



HAL
open science

Amperometric detection of the herbicide mesotrione based on competitive reactions at nitroreductase@layered double hydroxide bioelectrode

Slim Hidouech, Felipe Bruna, Isabelle Batisson, Pascale Pascale Besse, P. Besse-Hoggan Besse-Hoggan, Vanessa Prevot, Christine Mousty

► To cite this version:

Slim Hidouech, Felipe Bruna, Isabelle Batisson, Pascale Pascale Besse, P. Besse-Hoggan Besse-Hoggan, Vanessa Prevot, et al.. Amperometric detection of the herbicide mesotrione based on competitive reactions at nitroreductase@layered double hydroxide bioelectrode. *Journal of Electroanalytical Chemistry*, 2019, 835, pp.324-328. 10.1016/j.jelechem.2019.01.054 . hal-02175349

HAL Id: hal-02175349

<https://hal.science/hal-02175349>

Submitted on 21 Oct 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Amperometric detection of the herbicide mesotrione based on competitive reactions at nitroreductase@layered double hydroxide bioelectrode.

Slim Hdiouech^a, Felipe Bruna^a, Isabelle Batisson^b, Pascale Besse-Hoggan^a, Vanessa Prevot^a,
Christine Mousty^{a*}

^a*Université Clermont Auvergne, CNRS, Sigma Clermont, Institut de Chimie de Clermont-Ferrand, F-63000 Clermont-Ferrand, France*

^b*Université Clermont Auvergne, CNRS, Laboratoire Microorganismes : Génome et Environnement, F-63000 Clermont-Ferrand, France*

*Corresponding author: Christine.Mousty@uca.fr

Highlights

- The detection of mesotrione using a nitroreductase biosensor is described.
- The biosensor was prepared with a nitroreductase@layered double hydroxide biohybrid.
- The current response is due to a competitive enzymatic reaction.

Abstract

A mesotrione monitoring biosensor was prepared using a nitroreductase (NfrA2) immobilized on a glassy carbon electrode by adsorption on nanoparticles of Mg₂Al-NO₃ layered double hydroxides (MgAl-NPs). The amperometric transduction process was based on a competitive enzymatic reduction between Fe(CN)₆³⁻ and mesotrione, both substrates of NfrA2. Indeed in the presence of the NADH cofactor, Fe(CN)₆³⁻ was enzymatically reduced and the Fe(CN)₆⁴⁻ released was then oxidized at the electrode. When mesotrione was added to the electrolyte solution, a competitive enzymatic reaction took place at the NfrA2@MgAl-NPs modified electrode leading to a decrease of the electrocatalytic anodic current. Mesotrione can thus be detected by chronoamperometry at 0.375 V/SCE with a sensitivity of 18.4 mA M⁻¹ cm⁻² over a linear concentration range between 5 and 60 μM (R² = 0.998) and a detection limit (3σ) of 3 μM.

Keywords: Mesotrione, Nitroreductase, Biosensor, Layered double hydroxides, Amperometry

1. Introduction

Mesotrione, 2-(4-methylsulphonyl-2-nitrobenzyl)cyclohexane-1,3-dione, is a selective herbicide belonging to the triketone family. This nitro aromatic derivative was marketed in 2001 by Syngenta, under the brand name Callisto[®], as a new selective pre- and post-emergence weed control in maize crops [1]. Like many other pesticides, mesotrione may be washed off from soil or leaves by rain and reach surface water and groundwater by leaching. Therefore, this molecule has been detected in all these environmental compartments. The need of effective analytical tools to monitor mesotrione concentration is therefore justified. Its detection is generally possible using conventional analytical methods, namely liquid chromatography coupled with UV-Vis or fluorescence *via* the metabolite 2-amino-4-methylsulfonylbenzoic acid (AMBA) and mass spectrometry [2]. Sophisticated fluorometric immunosensors for mesotrione determination in water samples and in the commercial formulation (Callisto[®]) were also optimized with a limit of detection (LoD) of 0.1 nM [3, 4]. A colorimetric whole cell bioassay was developed with 4-hydroxyphenylpyruvate dioxygenase (HPPD) expressed in *E. coli* immobilized in microplates with a LoD of 69 nM [5]. Unfortunately since HPPD is the target enzyme of β -triketonic herbicides, the latter bioassay is not selective towards mesotrione. Although these analytical methods are highly sensitive, they are quite expensive and cannot be easily transported to an external experimental area. The development of fast and inexpensive approaches is thus of great interest. Miniaturized devices, such as chemical sensors or biosensors, can be proposed as possible alternatives to the conventional analytical methods.

