engineering proceedings

MDPI

Proceedings

Development of a Portable Electrochemical Platform with Chip-Integrated Gold Electrodes for Detection of Pharmaceutical Pollutants.

Miguel Tavares[1], Álvaro Torrinha[1], Raquel Queirós[2], João Piteira[2], Cristina Delerue- Matos[1] and Simone Morais[1]*

[1] REQUIMTE/LAQV, ISEP, Polytechnic of Porto, rua Dr. António Bernardino de Almeida, 4249- 015 Porto, Portugal
[2] International Iberian Nanotechnology Laboratory, Avenida Mestre José Veiga, Braga, 4715-330, Portugal
* Correspondence: sbm@isep.ipp.pt (S.M.)

Abstract: Electrochemical portable sensing systems can offer a viable solution in the analysis of environmental contaminants due to the down-size capability of the electronic components and, overall, to the simplicity of the detection principles. In the present work, a new electrochemical portable platform (EPP) with miniaturized chip-integrated gold electrodes was developed and applied for the determination of the drug acetaminophen (APAP) as a model analyte. The produced miniaturized chip-integrated gold electrodes were firstly characterized via atomic force and scanning electron microscopy, integrated in the EPP and subsequently the complete set-up was tested for electrochemical detection of APAP. The results showed adequate performance of the developed EPP when compared to a traditional electrochemical system under optimal conditions (pH 8, deposition potential 0.1 V, deposition time 240 s and scan rate of 50 $mV.s^{-1}$), with a sensitivity of 1.6 $\mu A.mM^{-1}$ and limit of detection of 67 µM. The EPP was validated in river and wastewater samples, achieving recoveries ranging from 93.0 to 96.6%.

Keywords: contaminants of emerging concern; pharmaceuticals; electrochemical portable platform; electroanalysis

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Lastname

Published: date

1. Introduction

The development of small, versatile, and portable analytical systems is of uttermost importance for the analytical and environmental sciences fields in order to accomplish simple, cheap and on-site determinations. Electrochemical systems can offer a viable solution in this matter due to the down-size capability of the electronic components that compose the required equipment (potentiostat) and, overall, to the simplicity of the detection principles [1, 2]. Moreover, nanotechnology has been revolutionizing the development of sensors by allowing a wide diversification in the design of the transducers as well as their miniaturization capacity and integration in small devices. Besides the improvement in versatility, a miniaturization approach translates also in lower environmental impacts through reduction of the use of materials and reagents and decrease in the generated wastes, thus sensors are becoming interesting alternatives to more traditional analytical techniques [1]. The main objective of the present work was the development and characterization of new miniaturized chip-integrated gold (ECCs) electrodes applied on a portable platform (EPP) for the determination of the drug acetaminophen (APAP). APAP was chosen as a model analyte for the validation since it is one of common prescribed drugs being frequently detected in the environment [3, 4]. As known, pharmaceutical compounds are an important class of contaminants of emerging concern due to their potential persistence, bioaccumulative and toxic nature, causing negative effects on ecosystems and consequently to human health [5]. Although several sensors

have been described for APAP, gold-based transducers have been limitedly developed for this drug and usually consist in the bulkier and independent three-electrode configuration system with a conventional electrochemical equipment [6-9].

