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 1.2.2

Miniaturized analytical devices based on new transducers

Lead Beneficiary	Work package	Delivery month
UNIOVI	**1**	**36**

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 1.2.2 is intended to provide information about innovative analytical devices for emerging contaminants (ECs) determination. The details of the development of miniaturized platforms based on novel low-cost transducers and the main results achieved are outlined in this report.

2. Task description

WP1 refers to the development of electrochemical sensors for in situ electrochemical control, using miniaturized platforms for the determination of environmental pollutants (pharmaceuticals and microplastics) in continental waters using established and novel methods. Task 1.2 focuses on the development of innovative analytical devices for screening drugs and molecules related to plastics. It consists of: **i)** selection of the most interesting pharmaceutical products and study of their electrochemical behavior on low-cost transducers; **ii)** design of a floating electroanalytical platform for monitoring the selected drug using low-cost materials; **iii)** development of a methodology for drug determination using the floating platform; **iv)** evaluation of the floating platform in a water pool for creating spatiotemporal maps.

3. WP1 - Task 1.2 team members

The Team members in WP1, Task 1.2, regarding the development of miniaturized analytical sensors, are:

Name	Organization	Role
Teresa Fernández-Abedul	UNIOVI	Researcher
Estefanía Costa Rama	UNIOVI	Researcher
María Cerrato-Álvarez	UNIOVI	Researcher
Javier Menéndez	UNIOVI	Researcher

4. Developed activities

Development of a miniaturized low-cost cell for the analysis of venlafaxine

Venlafaxine (VFX) is one of the most widely prescribed selective serotonin and norepinephrine reuptake inhibitor antidepressants worldwide, making it one of the drugs with the highest concentration in aqueous matrices such as fresh water, marine water, and groundwater. High human consumption combined with its incomplete elimination in wastewater treatment plants (WWTPs) causes it to persist in treated water and be released into the environment. In addition, this drug appears on the Watch List of substances for European Union-wide monitoring in the field of water policy (2022) and, for this reason, it has been selected for this project. Its electrochemical behavior is described in the literature, so the signals obtained by cyclic voltammetry (CV) on screen-printed carbon electrodes in media with different pH values were reported, and the improvement in sensitivity through the use of nanomaterials was also studied.

In this project, an electrochemical cell was developed using pencil leads as electrodes, selected not only for their low cost and easy availability but also for their advantageous cylindrical geometry, which promotes radial diffusion and allows adjustment of the

electrode area, in addition to their high electrical conductivity. Faber-Castell B leads with a diameter of 1.4 mm were chosen for this purpose. As shown in Figure 1, the cell consists of a disposable three-electrode configuration, where pencil leads serve as the working electrode (WE), pseudo-reference electrode (RE), and counter electrode (CE), housed within a microcentrifuge tube acting as the electrolyte container. The RE and CE were used without any pretreatment or surface renewal during measurements, whereas the WE was modified with functionalized multiwalled carbon nanotubes to enhance sensitivity. Electrical contact with the pencil leads was achieved using hook clips, ensuring a stable and reproducible connection.

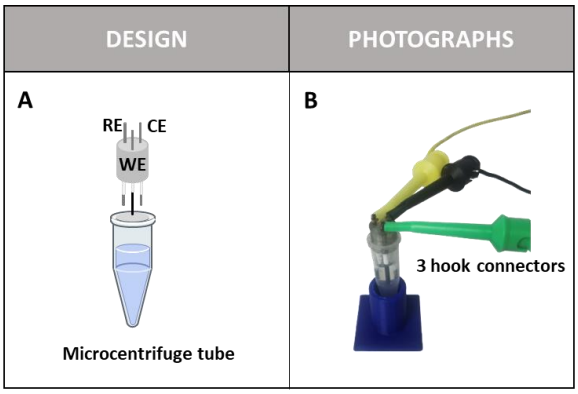

Figure 1. Design of the electrochemical cell.

Furthermore, a low-cost floating electroanalytical platform was also designed for the monitoring of the selected pharmaceutical compound. This floating cell was also based on pencil-lead electrodes, and a support was designed to allow the cell to float when coupled to a miniaturized (USB-sized) potentiostat.

