Reviewer response

Reviewer: M. Teresa Fernández Abedul, University of Oviedo PhD

Candidate: Juan Santiago Hidalgo Viteri, University of Pannonia PhD

Dissertation Title: Electrochemical techniques of investigation – a tool for different applications

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1. **The inclusion of exhaustive protocols must be only made when required for experimental tasks but not for a PhD Dissertation, especially when they are not going to be used or are far from the topic. In some cases, the level of detail is excessive, including reagent suppliers or instrumentation models reported in the literature. While techniques and methodologies are discussed, some electrodes (e.g., boron-doped diamond electrode), or techniques (e.g., linear polarization or open-circuit potential measurements, as well as specific plots such as Nyquist plots in electrochemical impedance spectroscopy), are not included in the experimental part.**

Thanks for the comment. I decided to include detailed information in the case of the first chapter (Drug Delivery System (DSS)) because at the time that we prepared the experimental part, there was not any information related to the use of electrochemical impedance spectroscopy as an analytical technique to study the drug release profiles. Therefore, it was necessary to contemplate all the possible alternatives. For example, the composites that can form the DSS, the model drugs, the kinetics of the delivery, and the mathematical models applied study the delivery. Moreover, we agree that boron-doped diamond electrodes are not discussed during the literature review, but I encountered that the use of this electrode was not a central part in our research, and we only use them as a method to probe our technology (the use of electrochemical impedance spectroscopy (EIS) as an alternative method for analyzing paracetamol (PA) released profile). Thus, it was not necessary to extend the information regarding this. Regarding the inclusion of other electrochemical techniques such as linear polarization. I included it in the previous version of the thesis, but due to the extension of the literature review, I decided not to include it in the last version. Anyways, the experimental part, especially in the case of corrosion studies (Chapter 3.2), explained their use and the principal information that we obtained from them. Overall, I improved the literature review part, and I try to emphasize

the changes relating to the most important information which served during the experimental part.

2. **The extent of repetition is notable in the introduction and literature review. Also, electrochemistry could have been used as a unifying theme, connecting the different applications. Thus, comparison of electrochemical cells and processes could have been made as well as the inclusion of the main trends.**

Thanks for the comment. I rewritten the introduction to state the principal chapters of the thesis dissertation and during the literature review, I extended and studied in deep the points highlighted during the introduction to have a proper perspective of the current state of art. I tried to minimize the repetition of the concepts anyways it is still some overlapping due to the extension of the chapters. I studied each electrochemical technique separate in each chapter, because as it is stated, the electrochemical techniques were used for different purposes (e.g. for DDS they were used to electropolymerized and release profile analyze, while for DS detection they were used to study the redox processes of the DS). Anyways, you are right, that a unifying theme can give another perspective of the electrochemical methods connected for different applications. Thus, the differences in electrochemical cells depend on the type of the produced technology, the purpose of the analysis and the material uses. Also, the inclusion of the main trends was stated in each subsection of the literature review. Thus, I included the principal DDS (see section 2.1.2.), the trends in corrosion protection by green inhibitors (2.2.4.2.1.), and the chemically modified electrodes to detect DS in different real samples (2.3.5.2.).

3. **The candidate comments in all the experimental sections of the Dissertation the general objective and the specific ones. However, as commented before, a junction is required, also in the objective. A well-defined general objective for the entire Dissertation, that could be later specified in each of the parts, as is done appropriately by the candidate would improve it.**

Thanks for the comment. You are right, a connection was not established as a general objective in the thesis. Anyways, I included the general objective in the thesis booklet which is going to be presented during the dissertation. I stated as general objective: “To investigate and demonstrate the versatility of electrochemical techniques as fundamental tools for the characterization, analysis, and evaluation of diverse systems and materials,

emphasizing their applicability across different scientific and technological fields, including corrosion studies, sensor development, and environmental monitoring". Therefore, this objective connected the chapter studied during the experimental part.

