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Review of the doctoral dissertation prepared by
Mr. Shakeel Ahmad
entitled „Deciphering the role of antibiotic exposure at the sub-minimum inhibitory concentration on bacterial heterogeneity at single-cell level"

1. Introduction

This review was carried out based on the resolution of the Scientific Council of the Institute of Physical Chemistry of the Polish Academy of Sciences of August 1, 2025. The subject of the evaluation is the doctoral dissertation of Mr. Shakeel Ahmad, entitled "Deciphering the role of antibiotic exposure at the sub-minimum inhibitory concentration on bacterial heterogeneity at single-cell level", which was prepared under the supervision of the supervisor, prof. dr hab. Piotr Garstecki and the co-supervisor, dr Ilona P. Foik.

2. Evaluation of the doctoral dissertation

Droplet microfluidics plays a crucial role in single-cell research, revolutionizing biology and medicine by enabling unprecedented precision, high throughput, and miniaturization of research instruments. This type of microsystem is a powerful tool for probing the complexity of biological processes at the most fundamental level, opening new possibilities in diagnostics, therapy, tissue engineering, and cell biology. It has groundbreaking significance in the study of the effects of antibiotics on bacteria, offering a rapid, highly precise, and automated alternative or complement to traditional laboratory methods (such as the standard antibiogram). Droplet microfluidics is therefore a rapidly developing tool with the potential to transform the clinical diagnosis of antibiotic resistance and accelerate the search for new, effective drugs.

The work presented for review, concerning the study of the effect of short exposures of selected cells to antibiotics at concentrations below the MIC value using microfluidic droplet systems, is therefore part of the global research trend related to the use of microfluidic systems to study antibiotic resistance at the level of a single bacterium.

2.1. Overall rating

The doctoral dissertation submitted for evaluation is written in English, is 123 pages long, and contains six chapters and a bibliography. The work is research-based and experimental in nature. The thesis statement is unstated, but the PhD candidate states the purpose of the work in Chapter 2. This purpose (p. 62) is sufficiently clear.

In the first chapter, the author introduces the reader to the research topic discussed in the dissertation, including in particular a literature review related to droplet microfluidic systems and their use in determining AST and heteroresistance detection.

The second chapter describes the purpose of the work, with a short introduction to the topic of the dissertation and the potential consequences of the observed results.

The third and fourth chapters are a traditional description of the materials and methods used in the research, in the format known from a scientific article.

The fifth chapter contains a comprehensive description of the results of the author's own research, including the determination of MICs and sub-MICs, the resistance profile for given antibiotic doses, a description of the effect of pre-exposure on heteroresistance and an analysis of the results obtained for two cell lines – Escherichia coli MG1655 and Pseudomonas aeruginosa PA14.

Chapter six is devoted to a discussion of the results and conclusions. In the final section of the dissertation, the author summarizes the results of his work, implicitly stating that the work's goal was achieved, a conclusion with which the reviewer fully agrees. The author also suggests further research directions, stating that droplet microfluidics is a powerful tool for unravelling bacterial heterogeneity. The PhD student's discussion of the obtained results is comprehensive and confirms the author's knowledge of the research topic. The PhD student addressed the posed problem, used appropriate methods, and made valid assumptions.

In his dissertation, the author refers to 246 literature items – scientific articles. The vast majority of these were written in the last 10–15 years. These are therefore current publications and reflect the current state of knowledge and technology. The dissertation provides a thorough analysis of sources, including world literature, the state of knowledge, and applications.

The thematic organization of the dissertation is correct, clear, and logical. It resembles a comprehensive scientific article. The work is written in concise language without significant editorial errors. However, in parts of the dissertation (Chapters 1 and 4), there are no references to the included figures in the text. The figures are prepared correctly, are mostly legible, and are well-labelled. Tables also complement the content. The author has demonstrated the ability to present her results accurately and convincingly.

2.2. Original elements of the dissertation

This doctoral dissertation presents the author's original contribution to the chemical sciences in the field of droplet microfluidic systems, research methods utilizing such microsystems, and the processing and interpretation of research results. I consider the development of a research methodology for the use of droplet microfluidic systems to study bacterial heteroresistance at the single-cell level to be my most important achievement.

2.3. Content and editorial comments and remarks

During the reading of the doctoral dissertation, no substantive errors or controversial conclusions were found, but my comments/remarks are as follows:

1. The chapter describing the research methodologies used is missing, for example, a pictorial figure illustrating the research protocol, with particular emphasis on the use of two microfluidic systems.

2. Please provide the source of Figure MF2; the phrase "published from the article by Pacocha et al." is insufficient.

3. Please provide the dimensions of the microfluidic chip used to visualize cell droplets (overall dimensions, height of the microfluidic system). Is this your own work?

4. What were the parameters of the droplet generation process – phase flows (Section 4.4.1)?

5. Section 5.1.1 describes the determination of MIC and sub-MIC parameters based on observations of bacterial growth kinetics depending on antibiotic concentration. Is it possible to more precisely determine the MIC in the concentration range of 8-12 ng/ml (Figure RF1a) for ciproflaxacin, and similarly for strepthomycin in the range of 2-3 ng/ml (similarly for the results described in section 5.2.1). How will a more precise determination of these parameters affect further conclusions?

3. Conclusion

I certify that the doctoral dissertation of Mr. Shakeel Ahmad, entitled "Deciphering the role of antibiotic exposure at the sub-minimum inhibitory concentration on bacterial heterogeneity at the single-cell level" represents an original solution to a scientific problem and meets the requirements for doctoral dissertations, as defined in the Law on Higher Education and Science (Article 187, sections 1-2).

Based on my review of Shakeel Ahmad's doctoral dissertation, I certify that he possesses systematic general knowledge within the discipline of chemical sciences and the competence to conduct scientific research (as confirmed by published scientific article in Scientific Reports). The dissertation's usefulness for chemical sciences has therefore been demonstrated and is significant.

Considering the research results presented in the dissertation, my final assessment of Mr. Shakeel Ahmad's doctoral dissertation is positive. I therefore request that the dissertation be accepted and allowed to be publicly defended.