Taking into account that mesotrione can be reduced by electrochemistry, its electrochemical quantification was reported at a hanging mercury drop electrode [6], a glassy carbon electrode

[7], an organo-clay modified electrode [8] or using a carbon black electrode. This latter electrode gives the best LoD of 26 nM [9].

Recently, Carles *et al.* [10] have identified and purified two nitroreductases (called NfrA1 and NfrA2/YcnD from *Bacillus megaterium* Mes11 strain) able to biotransform mesotrione. These enzymes, which use nicotinamide adenine dinucleotide phosphate (NADPH) and/or nicotinamide adenine dinucleotide (NADH), respectively, as co-factors, transform mesotrione into an intermediate hydroxylamino derivative and then into 4-methylsulfonyl-2-aminobenzoic acid (AMBA). In the literature, other nitroreductases using the same co-factors were used into amperometric biosensors for the detection of the explosive trinitrotoluene (TNT) [11-14]. To the best of our knowledge no nitroreductase-based biosensor has been described for mesotrione detection.

The development of efficient biosensors usually requires the immobilization of enzymes. Recently in our group, the immobilization of NfrA2 on Mg₂Al-NO₃ layered double hydroxide (LDH) nanoparticles (MgAl-NPs) was optimized [15]. This inorganic lamellar material, also called anionic clay, exhibits intrinsic properties such as adsorption behavior, anionic exchange capacities and good biocompatibility, favorable for interactions with enzymes. Generally, enzyme@LDH biohybrid materials are also able to preserve enzyme activity [16]. Hence, the optimized NfrA2@MgAl-NPs biohybrid material displays a stable and reproducible enzymatic activity [15]. With the aim of developing an alternative method to detect mesotrione, an original biosensor was further developed using the NfrA2@MgAl-NPs biohybrid coated on a glassy carbon electrode (GCE) as recognition membrane. Interestingly, mesotrione and hexacyanoferrate, a well-known redox mediator, are both substrates of NfrA2. Therefore, a new

concept of amperometric transduction process was proposed based on the competitive affinity of NfrA2 towards mesotrione and hexacyanoferrate (Fig.1).

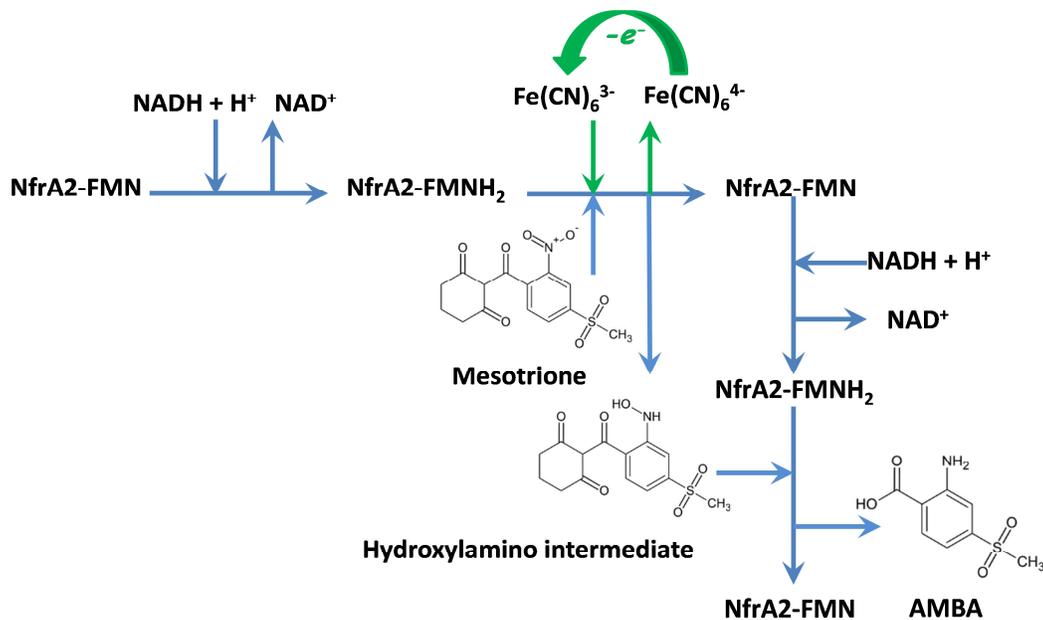


Figure 1: Proposed mechanistic scheme for mesotrione detection with a NfrA2 biosensor (in green the electron transfer occurring at the electrode surface).

