the Febros WWTP (Portugal). During part of this work, Patrícia Rebelo from REQUIMTE (Portugal) visited UNIOVI (Spain) to work and discuss the development of MIP sensors.

Development of a MIP sensor for the analysis of carbamazepine

Carbamazepine (CBZ) is an antiepileptic and mood stabilizing drug that is widely prescribed. It is recalcitrant to conventional wastewater treatments, so it ends up being released into the environment. The MIP sensor was prepared through surface imprinting on a screen-printed carbon electrode (SPCE) by electropolymerization (CV, 0.0 to 1.4 V at 100 mV/s during 10 cycles). 3,4-ethylenedioxythiophene (EDOT) was used as the functional monomer and CBZ as the template. Various experimental parameters were optimized: monomer and CBZ concentrations for the MIP synthesis, extraction solvent and time, and incubation time (for rebinding of CBZ to the MIP). After the rebinding of CBZ, its oxidation current was recorded by differential pulse voltammetry (DPV, 0.4 to 1.4 V at 50 mV/s). As in the other studies a NIP sensor was also constructed.

This work was performed by Verónica Poza-Nogueiras, a postdoc researcher from UVIGO (Spain), between October 2022 and September 2023 during an internship at REQUIMTE (Portugal).

5. Results

Validation of an electrochemical MIP sensor for the analysis of atorvastatin

The results of the analysis of ATV in surface- and WW samples using the MIP sensor and UHPLC-MS/MS are shown in Table 1.

Table 1. Results of the analysis of ATV in wastewater samples using the MIP sensor and UHPLC-MS/MS.

Water sample	[ATV] (nM)* HPLC	CV (%)	[ATV] (nM)* MIP sensor	CV (%)
São Jacinto	n.d	-	n.d	-
Ribeira de Frades Montante	0.174	5.4	n.d	-
Ribeira de Frades Jusante	1.33	5.1	3.10	22
Effluent São Jacinto	0.179	7.4	n.d	-
Effluent Ribeira de Frades	3.15	11	3.43	19
Effluent T0_setembro	1.66	7.7	4.97	50
T0_julho	1.07	8.4	2.03	20
UFT0_setembro	0.737	14	3.89	24
UF502040	n.d	-	n.d	-
18W60	n.d	-	n.d	-
18W30	n.d	-	n.d	-
7W60	n.d	-	n.d	-

* n.d. – not detected

As can be observed, the MIP sensor was able to quantify ATV concentrations that are comparable with UHPLC-MS/MS, showing that it can be very useful for screening purposes. However, the concentrations obtained with the MIP sensor were generally higher. This could be attributed to a range of factors, including differences in the sample preparation method for both analysis methods.

Development of a MIP sensor for the analysis of atenolol

In the potential range from -0.1 V to +1.4 V, differences were observed in the first scan of the synthesis of the MIP and the NIP; in the MIP synthesis a slight peak was recorded at +1.1 V, while it did not appear in the NIP synthesis. As expected, these results suggest the oxidation of ATL molecules that are being incorporated in the MIP film. Following the preparation of the polymeric film, the imprinting effects on sensor performance were evaluated using DPV (Figure 2).

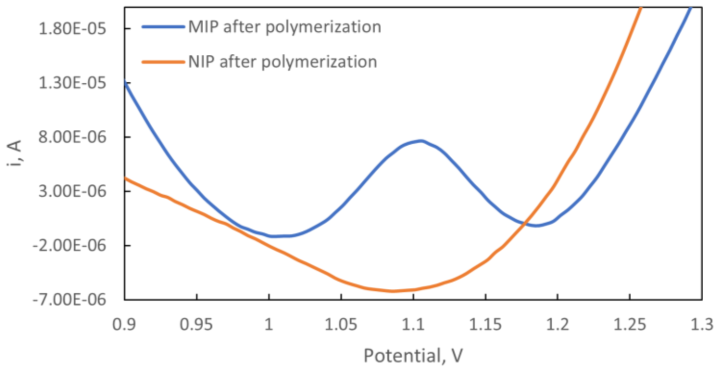

Figure 2. DPV voltammograms of the MIP and the NIP sensor after polymerization.

As can observed in Figure 2, a remarkable difference is noticeable between the MIP and the NIP sensor. An oxidation peak at +1.1V was exclusively observed for the MIP sensor, confirming the successful incorporation of ATL inside the MIP layer. Studies are still ongoing to find the best solvent to remove the ATV molecules in order to ensure the stability of the prepared polymer, and to reach the best sensitivity of the sensor.

Development of a MIP sensor for the analysis of venlafaxine

Regarding the pencil-lead based cell, after the evaluation with the well-known redox probe, studies with VFX were made. The optimization of accumulation potential and time was made, resulting in 0 V and 180 s as appropriate values for precise measurements. The electroactive geometrical surface of the WE was evaluated by varying the pencil lead length between 0.5 and 2 cm. The optimum value chosen was 1 cm. A calibration straight was established between 0.8 and 10 µmol/L with the following equation: i (µA) = (1.477 ± 0.035) [VFX] (µmol/L – (0.868 ± 0.195) (n = 7) with a good determination coefficient (R^2 = 0.996). The method offered a limit of detection of 0.4 µmol/L. Recoveries for the analysis of VFX in the influents and effluents of a WWTP were 94 - 96 % and 80 - 90 %, respectively. This cell is going to be used in a floating platform for continuous monitoring of redox substances in waters, confirming the presence of VFX using the MIP-based sensor on screen-printed electrodes. Their concentrations were optimized with 3 and 6 mM as optimum values, respectively. Likewise, the number of cycles during electropolymerization (15) and the incubation time (10 min) wereas optimized. In Figure 3 the signals obtained by square wave voltammetry (SWV) with MIP and NIP sensors after incubation with VFX are presented.