4. **Presentation of the scientific data and significant figures: The candidate includes the mean and the standard deviation, but the mean values are not rounded using the value of the standard deviation (e.g., mean = 378.00, SD = 30.37). It seems that the candidate uses always two decimals as criterion.**

Thanks for the comment. I used two decimals as criterion for all the reported data. The only exception that I made was the coefficient of correlation, and regression in which I used 4 decimals.

5. **Linearity: The correlation coefficient indicates if linearity exists and, in some cases, this is very low. The way the equations are represented should have been revised (e.g., 31 and subsequent).**

Thanks for the comments. I agree in the case of the pH studies and controlled process; the coefficient of correlation (R) lies between 0.9575 to 0.9875 which indicates a low linearity. I prepared at least 3 measurements for each experiment, but the tendency was almost constant. However, this could be explained by the addition of the biofilaments, whose electrochemical behavior is not well understood and is greatly influenced by the change in the pH of the medium. Thus, some points were not considered but the problem persisted. Anyway, when the calibration curves were built, the linearity in all cases reported R values higher than 0.9900. This indicates a proper response of the modified electrodes in the presence of sodium diclofenac salt. Overall, the electrode good analytical performance enables us to discuss that the proposed objectives were achieved, and the prepared device can be used for the monitoring of the DS in different environmental samples.

6. **Interpretation of raw data (e.g., CV in Figure 31. In A, there is a redox process. In B there are more than two processes in bioF and CNTs modified electrodes). The choice of the electrode modified with bioF is not justified. The more intense process is affected by the preceding one and the intensity of the peak current does**

not seem to be higher than these containing just MWCNTs. Therefore, the conclusion seems not sound.

Thanks for the comments. The following points can be discussed:(a) the redox mechanism is different when TiO_2NPs (Anatase and A.G.) and the f-MWCNTs are added. Thus, a two-step irreversible mechanism is reported in the case of TiO_2NPs (step a. formation of the radical, b. oxidation or product formation), as indicated by the only anodic peak reported. While, in the case of f-MWCNTs, it is reported that a multistep reversible mechanism, which involves the formation of one or two quasi-reversible or irreversible pairs of peaks (Ic and IIc), appears. This is confirmed by other authors [1, 2] when MWCNTs are used as modifiers. The use of the bioF was highlighted in the conclusions; anyways, the idea behind the bioF consisted in the modification of the biofilaments by genetic procedures (vector changes). So, the results show that (a) the bioF can resist harsh conditions of pH (in our case we used acid conditions, which for most of the biomolecules is not proper), (b) the genetic modification of the filament can be extended towards other analytes (such as metals, pharmaceutical compounds, pesticides, etc.), and (d) there is the possibility to miniaturize the technology and apply the modified electrodes in online measurements.

The intensity of the signal increases notably when f-MWCNTs are used, but if the rest of the electrochemical characteristics are considered, such as electrochemical active area, charge transfer resistances, or charge transfer rate, the best performance was reported by the TiO_2A.G+bioF+Chit/GCE. Then, in terms of limit of detection (LOD) and sensitivity, the mentioned electrode also reported higher characteristics compared to the other modified electrodes, and the fMWCNTs+Chit/GCE reports a considerably higher LOD and sensitivity. Thus, the synergetic effect between the bioF and the NPs allows for improved analytical performance of the electrodes, as the ANOVA demonstrates.

7. **Study design: Once optimization is made, calibration curves and real sample analysis should be made with the best modified electrode. Precision studies should be made at a concentration level that is inside the concentration range.**

Thanks for the comment. The optimization was made for each concentration of nanoparticles (TiO_2, TiO_2AG, and f-MWCNTs) and the bioF, so that is why we continued the experiments for the optimal concentration of NPs and bioF. Therefore, a future study should be made to compare the optimal electrode which most probably is the

TiO_2A.G+bioF+Chit/GCE. Precision studies (e.g. repeatability and reproducibility) were performed at 1.50 μM of DS in 0.025 M PBS which is inside in the concentration linear range. It was a mistake during the report of the data in the thesis, but the precise data is reported in the published articles [3].