2. Material and methods

Nicotinamide adenine dinucleotide (NADH), mesotrione, 3-(N-morpholino)propanesulfonic acid (MOPS) were purchased from Sigma-Aldrich. Magnesium and aluminium nitrate salts, Mg(NO₃)₂·6H₂O, Al(NO₃)₃·9H₂O and potassium hexacyanoferrate(III) (K₃Fe(CN)₆) and phosphate buffer components (K₂HPO₄ and KH₂PO₄) were of analytical grade (Acros Organics, Merck) and were used without further purification. Deionized water was used for all experiments. Recombinant NfrA2 was expressed in *E. coli* BL21 (DE3) pLys cells and then extracted and purified as previously described [15]. The purified enzyme was lyophilized and stored at 4°C for more than one month without losing activity.

The activity of NfrA2 towards 0.1 mM mesotrione was determined by HPLC. The enzymatic reaction was carried out in the presence of 0.1 mM mesotrione, 0.7 mM NADH and 12.5 or 25 $\mu\text{g/mL}$ of free or immobilized enzyme, respectively, in 50 mM of MOPS buffer solution, at an optimum pH 6.5, under stirring for 1 h. The disappearance of mesotrione was linear in the whole time interval. One unit of nitroreductase activity (U) was defined as the amount of enzyme which transforms 1 μmol of mesotrione equivalent per minute. The specific activity of the purified lyophilized NfrA2 was 0.06 U mg^{-1} of protein.

With $\text{K}_3\text{Fe}(\text{CN})_6$, the assays were monitored by a spectrophotometry at 420 nm ($\epsilon_{420} = 1040 \text{ M}^{-1} \text{ cm}^{-1}$) using a Cary 300 UV-Vis (Agilent). The reaction was carried out in a 0.1 M phosphate buffer solution (PBS pH 6.5) containing 1.5 mM $\text{K}_3\text{Fe}(\text{CN})_6$, 1.5 mM NADH and 50 μg of NfrA2. The resulting activity was 2.68 U mg^{-1} protein (1 U = 1 μmol of $\text{K}_3\text{Fe}(\text{CN})_6$ reduced/min).

$\text{Mg}_2\text{Al-NO}_3$ LDH nanoparticles (MgAl-NPs) were prepared by a flash coprecipitation, followed by a hydrothermal treatment as previously described [17]. The whole procedure was carried out under N_2 atmosphere. A metal nitrate solution (100 mL, 0.4 M $\text{Mg/Al} = 2$) was rapidly added to a 0.25 M NaOH solution (300 mL) under vigorous stirring. The dispersion was first aged for 30 min at pH 10.0 at 0°C and then hydrothermally treated at 150°C for 4 h. The sample was centrifuged at 18500 g for 30 min, washed three times with deionized water. The resulting MgAl-NPs were re-dispersed in deionized water (50 mL) and stored as a colloidal dispersion (26 % w/w) at 4°C . The formation of LDH structure was confirmed by powder X-Ray Diffraction and Fourier-Transformed Infrared spectroscopy. The Zeta-potentials (ζ) of MgAl-NPs, measured at pH 6.5 with a Zetasizer nano ZS (Malvern instruments) apparatus, was positive ($\zeta = +32 \text{ mV}$). The isoelectric point of NfrA2 was determined at pH 5.7, and the protein was negatively charged

at pH 6.5 ($\zeta = -10$ mV). The adsorption of NfrA2 on MgAl-NPs was carried out by mixing the enzymatic and the LDH suspensions. The optimized biohybrid corresponds to an Enz/LDH ratio of 1:1 leading to an enzyme adsorption > 80 % and a 40 % of remaining activity, compared to the same amount of free enzyme. Pristine MgAl-NPs and NfrA2@MgAl-NPs biohybrid (1:1) were characterized in Transmission Electron Microscopy (TEM). TEM images were taken using a Hitachi 7650 microscope at an acceleration voltage of 80 kV. One droplet of the aqueous colloidal suspensions was deposited on a 400 mesh holey carbon-coated copper grid and dried at room temperature.