5. Results

Development of a miniaturized low-cost cell for the analysis of venlafaxine

After validating the pencil-lead-based cell with a well-known redox probe, studies were conducted with VFX. An analytical methodology was developed for determining VFX, which involves an anodic process at 0.8 V in a phosphate buffer solution with a pH of 7 (CV). To this end, the effect of different variables (use of nanomaterials, electrode area, potential, and accumulation time) was evaluated. Calibration curves were then constructed with VFX standards of increasing concentration from 0.8 to 10 µmol L^{-1}, obtaining a linear relationship with the equation ip/µA = 1.48 [VFX]/µmol L^{-1} - 0.9, for n = 7, with a good coefficient of determination (R^2 = 0.996), Figure 2 (left). The method, with a detection limit of 0.4 µmol L^{-1}, was evaluated by determining VFX in wastewater collected at the Febros WWTP (Portugal) and fortified with VFX, finding recoveries of approximately 95% and 85% for the plant inlet and outlet, respectively [1].

Studies were also conducted for its main metabolite, desmethylvenlafaxine (DVFX), that can be present in wastewater and the Watch List 2022 of the EU recommended analyzing both drugs. Therefore, the behavior of DVFX was evaluated in case this could interfere the process of the parent compound. Thus, the procedure for determination of VFX was also followed for DVFX using the same optimized variables. Voltammograms were recorded in 3 µmol L^{-1} VFX and DVFX (each) solutions at different BR buffer pH 7 using standard DVFX with concentrations comprised between 0.8 and 10 µmol L^{-1}. A linear response between the peak current intensity and the concentration of DVFX, according to the linear equation ip/µA = (1.64 ±0.07) [DVFX]/µmol L^{-1} – (1.1 ±0.4) (n = 6), was achieved. The method offers a good determination coefficient (R^2 =0.997) and a detection limit of 0.6 µmol L^{-1}, Figure 2 (right).

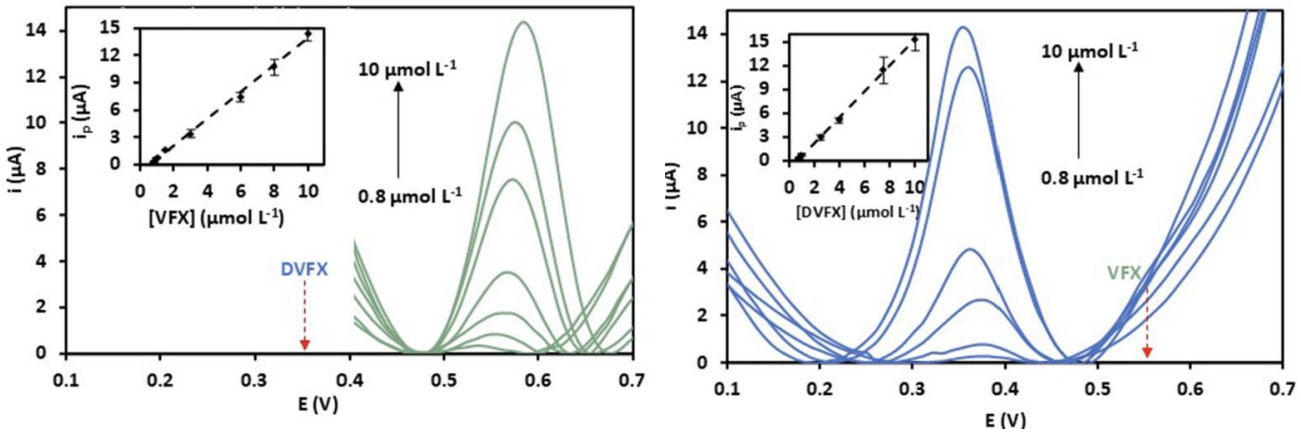

Figure 2. Voltammograms recorded for different VFX (left) and DVFX (right) concentrations and linear dynamic range of the calibration curve obtained representing the peak current intensities against concentrations. The potential of the process of the metabolite is indicated in VFX square wave voltammograms, and this of the main drug in DVFX SWVs highlights the selectivity of electrochemical techniques.