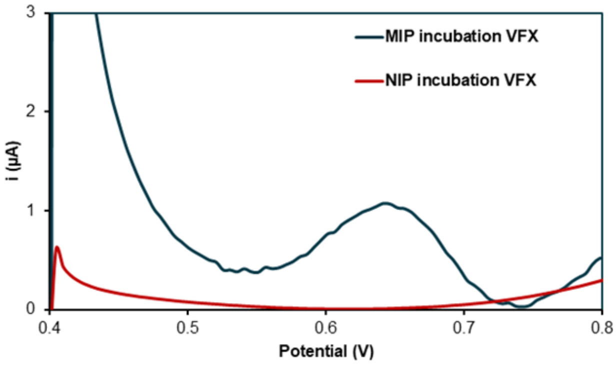

Figure 3. SWV voltammograms after incubation of 0.1 µM venlafaxine for 40 min.

Development of a MIP sensor for the analysis of carbamazepine

Figure 4a shows the electrochemical polymerization process. The peak current intensity decreased as PEDOT (poly(3,4-ethylenedioxythiophene)) was formed, both for the production of the NIP and the MIP sensor. However, differences were observed: whereas two peaks were detected during the formation of the MIP (at ca. 1.0 V and 1.2 V, ascribed to the oxidation of EDOT and CBZ, respectively), only the oxidation peak of the EDOT was observed on the NIP, with a reduced intensity. The successful incorporation of the template on the MIP sensor during the polymerization step was corroborated by DPV, as demonstrated by the CBZ oxidation peak observed in Figure 4b, which is not present in the case of the NIP. The extraction of CBZ from the MIP is key to ensure the formation of the specific cavities. As depicted in Figure 4b, the CBZ peak completely disappeared after the extraction, indicating that this step was successfully achieved.

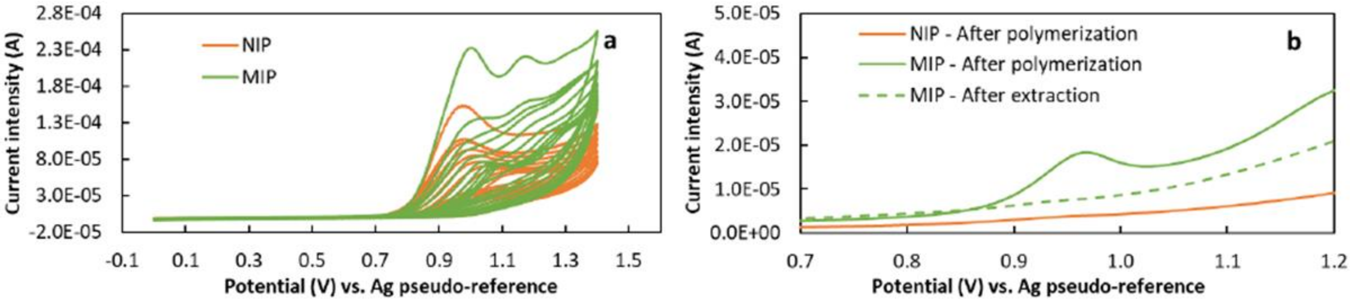

Figure 4. (**a**) CV voltammograms obtained during the production of the NIP and MIP sensors (**b**) DPV voltammograms of the NIP and MIP sensors after polymerization and extraction with NaOH 0.1 M.

The feasibility of fabricating MIP-based SPCEs sensors for the detection of CBZ was demonstrated and key process parameters were optimized. These promising results pave the way for the future development of a suitable sensor for environmental water analysis.

6. Associated indicators

Communications

1. Pacheco, J.G., Seguro, I., Rebelo, P., Delerue-Matos, C., *Molecularly imprinted electrochemical sensors for pharmaceuticals monitorization in water*, XXV Meeting of the Portuguese Electrochemical Society, August 30 - September 1, 2023, Coimbra, Portugal. (Oral presentation)
2. Cerrato-Álvarez, M., Menéndez-Menéndez, J., Rioboó-Legaspi, P., Costa-Rama, E., Fernández-Abedul, M.T., *Electrochemical molecularly imprinted polymer sensor for selective determination of emerging contaminants in water*, II Reunión Científica del Grupo de Ciencia y Tecnologías (Bio)Analíticas - GCTbA2023, June 27-28, 2023, Zaragoça, Spain. (Poster presentation)
3. Cerrato-Alvarez, M., Rioboó-Legaspi, P., Costa-Rama, E., Fernández-Abedul, M.T., *Electroanalytical platform based on pencil graphite electrodes for detecting antidepressant drugs in inland waters*, XXXIX Reunión Bienal de la Real Sociedad Española de Química, June 25-29, 2023, Zaragoça, Spain. (Poster presentation)
4. Poza-Nogueiras, V., Pacheco, J.G., Delerue-Matos, C., *Towards the development of a simple molecularly imprinted electrochemical sensor for the detection of carbamazepine*, 5th Doctoral Congress in Engineering - DCE23, June 15-16, 2023, Porto, Portugal. (Poster presentation)

References

[1] Rebelo, P., Pacheco, J.G., Voroshylova, I.V., Melo, A., Cordeiro, M.N.D.S., Delerue-Matos, C. (2022). *A simple electrochemical detection of atorvastatin based on disposable screen-printed carbon electrodes modified by molecularly imprinted polymer: Experiment and simulation*. Analytica Chimica Acta 1194, 339410.