

Opinion of opponent Dr. chem. Laura Andze on the dissertation thesis of Ing. Leontýna Varvařovská, a student of the doctoral program Biomedical Engineering.

Overall opinion

At first glance, the dissertation by *Ing. Leontýna Varvařovská*, titled “Functionalized Nanofiber Membrane for Ultrasensitive Multifunctional Bionanosensor,” may appear relatively concise for a doctoral work. However, a deeper reading reveals that the author presents her research in a highly concentrated and logically organized manner, avoiding unnecessary repetition and focusing on the essential scientific content. The work demonstrates clear structure, solid experimental design, and convincing results, reflecting the candidate’s ability to work independently on complex interdisciplinary problems at the interface of nanotechnology, materials science, and biomedical engineering. Overall, the dissertation represents a high-quality scientific contribution with both academic and practical relevance.

Literature review

The theoretical part of the thesis is well written and thematically coherent. Although the literature review appears relatively short at first, a closer look reveals it is dense with relevant information and reflects the author’s strong understanding of the field. The chapters on nanofibers, carbon nanodots, and biosensors cover the key theoretical and technological aspects necessary for understanding the presented research. Nevertheless, the review could be improved by including slightly more detailed fundamental explanations – for instance, of nanofiber–biomolecule interactions or signal transduction mechanisms – to help readers from related disciplines. Despite these minor remarks, the literature review effectively supports the dissertation's aims and conceptual framework.

Aims of the thesis

The aims are clearly formulated and realistic, focusing on the development of a functionalized nanofiber membrane combined with carbon nanodots for bacterial detection in air and liquid media. The objectives are consistent with the current trends in biomedical sensor research and are fully aligned with the declared field of *Biomedical and Clinical Technology*. The author has successfully maintained the balance between fundamental material research and practical application, ensuring the aims are both scientifically sound and societally relevant.

Methodology

The methodology is one of the dissertation's strongest aspects. The author employs appropriate, modern, and reproducible experimental techniques, including needleless roller electrospinning for nanofiber fabrication, surface functionalization with specific antibodies, microwave synthesis of carbon dots, and advanced analytical methods (SEM, TEM, FTIR, fluorescence spectroscopy). The experimental design is systematic and logically sequenced, showing a good understanding of

process optimization and material–property relationships. A slightly more detailed description of experimental parameters and statistical analysis could further enhance reproducibility, but the presented methods are fully adequate for doctoral-level research.

Results

The obtained results are clearly presented, well illustrated, and scientifically convincing. The author demonstrates the successful fabrication and functionalization of PAN nanofiber membranes, the synthesis of fluorescent carbon dots, and the integration of both components into a biosensing platform capable of detecting *E. coli* and *S. aureus*. The results show high sensitivity, specificity, and rapid response, confirming the effectiveness of the proposed approach. The author appropriately treats the experimental data, clearly showing standard deviations and p-values in the figures, and she correctly compares her biosensor performance with unmodified control membranes. In the Discussion, the author also places her findings in the context of other published biosensor studies, highlighting similarities and advantages of her system. However, these comparisons are primarily qualitative. A more detailed quantitative benchmarking, for example, a concise table summarizing detection limits, response times, or selectivity factors of comparable biosensors, would further emphasize the competitiveness and technological value of the presented system. Nevertheless, the achieved outcomes convincingly demonstrate the concept's originality and feasibility.

Discussion and conclusion

The discussion is concise and focused, linking experimental findings with theoretical background and demonstrating the author's critical thinking. The presented data well justify the conclusions and highlight the scientific contribution and practical potential of the developed sensor. The discussion could benefit from a more detailed interpretation of the underlying detection mechanisms and a more explicit reflection on the limitations and long-term stability of the functionalized nanofiber membranes. Also, while the future outlook is well outlined, it would be beneficial to indicate more specific steps toward practical implementation or possible integration into existing diagnostic frameworks. Overall, the discussion and conclusions reflect scientific maturity and a comprehensive understanding of the research problem, providing a convincing closure to the work.

