Review Report on PhD Thesis

Faculty: **Central European Institute of Technology**
Brno University of Technology in Brno

Academic year: **2024/2025**

Student: **Sanam Garehbaghi**

Doctoral study program: **Advanced Materials and Nanosciences**

Supervisor: **doc. RNDr. Lukáš Richtera, Ph.D.**

Reviewer: **Prof. Dr. Kledi Xhaxhiu**

PhD thesis title: Novel approaches in catalytic biosensors

Topicality of doctoral thesis:

The thesis is interesting and explores a relevant topic in the field of catalytic biosensors, with a focus on enhancing their selectivity, stability, and practical usability for practical applications in both food safety, specifically mastitis diagnosis and early-stage cancer detection. The present study shows significant progress in biosensor development through the use of enzymatic cascade systems and nanozyme-based platforms. The initial part of the study introduces a second-generation enzyme-based biosensor that employs a cascade catalytic reaction involving two natural enzymes, offering improved analytical performance. In the later stages, the focus shifts toward the use of nanozymes as enzyme alternatives. These are comprehensively discussed in a review that highlights advanced surface modification strategies designed to improve their catalytic efficiency for bio-sensing applications. The nanozyme-based biosensor developed in this work exhibits strong selectivity and sensitivity for miRNA-21 detection, showing competitive or superior performance when compared to other miRNA detection methods reported in the literature.

Meeting the goals set:

The objectives presented in the thesis are clearly defined and consistently addressed throughout the work. The PhD candidate successfully develops and investigates two separate biosensing platforms: a) An enzyme cascade system for detecting lactose in milk, and b) DNA-conjugated nanozyme-based optical sensor for detecting miRNA-21. Both systems are thoughtfully designed, synthesized, and characterized using appropriate analytical techniques. The experimental results meet and in some aspects even exceed the initial goals set by the study.

In the first system, PhD candidate employs a cascade enzymatic reaction using β-galactosidase and glucose oxidase to detect lactose. This bio-sensing method shows potential for practical use in diagnosing mastitis infections in milk products. The thesis also includes a literature-based review highlighting how the catalytic properties of gold nanoparticles qualify them as "nanozymes" suitable for various bio-sensing applications. These strategies include surface modification techniques tailored for biosensor development.

In the second system, an optical biosensor is designed using a nanozyme–cDNA conjugate to detect miRNA-21. Functionalizing the nanozyme surface with complementary DNA (cDNA) enhances the sensor's selectivity and sensitivity toward miRNA-21. This approach offers a promising, fast, and selective method for the early diagnosis of cancer-related biomarkers.

Problem solving and dissertation results:

The dissertation introduces innovative strategies to address common limitations found in traditional catalytic biosensors. PhD candidate presents two distinct systems, an enzymatic biosensor and a nanozyme-based sensor, both designed to achieve high sensitivity, selectivity, and reproducibility. In the enzymatic system, a composite of ruthenium oxide (RuO_2) and multiwalled carbon nanotubes (MWCNTs) is used to enhance electron transfer and improve analytical performance. Two natural enzymes, β-galactosidase and glucose oxidase, are immobilized on screen-printed carbon electrodes to develop a precise electrochemical biosensor for lactose detection. This biosensor serves as a potential screening tool for mastitis infections in milk. The incorporation of flow injection analysis emphasis its possible integration into real-time monitoring systems in dairy production environments.

In parallel, the nanozyme platform utilizes platinum-tyrosine (Pt-Tyr) nanoparticles functionalized with capture DNA (cDNA) to enable the selective and reversible detection of miRNA-21. This optical biosensor demonstrates high sensitivity and low detection limits, offering a promising and affordable strategy for early-stage cancer diagnostics. Furthermore, the thesis includes a comprehensive review of gold nanozymes, categorized by surface modification techniques, which serve as a valuable reference for the design of future catalytic biosensors in both in vitro and in vivo applications. Both systems are thoroughly validated through experimental data, showing strong analytical metrics such as low limits of detection, excellent reproducibility, and successful recovery in real samples. The originality and scientific relevance of the work are confirmed by publications in high-impact journals.

Importance for practice or development of discipline:

The thesis makes considerable contributions to the advancement of nano-biosensors, offering practical applications in both industrial and medical fields. The enzymatic cascade biosensor developed for lactose detection reveals strong potential for use in the dairy industry, particularly for quality control and the early screening of mastitis infections in milk products. Its high precision and compatibility with flow based systems suggest that it could be adapted for routine monitoring in factory settings. On the other hand, the colorimetric biosensor based on a Pt-Tyr nanozyme conjugated with capture DNA (cDNA) presents a promising approach for the detection of miRNA-21, a key cancer biomarker. This biosensor is designed for point-of-care applications and has the potential to be integrated into rapid diagnostic kits for early-stage cancer detection. Both bio-sensing platforms emphasize important trends in the field: miniaturization, improved robustness, and adaptability to applications. Together, they reveal how nanotechnology can be used to create innovative and accessible solutions for health diagnostics and food safety monitoring.

Formal adjustment of the thesis and language level:

The thesis is clearly written and well-organized, following a logical structure that includes all essential components: introduction, research objectives, literature review, methodology, experimental results, discussion, and conclusions. Figures and tables are well-designed and effectively support the content, although minor improvements in grammar and phrasing are recommended. The scientific language is appropriate, and terminology is used accurately. The reference list is comprehensive and includes current and relevant sources. The work demonstrates the PhD candidate's understanding of biosensor development using both natural enzymes and nanozymes. The experimental design is thorough and supported by solid theoretical foundations. The study introduces innovative approaches in nano-biosensing and presents original research results of high quality, which is published in high-quality Q1 journals, such as a review in Materials Today Bio with an impact factor of 10.2, and a research article in the Microchemical Journal, a Q1 journal with an impact factor of 5.1. Some results have also been presented at international conferences.

Questions and comments:

1. Could you clarify the detection mechanism for lactose using MWCNT and RuO_2? How is the electrochemical signal generated in response to lactose?
2. How might the bio-sensor be further optimized for accurate detection in dairy products that already contain glucose and hydrogen peroxide?
3. Does the study address the influence of pH and ionic strength on the assay signal or on the long-term stability of the sensor?
4. Is there potential to adapt the developed miRNA-21 biosensor into a paper-based colorimetric test kit for point-of-care applications?
5. It appears that Figure 1.1 in Chapter One is duplicated. Could this be corrected for more clarity?
6. Should the term "nanozyme" be abbreviated (e.g., NZ) after its first full use for consistency throughout the text?
7. Several paragraphs in the introduction are quite long. Would it be possible to split them into shorter segments to enhance readability?
8. If human serum samples were used in the experiments, have you included a statement regarding ethical approval or IRB (Institutional Review Board) compliance?

9. There are several abbreviations used without definition, such as SPE, FIA, and DPV. Could you define these terms at first mention for clarity?

Conclusion:

This dissertation demonstrates strong scientific merit and originality. The PhD candidate presents a clear understanding of advanced materials and biosensor design, supported by well-performed experiments and good results. The work is further validated through publications in reputable peer-reviewed journals, reflecting the relevance and impact of the research. The thesis meets the academic and scientific standards required for the award of a PhD degree. It also reflects the candidate's readiness for independent research in the field of nanotechnology and biosensing. Considering the novelty of the methods, the reliability of the results, and the overall quality of the work, I recommend a final grade of A according to the ECTS grading system.

In my opinion, the thesis is complete and well-prepared for oral defense before the appropriate examination committee.

In Tirana, date 18/07/2025

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Prof. Dr. Kledi Xhaxhiu