Glassy carbon electrodes (GCE, $A = 0.196$ cm²) were used to prepare the biosensors. Before the deposition of the NfrA2@MgAl-NPs biohybrid, the GCE was polished with a diamond paste (1 μ m), washed with acetone, then polished with alumina slurry (0.04 μ m), and finally rinsed with ethanol and water. A drop (50 μ L) of the suspension containing 100 μ g of NfrA2@MgAl-NPs biohybrid (Enz/LDH 1:1) was deposited on the surface of GCE and the bioactive layer was dried under vacuum for 1 h. The NfrA2@MgAl-NPs/GCE and MgAl-NPs/GCE modified electrodes were imaged by Scanning Electron Microscopy (SEM) using a Zeiss supra 55 FEG-VP operating at 3 keV. The activity of the dried NfrA2@MgAl-NPs biohybrid was fully maintained at least for one month of storage.

Cyclic voltammetry (CV) was recorded using a PGSTAT 204 controlled by the NOVA software (Metrohm). Chronoamperometry experiments were recorded with a potentiostat EA161 (EDAQ), under dynamic conditions using a rotating disk carbon electrode (500 rpm) in a thermostatic cell (25°C) at an applied potential fixed at 375 mV/SCE. Before use, the NfrA2@MgAl-NPs/GCE modified electrodes were rehydrated in 0.1 M phosphate buffer solution (PBS, pH 6.5) for 15 min

and then transferred into the three-electrode cell to carry out electrochemical characterization. The reference electrode was a saturated calomel electrode (SCE) and the counter electrode a Pt wire.

3. Results and discussion

A NfrA2 electrochemical biosensor was prepared by immobilization of the enzyme on glassy carbon electrodes (GCE) by entrapment within a layered double hydroxide (LDH) matrix. LDH are synthetic lamellar materials with a general formula $[M_{1-x}^{2+}M_x^{3+}(\text{OH})_2]^{x+}[(A^{n-})_{x/n,y} \text{H}_2\text{O}]$ (abbreviated as $M^{2+}M^{3+}\text{-A}$). The interactions between enzymes and LDH particles are promoted by the opposite charges of LDH layers and biomolecules. An adsorption process is a simple and convenient way to prepare enzyme@LDH biohybrids for biosensor applications [16]. Recently, we have shown that $\text{Mg}_2\text{Al-NO}_3$ LDH nanoparticles (MgAl-NPs) displayed high enzyme affinity **leading** to stable Enz@LDH biohybrid materials [15, 17]. TEM images evidenced the influence of NfrA2 on MgAl-NPs dispersion. A pristine colloidal suspension of MgAl-NPs displayed small polydisperse particles with an average size around 90 nm (Inset Fig.2A), whereas the electrostatic interactions between positively charged MgAl-NPs ($\zeta = +32$ mV) and negative NfrA2 ($\zeta = -10$ mV) induced particle aggregation (Fig.2A). Thin films were prepared using this NfrA2@MgAl-NPs biohybrid through a one-step deposition on a polished glassy carbon electrode. We have compared the surface topology of both NfrA2@MgAl-NPs and MgAl-NPs films cast on GCE by SEM. The SEM images clearly show the influence of the enzyme on the membrane morphology. While a homogeneous film (Fig.2B) in which it is possible to distinguish the individual MgAl-NPs is observed with biohybrid, pure MgAl-NPs lead to less dense and continuous film (Fig.2C). In the latter case, dispersed aggregates of MgAl-NPs are observed at the electrode surface. The

electrostatic interactions involved between the two components, *i.e.* LDH nanoparticles and NfrA2, allow the formation of reproducible biomembranes at the electrode surface in which the enzyme is irreversibly immobilized.