Specific opinion following criteria:

1. Topicality of the dissertation topic

The dissertation topic, “Functionalized Nanofiber Membrane for Ultrasensitive Multifunctional Bionanosensor,” is highly relevant to current scientific and technological challenges in biomedical engineering. The dissertation addresses a modern topic in biomedical engineering, the development of functionalized nanofiber membranes for ultrasensitive biosensing. The combination of nanofibers, surface functionalization, and carbon nanodots reflects current global

trends in rapid diagnostics and nanobiotechnology. The topic is scientifically progressive and practically significant, with clear potential for applications in medical and environmental monitoring

2. Fulfilment of the objectives of the dissertation thesis

The dissertation's objectives are clearly defined and fully achieved. The author successfully developed and characterized functionalized PAN nanofiber membranes, synthesized carbon nanodots, and integrated both into an effective biosensing system for bacterial detection in air and liquids. The presented results confirm that all planned research goals were met and demonstrate a coherent link between the aims, methodology, and achieved outcomes.

3. The methods and procedures of the solution

The applied methods are appropriate, modern, and well described. The author skillfully combines electrospinning, chemical surface functionalization, carbon dot synthesis, and advanced analytical techniques, including SEM, TEM, FTIR, and fluorescence spectroscopy. The experimental workflow is systematic and well-structured, demonstrating a good understanding of interdisciplinary research. Minor methodological details, such as extended reproducibility analysis, could be added, but overall, the procedures are sound and scientifically rigorous.

4. Results of the dissertation and specific contributions of its author

The dissertation presents clear, original, and well-documented results. The author successfully fabricated and functionalized PAN nanofiber membranes, synthesized fluorescent carbon nanodots, and integrated them into a biosensing platform capable of rapid and selective bacterial detection. Solid experimental data and proper statistical treatment support the results. The author's main contribution lies in the development of a multifunctional nanofiber-based biosensor with demonstrated potential for biomedical and environmental applications, representing a notable advancement in nanosensor design.

5. Relevance for the practice and development of the field of study Biomedical and Clinical Technology

Although I am not a specialist in biomedical or clinical technology, my background as a physical chemist and materials scientist at the *Latvian State Institute of Wood Chemistry, Cellulose Laboratory*, with research focused on electrospun needleless materials and wood-based implant composites, allows me to appreciate the materials science aspects of this work. From this perspective, the dissertation is highly relevant to the biomedical field, as it effectively translates advanced nanofiber and surface-functionalization techniques into practical biosensing applications. The presented concept of a functionalized nanofiber membrane shows strong potential for integration into diagnostic and environmental monitoring systems, thus meaningfully contributing to the development of Biomedical and Clinical Technology.

6. Formal arrangement of the dissertation thesis and its language level

The dissertation is well prepared and clearly organized, with a logical structure, high-quality figures, and consistent formatting. The text is written in clear and comprehensible English, making it easy to follow the author's ideas and results. Although I am not a language expert, the overall impression is that the language level is entirely adequate for a PhD dissertation, and the presentation meets the expected academic standards.

7. Comments and final evaluation of the dissertation thesis.

The dissertation by *Ing. Leontýna Varvařovská* represents a high-quality piece of scientific work that combines modern materials engineering with biomedical applications. The author demonstrates independent and systematic research, from the preparation and functionalization of nanofiber membranes to their integration into a biosensing platform and evaluation of performance. The results are convincing and supported by appropriate experimental evidence.

In my opinion, the work is concise yet well-focused, presenting information in a clear, logical manner. Minor remarks concern the limited depth of the quantitative comparison with other biosensor systems and the possibility of discussing in more detail the sensor's practical implementation and long-term stability. These, however, do not reduce the scientific value of the research.

Overall, I consider the dissertation an original and valuable contribution to the development of nanomaterial-based biosensing within Biomedical and Clinical Technology. I recommend the thesis for defense and support the awarding of the Ph.D. degree to the author.

Opponent Dr. chem. Laura Andze