8. **The candidate compares his results with bibliography, mostly in this electroanalytical section, as happens with the sensitivity and limit of detection. However, the calculation is not clear and in some cases is commented that the best methodology is the one with higher limit of detection. Additionally, a limit of detection of 17 nM with a linear range starting in 250 nM is not reasonable. Also, a LOD of 190 nM, with a much higher sensitivity requires explanation.**

The comparison between the prepared electrode and the ones reported in the literature is based on the LOD. Therefore, the Chit+TiO_2A.G+bioF/GCE reported the lowest limit of detection with respect to the other prepared electrodes (LOD = 17 nM) and lower than other electrodes reported in the literature, as is gathered in Table 17. This was demonstrated by the ANOVA studies in which we concluded that LOD is significantly dependent on the NPs ($p < 0.01$). Regarding the LOD calculation, the concentration linear range was established between 150 and 2500 nM and the LOQ, the lowest and smallest analyte signal that can be measured reliably and quantified with acceptable precision and accuracy against the instrumental noise background, is equal to 56.67 nM, which is three times lower than the first point. I agreed that it is not normal (because the LOQ should be inside the linear range), but there are examples in the literature that report the same tendency (https://doi.org/10.1016/j.jece.2025.117320). The authors explained that the lowest point was maybe omitted during the calibration curve. On the other hand, LOD of detection equal to 190 lies in the linear dynamic range (12% higher than the lowest point in the calibration curve), which is not suggested but it does not represent any problem in the determination. The sensitivity of this electrode was the highest but the error in the measurements was considerably higher than the other sensors. Thus, representing the not optimal performances of the Chit+f-MWCNTs/GCE is related to the not optimal electrochemical characteristics. This confirmed that the synergetic effect of the NPs and the bioF is crucial to increase the performance of the modified electrode.

9. **The candidate has written consciously the conclusions, partial and general, which is considered positively, although in some cases the number of pages resemble a discussion rather that a conclusion section.**

Thanks for the comment. I agreed with the referee. Anyway, the conclusions were extensive because of the bast number of numbers and in this way, it was avoided to omit some of the result and the explanation of it. However, the conclusions were synthesized and summarized in the thesis booklet which can be presented in the public defenses.

10. **Regarding the dissertation, the candidate has structured and organized the dissertation according to a numerical index, which, in general, is appropriate, but has some inconsistencies (e.g., indentation, or section levels as in e.g. 2.1.2.2 and 2.1.2.3, or 2.3 and 2.3.3, or 2.3 and 2.5 as well as 2.5 and 2.6). In which concerns the figures, the magnitude and units should have also been revised (e.g., current and peak current intensities are interchanged in some of them, as well as potential and peak potential). Bibliographic references should be revised and unified in format. Overall, the writing style is adequate, and the Dissertation reflects considerable effort.**

Thanks for the comment. The figures are revised and corrected to standardize. The bibliography was written following the APA sixth edition style by using Mendeley. However, the format is checked and standardized.

References

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[2] D. Minta, Z. González, G. Gryglewicz. (2024). Functionalized MWCNT-Au nanoparticles composite as sensing platform for the electrochemical detection of diclofenac at neutral pH. *Chem. Eng. Sci.* 292, 120009. doi: https://doi.org/10.1016/j.ces.2024.120009

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[4] C. Parat, E. Ricard, J. Lacoste, H. Pinaly, S. Le Faucheur, I. Le Hécho. (2025). Development of a flow analysis system based on an electrochemical sensor for high frequency on site monitoring of cobalt and nickel in a river water. *J. Environ. Eng.* 13, 117320. doi: https://doi.org/10.1016/j.jece.2025.117320