2. Materials and Methods

2.1. Reagents and solutions

Analytical grade chemicals were used without further purification. Britton-Robinson buffer (BRB, 0.1 M) was prepared using ortho-phosphoric acid (Sigma-Aldrich, Steinheim, Germany), boric acid, potassium chloride (VWR, Leuven, Belgium), sodium hydroxide (Labkem, Barcelona, Spain) and acetic acid glacial (Carlo Erba Reagents, Val-de-Reuil, France). Stock solution of 50 mM acetaminophen (Sigma-Aldrich, Steinheim, Germany) was prepared in BRB. 5 mM $[Fe(CN)6]^{3-/4-}$ ((Sigma-Aldrich, Steinheim, Germany) solution was prepared in 0.01 M phosphate buffer saline pH 7.4 using disodium hydrogen phosphate and sodium dihydrogen phosphate (Sigma-Aldrich, Steinheim, Germany). Piranha solution was prepared with a 3:1 (v/v) proportion of sulfuric acid 95% and hydrogen peroxide 130 vol. (Sigma-Aldrich, Steinheim, Germany). All aqueous solutions and electrolytes were prepared with ultrapure water obtained from a Milliporewater purification system (18 MΩ, Milli-Q, Millipore, Molsheim, France).

2.2. Fabrication and characterization of chip-integrated gold electrodes

2.2.1. Design and development of electrochemical cell-chips

The ECCs consist of an array of four independent electrochemical cells (ECs) with each cell composed of a 1000 μm diameter working electrode (WE), a pseudo-reference gold electrode (PRGE) and an auxiliary gold electrode, allowing multiplex and independent detection with statistical significance (Figure 1).

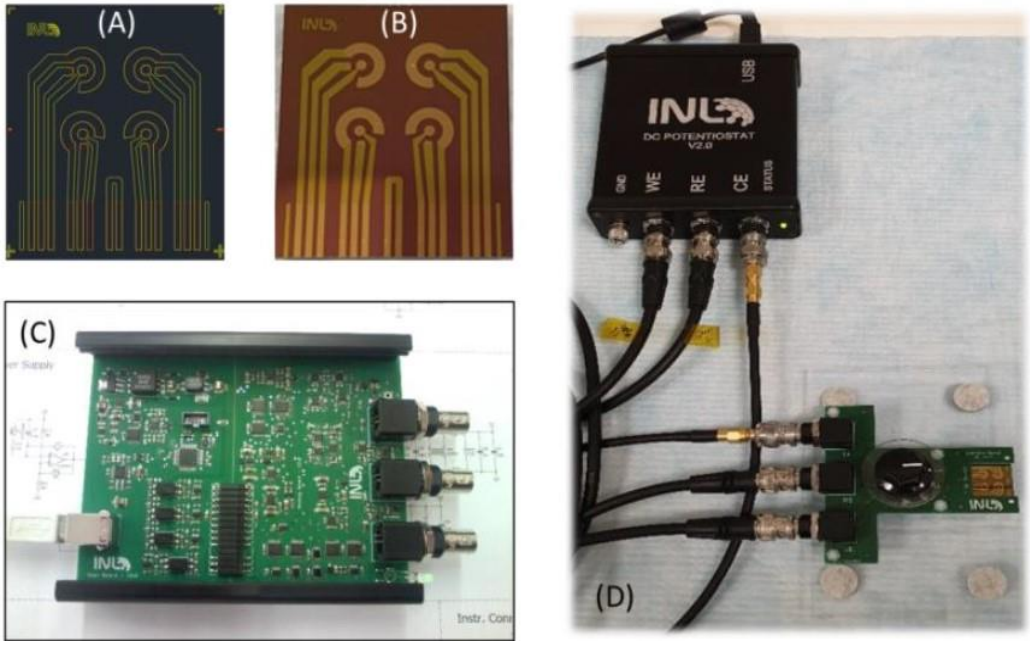

Figure 1. Electrochemical-Cell-Chips design (A) and fabricated (B) using microfabrication techniques; custom-made electrochemical portable platform (C) and complete set-up (D).

The ECCs were designed using AUTOCAD 2020 and produced using standard microfabrication technologies, onto 200 mm diameter silicon wafers (crystalline silicon coated with 100 nm thermal oxide from Si-Mat, Germany). The devices were fabricated on a thin layer of gold (100 nm) deposited by physical vapor deposition using a mul-

ti-target confocal sputtering tool (Kenosystec, Italy). The photolithography was performed by direct write laser lithography (Heidelberg DWL 2000, Germany) followed by ion milling etching process (Nordiko 7500, UK). An insulator Al_2O_3 layer of 200 nm was deposited by magnetron sputtering (Singulus Timaris FTM, Germany) on top of the devices. A second step of photolithography and etching defined the active area and the contacts [10]. In the end, the silicon wafer was diced to obtain 21 mm by 24 mm silicon chips.