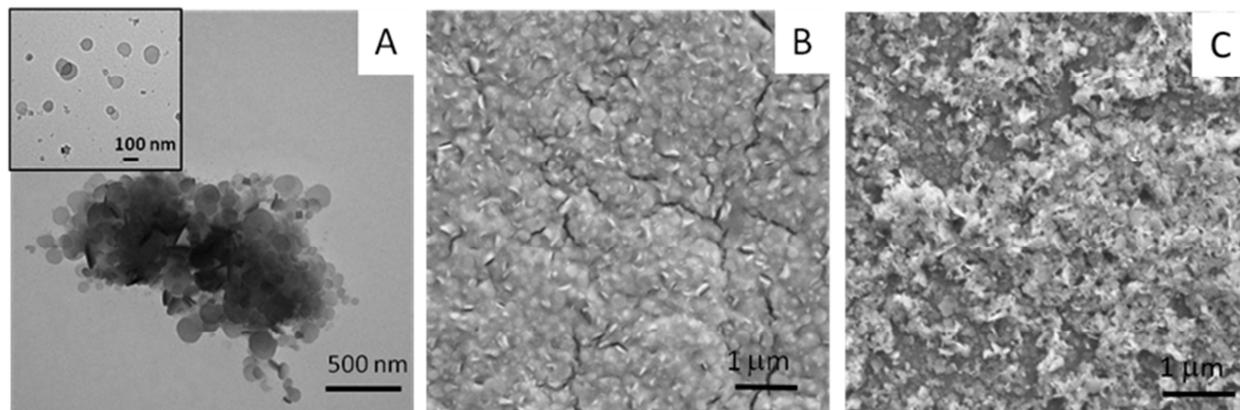


Figure 2: (A) TEM images of NfrA2@MgAl-NPs biohybrid and in the inset initial MgAl-NPs and SEM images of (B) the biomembrane NfrA2@MgAl-NPs/GCE and (C) the membrane MgAl-NPs/GCE.

NfrA2 belongs to the group of oxygen-insensitive (type I) nitroreductases using flavin mononucleotide (FMN) and NAD(P)H as cofactors. It reduces mesotrione according to a ping-pong Bi-Bi mechanism leading to the formation of hydroxylamino intermediate derivative and finally AMBA [10] (Fig.1). Those transformation products can be detected by electrochemistry. Unfortunately, electrochemical detection of AMBA cannot be taken into consideration since its oxidation potential (1.1 V/SCE) is more anodic than the oxidation potential of the NADH cofactor (0.7 V/SCE) [15, 18]. The kinetics parameters of NfrA2 for the reduction of mesotrione were determined in the presence of NADH and NADPH. In both cases, the Michaelis Menten constants (K_M) are high, 327 μM with NADPH and 68 μM with NADH and, a better catalytic efficiency ($k_{\text{cat}}/K_M = 0.37 \mu\text{M}^{-1} \text{min}^{-1}$) was obtained when NADH was used as the electron donor instead of NADPH ($k_{\text{cat}}/K_M = 0.07 \mu\text{M}^{-1} \text{min}^{-1}$) [10]. However, the specific activity of the

purified enzyme remained quite low (0.06 U mg^{-1}) for the reduction of mesotrione in the presence of NADH, as cofactor. Therefore, this data indicates that although NADH is an effective cofactor, mesotrione was converted with a very slow reaction rate which can be a limiting factor for the instantaneous detection of the enzymatic formed mesotrione metabolite, namely hydroxylamino intermediate, by electrochemistry. Indeed in a previous study [15], the amperometric response of NfrA2@MgAl-NPs bioelectrode in the presence of $200 \text{ }\mu\text{M}$ mesotrione and $400 \text{ }\mu\text{M}$ NADH was recorded by cyclic voltammetry. The current magnitude corresponding to the oxidation of the hydroxylamino intermediate, situated around 0.3 V/SCE , was very low ($0.8 \text{ }\mu\text{A cm}^{-2}$ at 10 mV s^{-1}) and prevented a sensitive detection of mesotrione. Under these conditions, the direct electrochemical detection of mesotrione at NfrA2@MgAl-NPs biosensor is thus impossible.

Interestingly, type I nitroreductases are able to reduce not only nitro-compounds but also a single electron acceptor, i.e. hexacyanoferrate [19-22]. For instance, NR from *Enterobacter cloacae* (EC 1.6.99.7) reduced $\text{Fe}(\text{CN})_6^{3-}$ with a K_M value of $80 \text{ }\mu\text{M}$ and an enzymatic rate constant ($k_{\text{cat}}/K_M = 3 \cdot 10^7 \text{ M}^{-1} \text{ min}^{-1}$) [22]. The enzymatic reaction rate was tenfold faster than with nitrobenzene ($k_{\text{cat}}/K_M = 1.8 \cdot 10^6 \text{ M}^{-1} \text{ min}^{-1}$) [23]. Similarly, Zenno *et al.* reported that NRs (NfrA1 and NfsA) have reductase specific activities towards $\text{Fe}(\text{CN})_6^{3-}$ ($\approx 200 \text{ U mg}^{-1}$) six or tenfold faster than nitro-compounds, such as methyl-nitrobenzoate ($20\text{-}30 \text{ U mg}^{-1}$) [20]. Therefore, we studied the NfrA2 enzymatic activity towards the reduction of hexacyanoferrate. We found, in this case, a specific activity of 2.68 U mg^{-1} and a K_M value of $90 \text{ }\mu\text{M}$. Moreover, hexacyanoferrate has been widely used as redox mediator in amperometric biosensing systems because of its reversible electrochemical behavior [24].

