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Review of the doctoral dissertation by Karthika Kappalakanda Valapil

"ITO Microelectrodes and Microelectrode Arrays for the Analysis of Cell Cultures and Biomedical Applications"

M.Sc. Karthika Kappalakanda Valapil completed her Ph.D. dissertation, "ITO Microelectrodes and Microelectrode Arrays for the Analysis of Cell Cultures and Biomedical Applications," at the Institute of Physical Chemistry, supervised by Prof. dr. hab. Martin Jönsson-Niedziółka and Dr. inż. Emilia Witkowska-Nery.

The thesis of M.Sc. Valapil focuses on the development and application of microelectrodes and electrode arrays in biomedical and cell culture applications. These systems can be used in a variety of cellular experiments, including drug screening, cell signaling, and ion channel activity. Pharmaceutical and clinical applications may benefit from the application of impedance-based cellular biosensors. The fact that there is currently little research in this area in the literature shows how the PhD thesis presents a significant and up-to-date approach.

The dissertation is divided into six chapters and is 114 pages long with 232 references. The references provided are current and carefully chosen. The doctoral student has co-authored a total of 3 scientific publications in renowned journals with international recognition and high impact factors, with one publication directly related to the presented dissertation. Those publications have received a total of 30 citations to date, demonstrating the significance of the

research conducted. The thesis is organized in a logical and well-structured manner, with a brief introduction explaining the research at the beginning of each chapter and a summary at the end, which improves the readability. Furthermore, Chapter 6 provides a future outlook into potential applications of the author's developed solutions in the advancement of cellular sensors and biomedical applications. The doctoral candidate underscores the importance of additional research to enhance the efficacy of the recommended strategies and identifies potential areas for further investigation.

The first chapter of the PhD thesis provides an overview of the research topic and discusses the factors associated with electrode fabrication as well as the application of biosensors in cell culture analysis. The methods for measuring glucose and those employed in the PhD thesis are discussed as well in this chapter. The second chapter discusses the fabrication and assessment of conductive platinum and ITO substrates for application in biosensing. ITO microelectrodes and electrode arrays were fabricated using a laser-assisted technique. Several techniques were used to characterize the size and morphology of the substrates, including electrochemistry, EDX, and SECM. The results indicated that for biosensing applications, CO_2 laser-cut ITO microelectrodes and in situ-prepared microelectrode arrays were advantageous, and the author used them to analyze the properties of cell cultures in the following chapter. A circular electrode array was created and discovered to be a practical system to use for EIS-based cell culture characterization. It is worth noting that the author herself constructed microelectrodes and microelectrode arrays, evaluating their suitability for utilization in biological systems. In Chapter 3, the PhD candidate discusses the practical application of the electrodes she developed in cellular research, confirming their usefulness in this field. I consider this aspect of the research to be the most significant. The author effectively utilized electrode arrays to monitor the adhesive properties and proliferation of hepatocyte carcinoma (HepG2) cells. Impedance measurement used in the thesis allowed to monitor the cell properties. The method proposed has the potential to be useful for researching cellular processes in non-invasive ways, providing valuable insights into biological processes. In Chapter 4, the Author outlines the synthesis of novel mediators designed for glucose sensing in biological systems like cell cultures. The chapter describes the methodologies utilized in the synthesis of potential

glucose oxidation mediators, containing i) the construction of an osmium-based polymer characterized by the general formula cis-[(Os(N-N)2(PVI)n+1Cl]+(n = 9), and ii) the generation of Prussian Blue analogs represented by the general formula Ax[R(CN)6]1-y·wH2O. Furthermore, a novel technique for synthesizing Prussian Blue analogs involving the induction of the reaction by high-intensity light pulses, was introduced. Unfortunately, data supporting the application of these compounds for glucose determination—which is mentioned in the work's stated objective—are lacking. However, the research findings included in the dissertation are interesting and relevant. The work was completed according to high methodological standards, the analyses were performed correctly, and the results provided an extensive understanding of how electrochemical techniques can be utilized in cell culture research and biomedical applications. The obtained results are valuable and make a new contribution to the use of electrochemical methods for cellular research and biomedical applications. The presented discussion of the results obtained is substantively correct and accurate. The utilization of self-constructed electrode arrays and electrochemical techniques for the examination of cellular processes presents a promising alternative to *in vitro* and *in vivo* experiments for therapeutic validation, underscoring the significance of this work. The PhD candidate needed to synthesize new compounds to complete the tasks outlined in the doctoral thesis. The compounds, which included hydrogels containing osmium complexes and Prussian blue derivatives (using the Flash Light Sintering method), were synthesized. The PhD candidate characterized the resulting products using mass spectrometry, NMR spectroscopy, UV-vis spectrometry, and electrochemical techniques. The candidate also gained expertise in electrode array and microelectrode fabrication and characterization techniques (SEM, SECM, CV). Furthermore, the doctoral candidate needed to conduct research with cancer cells and determine whether the developed electrode substrates were suitable for cell adhesion and proliferation tests employing electrochemical impedance spectroscopy. This information demonstrates that the doctoral candidate meets the requirements for doctoral dissertations and possesses the ability for independent scientific research and general theoretical knowledge in the natural sciences, in the discipline of chemistry.

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I have not identified any significant issues with the approach or the results obtained. However, there are several questions and comments that I would like to address.

1. Page 54 - The author analyzes the phase angle and impedance vs. frequency but does not accompany the discussion with the corresponding graphs. At what frequencies were the greatest differences in EIS spectra without and in the presence of cancer cells.
2. Chapter 3: The method section outlines the preparation of cell cultures for both the HeLa and HepG2 lines, although the presentation of results is limited to the HepG2 line in certain sections.
3. Has there been any attempt to evaluate how anticancer drugs affect cells?
4. The methodology for measuring impedance in the multielectrode array lacks clarity regarding whether the measurement was conducted simultaneously on all electrodes or through a switched process. I couldn't find information about the size of the auxiliary and working electrode.
5. The differences in impedance measured with and without cells are described on page 55. In order to model the impedance for the system with and without cells, which equivalent circuit was used?
6. The duration for the cells to detach from the surface and lose viability is not specified. Was this measurement conducted? Additionally, I am wondering if it is possible to test cell viability within 72 hours.
7. Fig. 47 does not specify the scan rate used to observe the catalytic process.
8. The calibration curve depicted in Figure 46 does not seem to be linear, so what is the correlation coefficient? There are no error bars.

Conclusion

The thesis submitted by M.Sc. Valapil presented an original solution to an important research topic and met the formal requirements for a PhD thesis. M. Sc. Valapil has demonstrated theoretical knowledge in the field of research and has proven her ability to conduct scientific research and solve scientific problems. In my opinion, the presented dissertation of M. Sc. Karthika Kappalakanda Valapil meets the requirements of a doctoral dissertation. Therefore, I confirm that the doctoral dissertation submitted for review meets the conditions specified in the

art. 187 ust. z dn. 20 lipca 2018r. Prawo o szkolnictwie wyższym i nauce (Dz. U. z 2023 r., poz. 742 ze zm.) and I recommend to the Scientific Council of the Institute of Physical Chemistry, Polish Academy of Science M.Sc. Karthika Kappalakanda Valapil admission to the subsequent stages of the doctorate process.

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