2.2.2. Characterization of the surface morphology

The surface morphology of the ECCs surface was analyzed by a commercial atomic force microscope (AFM) Dimension Icon, Bruker. The AFM measurements were performed in air using a standard tapping mode AFM probe (Nanosensors, spring constant of 42 $N.m^{-1}$, resonance of frequency of 240 kHz and nominal tip radius of 7 nm). The linear scanning rate was set as 0.7 Hz with scan resolution of 512 samples per line. The surface morphology of the electrode was also evaluated by scanning electron microscopy (SEM) using a FEI Quanta 650 FEG (FEI Europe B.V.), operated under high vacuum. The electron acceleration voltage used was 10 kV and a spot size of 5.0. Secondary electrons were detected by an Everhardt Thornley detector. The ECCs were characterized with cyclic voltammetry (CV) by a commercial potentiostat (Metrohm Autolab PGSTAT302N) and the developed EPP. Data acquisition and analysis were accomplished using NOVA software (version 2.0.2, Metrohm Autolab) and Electrolab software interface (developed by INL).

2.3. Electrochemical characterization and determination of acetaminophen

The ECCs were first pre-treated both chemically and electrochemically before their use to remove the photoresist film and to activate the electrodes. The photoresist layer is removed by washing ECCs sequentially with acetone and isopropanol (Sigma-Aldrich, Steinheim, Germany), followed by 60 min sonication in acetone. Next, ECCs are rinsed with acetone, isopropanol and lastly with water, and subsequently dried with a N_2 stream. Then, one drop of piranha solution was applied to each sensor of the ECCs and rinsed with water after 5 min. Electrochemical pre-treatment was then employed, through application of CV in 0.1 M sulfuric acid (20 µL covering the three electrodes) from -0.4 to 0.8 V at 100 $mV.s^{-1}$ for 15 scans. The ECCs were finally rinsed, N_2-dried and were ready to be used. An initial characterization of the ECCs was first made in $[Fe(CN)_6]^{3-/4-}$ solution using CV at 50 $mV.s^{-1}$. The calibration curve of APAP was obtained by CV at increasing concentrations of the analyte after a pre-concentration step performed at 0.1 V for 240 s.

The ECCs were validated in river (collected from Lis river, Portugal) and wastewater (collected from a wastewater treatment plant in Gaia city, North of Portugal) samples. Each sample was diluted 1:1 (v/v) with 0.2 M BRB pH 8 and 20 µL was dropped in one of the sensors composing the ECC. Then, CV was applied in the optimum conditions for APAP screening. In the absence of signal, the samples were spiked with known concentrations of APAP stock solution (6 mM for wastewater; 0.3 and 0.6 mM for river water).

3. Results and Discussion

3.1. Characterization of the the ECCs surface by AFM, SEM, and CV

The morphology of the ECCs were characterized by AFM (Figure 2A) and SEM (Figure 2B). The gold electrode surface in Figure 2A showed a very regular and homogeneous surface with an average roughness of 0.92 ± 0.02 nm. The black spots in Figure 2B suggest the presence of photoresist residues from the microfabrication process.