An original amperometric transduction process was therefore proposed based on the ability of NfrA2 to reduce $\text{Fe}(\text{CN})_6^{3-}$ (Fig.1). The voltammetric curves clearly showed a modified electrochemical signal for $\text{Fe}(\text{CN})_6^{3-}$ upon the electroenzymatic reaction (Fig.3). $\text{Fe}(\text{CN})_6^{3-}$ can be reversibly reduced at NfrA2@MgAl-NPs bioelectrode at a mean redox potential ($E_{1/2}$) of 0.1 V/SCE with a quasi-ideal current ratio ($I_{p_a}/I_{p_c} = 0.88$). The addition of NADH cofactor into the electrolyte solution caused an increase of the anodic peak with a concomitant decrease of the corresponding cathodic signal, leading to an I_{p_a}/I_{p_c} ratio of 5. Indeed, $\text{Fe}(\text{CN})_6^{3-}$ was mainly reduced by the immobilized NfrA2 and then $\text{Fe}(\text{CN})_6^{4-}$, released from the enzymatic reaction, was oxidized at the electrode surface (Fig.1). The addition of mesotrione into the same electrolyte solution led to a competitive enzymatic reaction which took place at the bioelectrode surface leading to a decrease of the electrocatalytic anodic current ($I_{p_a}/I_{p_c} = 2$) (Fig.3). This amperometric transduction concept is similar to the enzyme inhibition-based biosensors [25].

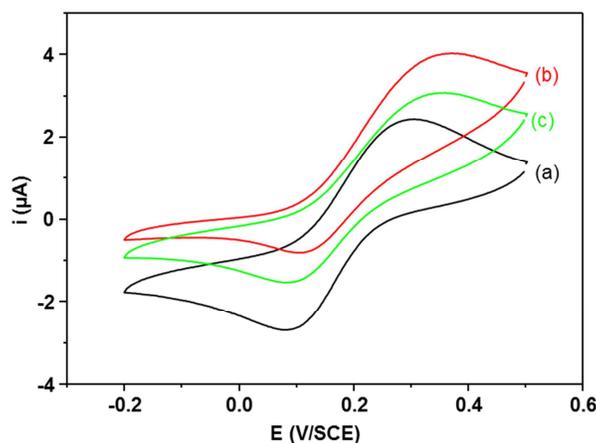


Figure 3: Cyclic voltammograms ($v = 10 \text{ mV s}^{-1}$) of NfrA2@MgAl-NPs/GCE (1:1) in 0.1 M PBS pH 6.5, containing $200 \mu\text{M Fe}(\text{CN})_6^{3-}$ before (a) and after additions of $400 \mu\text{M NADH}$ (b) and $200 \mu\text{M mesotrione}$ (c).

The NfrA2@MgAl-NPs bioelectrode was subsequently tested for indirect mesotrione determination. A steady state current was recorded in chronoamperometry at 375 mV/SCE using a rotating disk electrode. As shown in the inset of Fig.4, successive additions of mesotrione caused a decrease of the anodic current, with a response time of < 1 min. The mesotrione calibration curve was established in triplicate. A linear response, satisfying $Y = 0.00564 + 0.00361 X$ ($R^2 = 0.998$, $n = 7$), was obtained between 5 and 60 μM (Fig.4). The sensitivity, calculated from the slope of this equation, was $18.4 \text{ mA M}^{-1} \text{ cm}^{-2}$ and the detection limit (3σ) was 3 μM .

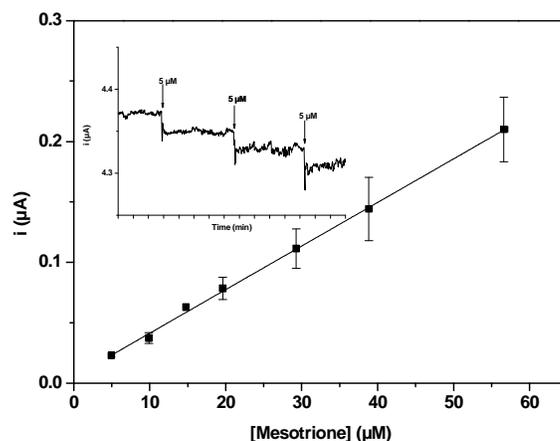


Figure 4: Calibration curve of mesotrione obtained with NfrA2@MgAl-NPs/GCE (1:1). Inset: Chrono-amperogram in 200 μM $\text{Fe}(\text{CN})_6^{3-}$ and 800 μM NADH adding 5 μM mesotrione. ($E_{app}=375 \text{ mV}$, 500 rpm, 20 mL 0.1 M PBS pH 6.5 at 25°C).