Paper, a low-cost material, has been combined with the pencil leads for different uses (Figure 3): i) as a filter for collecting samples that could have particulate material, ii) as a sampler acting by capillarity or ii) as an electrochemical cell that can store dry electrolyte. This would allow to observe the process of VFX where initial water sample pH would have impede it.

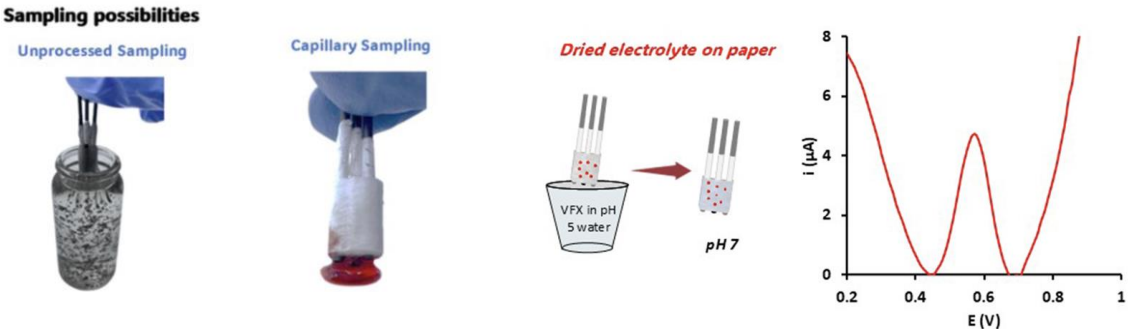

Figure 3. Pictures of different uses of the paper (glass fiber pad)-lead combined cell. Adsorptive SWV for 25 µmol L^{-1} VFX at pH 5.4, where this molecule is not electroactive. The dried electrolyte increases the pH to 7 and the process is seen.

Regarding the low-cost floating electroanalytical platform, it was successfully integrated into a water-filled container, enabling amperometric (i–t) monitoring for early detection of electroactive species. It was optimized by cyclic voltammetry using potassium ferrocyanide as a model redox probe. Its response to simulated injections of the redox probe and VFX, by means of controlled injections using a peristaltic pump to simulate spatiotemporal monitoring scenario, was also evaluated considering transport phenomena based on diffusion and agitation. The radial diffusion regime provided significantly higher current intensities compared to conventional planar screen-printed electrodes. Different studies on the influence of flow rate, stirring speed, analyte concentration, injection distance, and depth on the analytical response were performed.

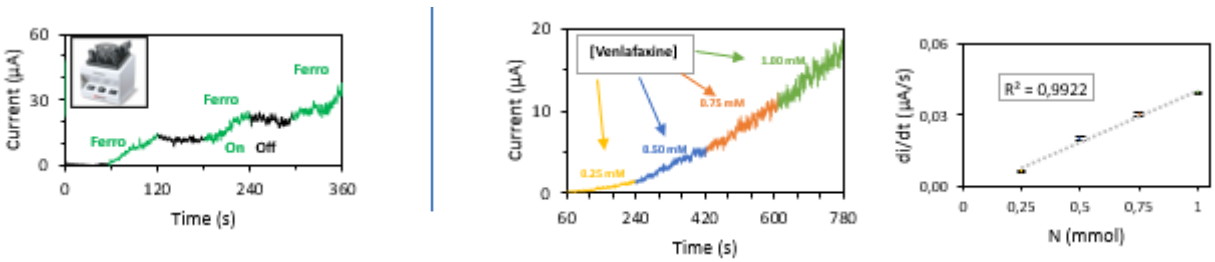

Figure 4, left. Chronoamperogram recorded with carbon leads applying 0.25 V, adding 5.2 mL min^{-1} of 10 mM ferrocyanide solution at 2, 4 and 6 min under magnetic stirring (100 rpm) magnetic stirring. **Right,** Chronoamperogram recorded with carbonleads applying 0.9 V, for discharges of different concentration of VFX.