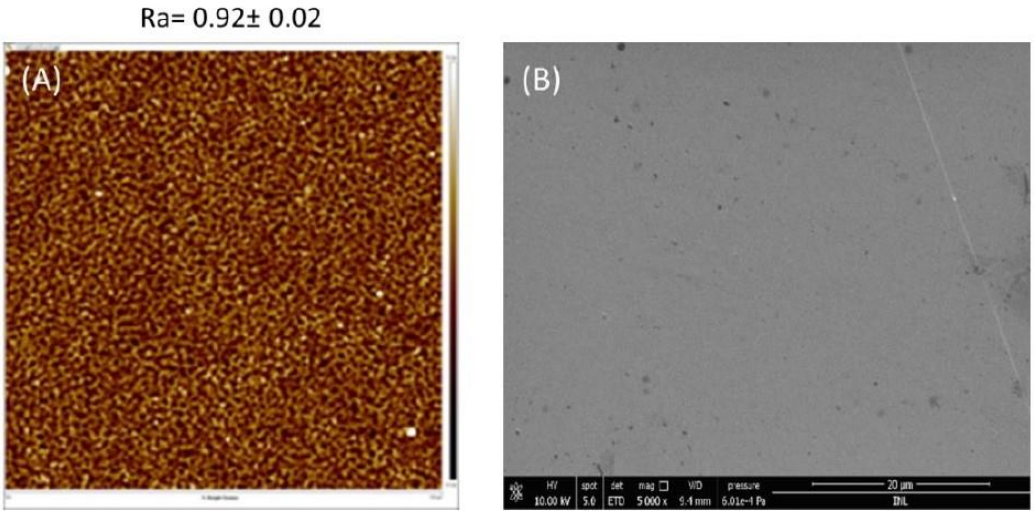

Figure 2. AFM image (A) and respective value of roughness (Ra) for the bare electrode and corresponding SEM image (B) of the WE surface, scale bar 20 μm. The roughness average (Ra, nm) is expressed as the average ± standard deviation (n=3).

Typical cyclic voltammograms of the ECCs obtained in a redox indicator with the commercial electrochemical platform and with the developed EPP are represented in Figure 3. The typical electrochemical behavior of an Au micro-fabricated electrode in $[Fe(CN)_6]^{3-/4-}$ can be seen in both Figure 3A and 3B thus confirming that the fabrication process was successfully performed. The voltammograms curves also show the similar signals obtained by the two systems. A slightly peak deformation can be seen for the reduction peak in Figure 3B, which may be due the solution decay. The results also confirm the adequate performance of the developed EPP, which was further used for the subsequent assays.