4. Conclusion

Recently, we have identified and purified a nitroreductase (NfrA2) able to reduce mesotrione. We took this opportunity to develop a new concept of biosensor to detect mesotrione. Specific interactions between NfrA2 and LDH-NPs allow the formation of homogeneous and reproducible biomembranes at the electrode surface by a simple adsorption process. An original amperometric

transduction process was proposed based on the competitive enzymatic reduction of $\text{Fe}(\text{CN})_6^{3-}$ and mesotrione, both substrates of NfrA2. The biosensor response shows a linear dependence on mesotrione concentration that was considerably enhanced compared to that recorded after the direct enzymatic reduction of mesotrione. We are convinced that this concept is of interest and could be applied to the development of biosensors based on various types of nitroreductases. Note that these results are preliminary and that further investigations would be necessary to understand the enzymatic reactions better and hence to improve the biosensor performance.

Acknowledgements

Authors acknowledge the Regional Auvergne Council, CNRS, the French Ministry for Higher Education and Research and the European Regional Development Fund for financial support. We would also like to express our gratitude to the Clermont Environmental Research Federation, UCA/CNRS FR 3467/INRA.

References

- [1] G. Mitchell, D.W. Bartlett, T.E.M. Fraser, T.R. Hawkers, D.C. Holt, J.K. Towson and R.A. Wichert, Mesotrione: a new selective herbicide for use in maize, *Pest. Manag. Sci.*, **57** (2001) 120-128.
- [2] E. Dumas, M. Giraud, E. Goujon, M. Halma, E. Khili, M. Stauffert, I. Batisson, P. Besse-Hoggan, J. Bohatier, P. Bouchard, H. Celle-Jeanton, M. Costa Gomes, F. Delbac, C. Forano, P. Goupil, N. Guix, P. Husson, G. Ledoigt, C. Mallet, C. Mousty, V. Prévot, C. Richard and S. Sarraute, Fate and ecotoxicological impact of new generation herbicides from the triketone family: An overview to assess the environmental risks, *J. Hazard. Mater.*, **325** (2017) 136-156.

- [3] C. Mastichiadis, I. Christofidis, M.A. Koupparis, C. Willetts and S.E. Kakabakos, Solid-phase fluoroimmunoassay for the determination of mesotrione - a novel triketone herbicide - in water with direct measurement of the fluorescence onto the solid support, *Analyst*, 128 (2003) 404-410.
- [4] C. Mastichiadis, S.E. Kakabakos, I. Christofidis, M.A. Koupparis, C. Willetts and K. Misiakos, Simultaneous Determination of Pesticides Using a Four-Band Disposable Optical Capillary Immunosensor, *Anal. Chem.*, 74 (2002) 6064-6072.
- [5] E. Rocaboy-Faquet, T. Noguier, S. Romdhane, C. Bertrand, F.E. Dayan and L. Barthelmebs, Novel bacterial bioassay for a high-throughput screening of 4-hydroxyphenylpyruvate dioxygenase inhibitors, *Appl. Microbiol. Biotechnol.*, 98 (2014) 7243-7252.
- [6] G. Erdoğan and S. Titretir, Voltammetric determination of mesotrione at hanging mercury drop electrode, *J. Anal. Chem.*, 62 (2007) 777-780.
- [7] H. Barchanska, P. Markowski and M. Strzebin, Electrochemical determination of mesotrione and its degradation products on glassy carbon electrode, *Int. J. Environ. Anal. Chem.*, 98 (2018) 493-505.
- [8] J. Kanga Wagheu, C. Forano, P. Besse-Hoggan, I.K. Tonle, E. Ngameni and C. Mousty, Electrochemical determination of mesotrione at organoclay modified glassy carbon electrodes, *Talanta*, 103 (2013) 337-343.
- [9] P.B. Deroco, B.C. Lourencao and O. Fatibello-Filho, The use of modified electrode with carbon black as sensor to the electrochemical studies and voltammetric determination of pesticide mesotrione, *Microchem. J.*, 133 (2017) 188-194.
- [10] L. Carles, P. Besse-Hoggan, M. Joly, A. Vigouroux, S. Moréra and I. Batisson, Functional and structural characterization of two *Bacillus megaterium* nitroreductases biotransforming the herbicide mesotrione, *Biochem. J.*, 473 (2016) 1443-1453.