Figure 4 left shows an example of the i-t curves obtained when a peristaltic pump is discharging a solution of ferrocyanide in a 500 mL container including the floating platform under stirring. When the discharge stops the current attains a plateau, being possible to detect the time of discharges. Figure 4 right shows the i-t curve for simulated discharges of venlafaxine, being the slope (charge) proportional to the concentration. These results are very promising for monitoring electroactive emerging contaminants in waters as a warning system. Confirmation could be made through MIP sensors.

6. Associated indicators

Communications

1. 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, Zaragoza, Spain. (Poster presentation)
2. 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, Zaragoza, Spain. (Poster presentation)
3. Cerrato-Álvarez, M., Rioboó-Legaspi, P., Costa-Rama, E., Fernández-Abedul, M.T., *Floating electrochemical platform based on pencil leads for the determination of venlafaxine in inland waters*, II PhD Multidisciplinary Chemical Congress, March 14-15, 2024, Gijón, Spain. (Poster presentation)
4. Costa Rama, E., Cerrato Álvarez, M., Rioboó Legaspi, P., Rebelo, P., Pacheco, J.G., Nouws Henri P.A, Delerue Matos, C., Fernández Abedul, M.T., *Low-cost Electroanalytical Platforms for the Determination of Emerging Contaminants*, 36th Topical Meeting of the International Society of Electrochemistry, May 26-29, 2024, Sibenik, Croatia. (Oral invited)
5. Cerrato Álvarez, M., Rioboó Legaspi, Costa Rama, E., Fernández Abedul, M.T., *A home-made electrochemical cell for venlafaxine determination in wastewaters*, 36th Topical Meeting of the International Society of Electrochemistry, May 26-29, 2024, Sibenik, Croatia. (Poster presentation)
6. Cerrato-Álvarez, M., Rebelo, P., Costa- Rama, E., Pacheco, J.G., Nouws, H.P.A., Delerue-Matos, C., Fernández-Abedul, M.T., *Electropolymerized molecularly imprinted polymer sensor for the simple voltammetric detection of venlafaxine in water samples*, 36th Topical Meeting of the International Society of Electrochemistry, May 26-29, 2024, Sibenik, Croatia. (Oral presentation)
7. Menéndez Menéndez, J., Cerrato-Álvarez, M., Rioboó-Legaspi, P., Costa-Rama, E., Fernández-Abedul, M., *Floating pencil lead-based electrochemical cells to monitor the presence of emerging contaminants in waters*, 36th Topical Meeting of the International Society of Electrochemistry, May 26-29, 2024, Sibenik, Croatia. (Oral invited)
8. Cerrato Álvarez, M., Rioboó Legaspi, Costa Rama, E., Fernández Abedul, M.T., *Carbon nanotube-modified graphite pencil leads as electrodes in a miniaturized platform for determining venlafaxine. póster. xi international congress on analytical nanoscience and nanotechnology*, XI IXI International congress on analytical nanoscience and nanotechnology, September 3-6, 2024, Santiago de Compostela, Spain. (Poster presentation)
9. Costa Rama, E., Cerrato Álvarez, M., Menéndez Menéndez, J., Rioboó Legaspi, P., Rebelo, P., Nouws Henri P.A, Delerue Matos, C., Fernández Abedul, M.T., *Decentralised electroanalysis of emerging contaminants: molecularly imprinted polymer sensor for the selective detection of the antidepressant venlafaxine in water*, 35th Anniversary world congress on biosensors, May 19-22, 2025, Lisboa, Portugal. (Poster presentation)
10. Costa Rama, E., Cerrato Álvarez, M., Torre, R., Rioboó Legaspi, P., Nouws Henri P.A, Delerue Matos, C., Fernández Abedul, M.T., *Hand-Made Carbon-Based Electroanalytical Platforms for the Affordable Determination of Pharmaceuticals and Biogenic Amines*, 76th Annual Meeting of the International Society of Electrochemistry, September 7-12, 2025, Mainz, Germany. (Oral presentation)

References

[1] Maria Cerrato-Alvarez, Pablo Rioboó-Legaspi, Estefania Costa-Rama, M. Teresa Fernández-Abedul (2025). *Field-deployable pencil lead-based electrochemical cell for the determination of the emerging contaminant and antidepressant drug venlafaxine in wastewater*. Biosensors and Bioelectronics 267, 116851.