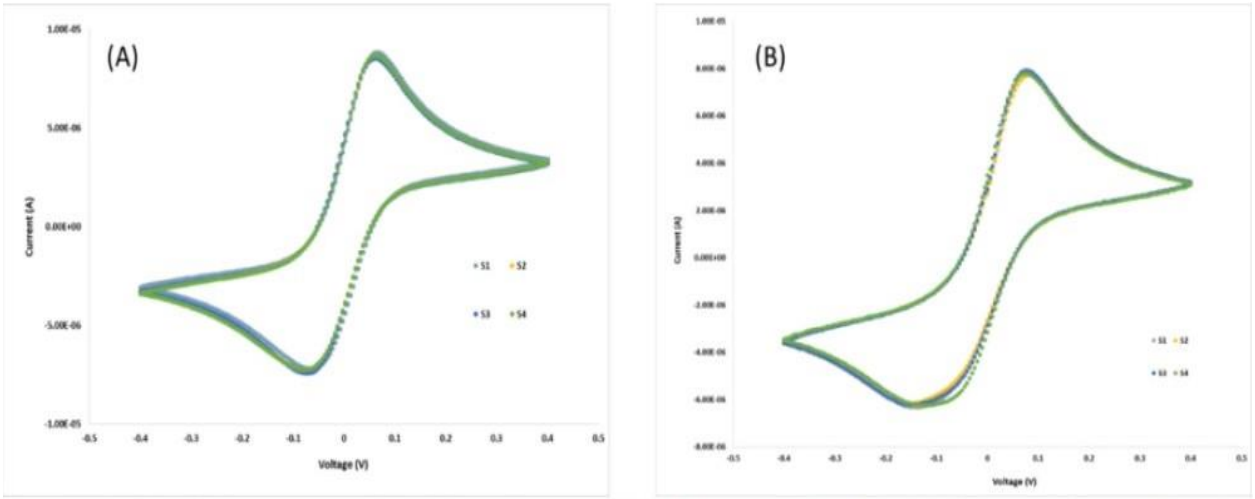

Figure 3. Cyclic voltammograms of the different sensors of an ECC acquired in AUTOLAB (A) and in the developed EPP (B) in 5 mM $[Fe(CN)_6]^{3-/4-}$ (PBS 10 mM, pH 7.4) at 50 $mV.s^{-1}$ using 5 mV voltage step.

3.2. Optimization of acetaminophen electroanalysis by the ECCs with the EPP

The APAP voltammetric behavior was firstly assessed at pH 7 based on the optimum pH identified in several works [11-13], where a well-resolved oxidation peak appeared at around +0.45 V (vs PRGE). However, accordingly to the literature, the optimum pH can vary greatly from acidic [14] to basic pH [15]. Our own pH investigations (Figure 4) indicated a maximum oxidation peak at pH 8 (Figure 4B). Also, APAP oxidation peak

shifts towards less positive potentials presenting a slope of -30 mV/pH (Figure 4C). An electrolyte pH 8 was selected as the optimum and used in further studies.

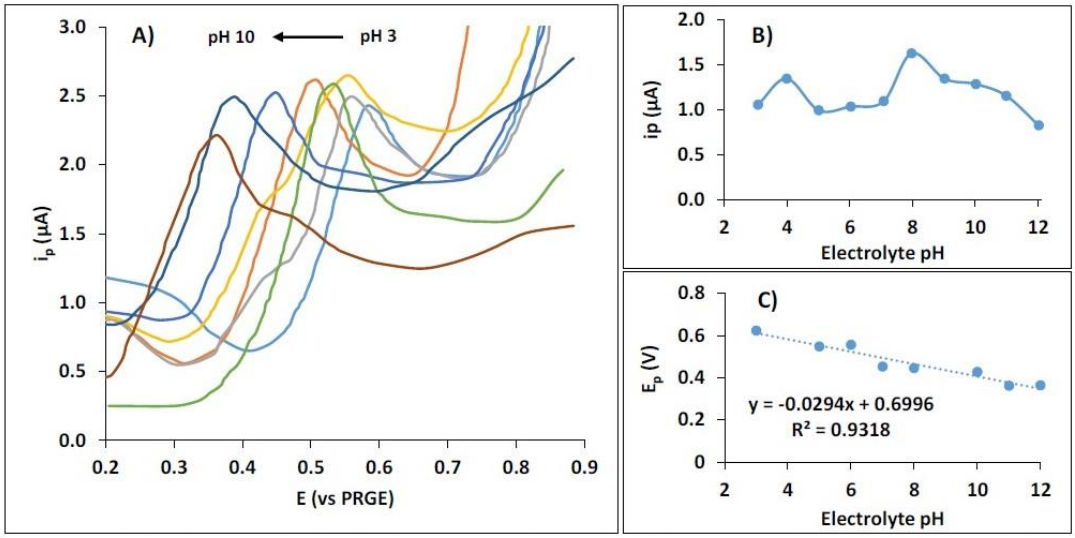

Figure 4. Optimization of electrolyte (0.1 M BRB) pH for 1 mM APAP. (A) CV forward scan at 50 mV.s^{-1}. (B) Peak intensity as a function of electrolyte pH. (C) Peak potential as a function of electrolyte pH.

The deposition potential and time were also studied and optimized to increase the APAP signal. It is possible to observe in Figure 5A that peak intensity reaches a maximum for an applied potential of 0.1 V (30 s) being thus selected as optimum value. The deposition time (Figure 5B) was varied from 0 to 360 s and performed at 0.1 V, revealed a maximum peak at 240 s being then chosen henceforth.