- [11] Z. Naal, J.H. Park, S. Bernhard, J.P. Shapleigh, C.A. Batt and H.D. Abruna, Amperometric TNT Biosensor Based on the Oriented Immobilization of a Nitroreductase Maltose Binding Protein Fusion, *Anal. Chem.*, 74 (2002) 140-148.
- [12] C.D. Gwenin, M. Kalaji, P.A. Williams and R.M. Jones, The orientationally controlled assembly of genetically modified enzymes in an amperometric biosensor, *Biosens. Bioelectron.*, 22 (2007) 2869-2875.
- [13] C.D. Gwenin, M. Kalaji, C.M. Kay, P.A. Williams and D.N. Tito, An *in situ* amperometric biosensor for the detection of vapors from explosive compounds, *Analyst* 133 (2008) 621-625.
- [14] J.S. Caygill, S.D. Collyer, J.L. Holmes, F. Davis and S.P.J. Higson, Disposable screen-printed sensors for the electrochemical detection of TNT and DNT, *Analyst*, 138 (2013) 346-352.
- [15] F. Bruna, C. Mousty, P. Besse-Hoggan, I. Batisson and V. Prevot, Assembly of nitroreductase and layered double hydroxides toward functional biohybrid materials, *J. Colloid Interface Sci.*, 533 (2019) 71-81.
- [16] C. Mousty and V. Prevot, Hybrid and biohybrid layered double hydroxides for electrochemical analysis, *Anal. Bioanal. Chem.*, 405 (2013) 3513-3523.
- [17] M. Halma, B. Doumèche, L. Hecquet, V. Prévot, C. Mousty and F. Charmantray, Thiamine biosensor based on oxidative trapping of enzyme-substrate intermediate, *Biosens. Bioelectron.*, 87 (2017) 850-857.
- [18] A. Radoi and D. Compagnone, Recent advances in NADH electrochemical sensing design, *Bioelectrochem.*, 76 (2009) 126-134.
- [19] S. Zenno, H. Koike, A.N. Kumar, R. Jayaraman, M. Tanokura and K. Saigo, Biochemical characterization of NfsA, the *Escherichia coli* major nitroreductase exhibiting a high amino acid sequence homology to Frp, a *Vibrio harveyi* flavin oxidoreductase. , *J. Bacteriol.*, 178 (1996) 4508-4514.

- [20] S. Zenno, T. Kobori, M. Tanokura and K. Saigo, Purification and Characterization of NfrA1, a *Bacillus subtilis* Nitro/flavin Reductase Capable of Interacting with the Bacterial Luciferase, *Biosci., Biotechnol., Biochem.*, 62 (1998) 1978-1987.
- [21] J. Rau and A. Stolz, Oxygen-Insensitive Nitroreductases NfsA and NfsB of *Escherichia coli* Function under Anaerobic Conditions as Lawsone-Dependent Azo Reductases, *Appl. Environ. Microbiol.*, 69 (2003) 3448-3455.
- [22] H. Nivinskas, S. Staskeviciene, J. Sarlauskas, R.L. Koder, A.F. Miller and N. Čenas, Two-electron reduction of quinones by *Enterobacter cloacae* NAD(P)H:nitroreductase: quantitative structure-activity relationships, *Arch. Biochem. Biophys.*, 403 (2002) 249-258.
- [23] H. Nivinskas, R.L. Koder, Ž. Anusevičius, J. Šarlauskas, A.-F. Miller and N. Čenas, Quantitative Structure–Activity Relationships in Two-Electron Reduction of Nitroaromatic Compounds by *Enterobacter cloacae* NAD(P)H:Nitroreductase, *Arch. Biochem. Biophys.*, 385 (2001) 170-178.
- [24] A. Chaubey and B.D. Malhotra, Mediated biosensors, *Biosens. Bioelectron.*, 17 (2002) 441-456.
- [25] A. Amine, F. Arduini, D. Moscone and G. Palleschi, Recent advances in biosensors based on enzyme inhibition, *Biosens. Bioelectron.*, 76 (2016) 180-194.