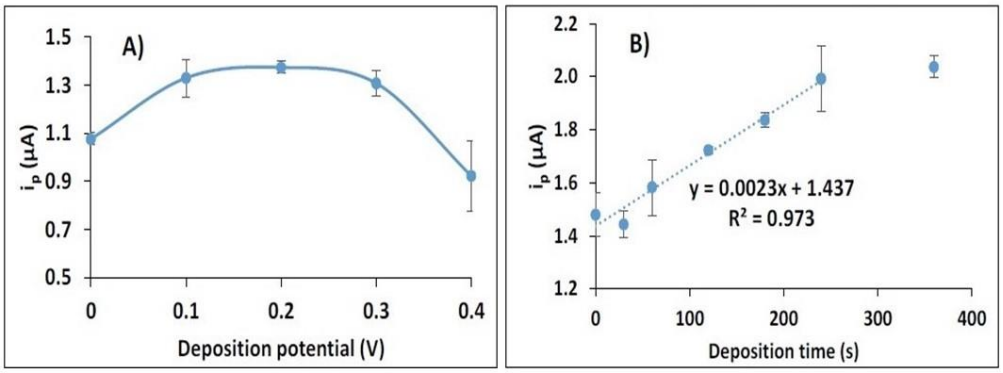

Figure 5. Optimization of APAP (1 mM in 0.1 M BRB pH 8) deposition. (A) Peak intensity as function of the deposition potential (applied for 30 s). (B) Peak intensity as a function of deposition time for an applied deposition potential of 0.1 V.

Under the optimum conditions obtained above, the ECCs were then applied for determination of APAP at increasing concentrations from 0.1 to 2 mM (Figure 6). The linear range of this sensor varied from 0.1 to 1.5 mM and the sensitivity retrieved from the calibration curve (Figure 6B) corresponds to 1.6 µA.mM^{-1} (203.8 µA.mM^{-1}.cm^{-2} based on the geometrical area of the working electrode of the sensor). The limit of detection (LOD) was calculated based on the standard deviation of the response and the slope of the calibration curve (LOD = 3.3$\sigma_{\text{residual-CC}}$/slope) [16], being 67 µM. This value is higher compared with other literature works that employed miniaturized systems with three integrated electrodes for detection of APAP, such as those based on screen-printed [17-19] or paper-based technology [20, 21]. Possible explanations for lower analytical performance of the EPP could be related with a very low geometrical area (0.0079 cm^2) of the WE in the

ECCs and the use of a lower sensitive technique as is the case of CV. Accordingly to the work of Fanjul-Bolado et al. [22], a comparison of electrochemical techniques used for APAP detection demonstrated a 5 times higher sensitivity for square wave voltammetry compared to CV. However, this technique was not available in the developed EPP.

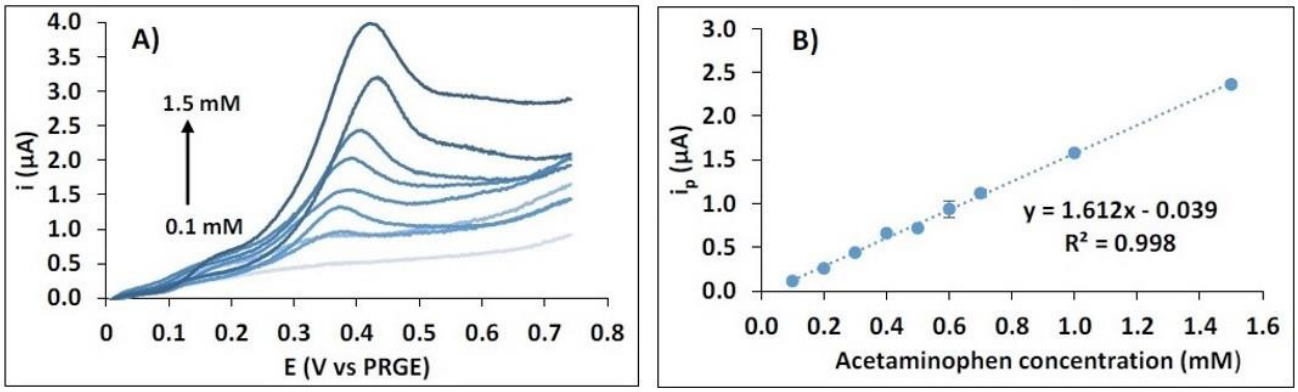

Figure 6. Analysis of APAP with the EPP. A) CV forward scan for various concentrations of APAP (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 1 and 1.5 mM) recorded at 50 $mV.s^{-1}$ in 0.1 M BRB pH 8, deposition time 240 s, deposition potential 0.1 V. B) Respective calibration curve.

The EPP was finally applied for the analysis of APAP in river water and wastewater samples. Since no signal was obtained when the samples were directly analyzed, spiking was performed at two levels 0.3 and 0.6 mM. The recoveries achieved are acceptable, varying from 93.0 % for river water to 96.6% for wastewater (Table 1).

Table 1. Analysis of spiked water samples with acetaminophen (n=3).

Sample	Spiking level (mM)	Recovery (%)	RSD (%)
Wastewater	0.6	96.6	4.1
River water	0.6	93.0	3.6
	0.3	93.6	9.4

4. Conclusions

The developed EPP with chip-integrated gold electrodes revealed adequate performance to be applied in the future for the detection of pharmaceutical pollutants, although some improvements (e.g., type of electrochemical techniques available) could be made to enhance sensitivity and operation of this new electrochemical platform. The development of portable and miniaturized electrochemical platforms may have a positive impact on the environmental field analysis not only by their versatility but also by their sustainable operation (e.g. reduced sample volume needed 20 µL) when compared with traditional analytical techniques.

Funding: The authors are grateful for the financial support from the project NATURIST 2022.07089.PTDC, funded by the National Funds through Portuguese FCT—Foundation for Science and Technology, Ministério da Ciência, Tecnologia e Ensino Superior (MCTES).
Acknowledgments: This work received support from UIDB/50006/2020, UIDP/50006/2020, and LA/P/0008/2020, from the FCT/MCTES. Miguel Tavares is grateful to FCT for his PhD grant (2022.13624.BD). Álvaro Torrinha thank FCT for funding through the Scientific Employment Stimulus-Individual Call (ref. 2022.04357.CEECIND).

References:

1. Kalambate, P.K., et al., *Electrochemical (bio) sensors go green.* Biosens Bioelectron, 2020. **163**: p. 112270.

2. Torrinha, Á. and S. Morais, *Electrochemical (bio)sensors based on carbon cloth and carbon paper: An overview.* TrAC Trends in Analytical Chemistry, 2021. **142**: p. 116324.
3. Verlicchi, P., M. Al Aukidy, and E. Zambello, *Occurrence of pharmaceutical compounds in urban wastewater: removal, mass load and environmental risk after a secondary treatment--a review.* Sci Total Environ, 2012. **429**: p. 123-55.
4. Koagouw, W., et al., *High concentrations of paracetamol in effluent dominated waters of Jakarta Bay, Indonesia.* Marine Pollution Bulletin, 2021. **169**: p. 112558.
5. Ebele, A.J., M. Abou-Elwafa Abdallah, and S. Harrad, *Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment.* Emerging Contaminants, 2017. **3**(1): p. 1-16.
6. Gholivand, M.B. and M. Amiri, *Simultaneous detection of dopamine and acetaminophen by modified gold electrode with polypyrrole/aszophloxine film.* Journal of Electroanalytical Chemistry, 2012. **676**: p. 53-59.
7. Jia, L., et al., *Determination of acetaminophen by square wave voltammetry at a gold electrode modified by 4-amino-2-mercaptopyrimidine self-assembled monolayers.* Journal of Analytical Chemistry, 2007. **62**(3): p. 266-269.
8. Menon, S., S. Jesny, and K. Girish Kumar, *A voltammetric sensor for acetaminophen based on electropolymerized-molecularly imprinted poly(o-aminophenol) modified gold electrode.* Talanta, 2018. **179**: p. 668-675.
9. Menon, S. and K.G. Kumar, *Simultaneous Voltammetric Determination of Acetaminophen and Its Fatal Counterpart Nimesulide by Gold Nano/L-Cysteine Modified Gold Electrode.* Journal of The Electrochemical Society, 2017. **164**(9): p. B482.
10. dos Santos, P.L., et al., *Enhanced performance of 3D printed graphene electrodes after electrochemical pre-treatment: Role of exposed graphene sheets.* Sensors and Actuators B: Chemical, 2019. **281**: p. 837-848.
11. Akhter, S., et al., *Enhanced amperometric detection of paracetamol by immobilized cobalt ion on functionalized MWCNTs - Chitosan thin film.* Anal Biochem, 2018. **551**: p. 29-36.
12. Afkhami, A., et al., *Preparation of $NiFe_2O_4$/graphene nanocomposite and its application as a modifier for the fabrication of an electrochemical sensor for the simultaneous determination of tramadol and acetaminophen.* Anal Chim Acta, 2014. **831**: p. 50-9.
13. Tadayon, F., S. Vahed, and H. Bagheri, *Au-Pd/reduced graphene oxide composite as a new sensing layer for electrochemical determination of ascorbic acid, acetaminophen and tyrosine.* Mater Sci Eng C Mater Biol Appl, 2016. **68**: p. 805-813.
14. Mulyasuryani, A., R.T. Tjahjanto, and R.a. Andawiyah, *Simultaneous Voltammetric Detection of Acetaminophen and Caffeine Base on Cassava Starch—Fe3O4 Nanoparticles Modified Glassy Carbon Electrode.* Chemosensors, 2019. **7**(4): p. 49.
15. Kang, X., et al., *A graphene-based electrochemical sensor for sensitive detection of paracetamol.* Talanta, 2010. **81**(3): p. 754-9.
16. Miller;, J.M.J., *Statistics and Chemometrics for Analytical Chemistry*. Sixth ed. 2010: Pearson Education Limited.
17. Serrano, N., et al., *Commercial Screen-Printed Electrodes Based on Carbon Nanomaterials for a Fast and Cost-Effective Voltammetric Determination of Paracetamol, Ibuprofen and Caffeine in Water Samples.* Sensors, 2019. **19**(18): p. 4039.
18. Deroco, P.B., et al., *Effect of Different Carbon Blacks on the Simultaneous Electroanalysis of Drugs as Water Contaminants Based on Screen-printed Sensors.* Electroanalysis, 2019. **31**(11): p. 2145-2154.
19. Saciloto, T.R., P. Cervini, and E.T.G. Cavalheiro, *Simultaneous voltammetric determination of acetaminophen and caffeine at a graphite and polyurethane screen-printed composite electrode.* Journal of the Brazilian Chemical Society, 2013. **24**(9): p. 1461-1468.
20. Camargo, J.R., et al., *Waterproof paper as a new substrate to construct a disposable sensor for the electrochemical determination of paracetamol and melatonin.* Talanta, 2020. **208**: p. 120458.
21. Lee, S.H., et al., *Determination of acetaminophen using functional paper-based electrochemical devices.* Sensors and Actuators B: Chemical, 2016. **232**: p. 514-522.
22. Fanjul-Bolado, P., et al., *Electrochemical study and flow injection analysis of paracetamol in pharmaceutical formulations based on screen-printed electrodes and carbon nanotubes.* Analytica Chimica Acta, 2009. **638**(2): p. 133-138.