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**Evaluation of the doctoral dissertation Ms. Neha Venkatesh Rangam, M.Sc.  
“Unraveling the chemistry behind the biological activity of green silver nanocomposites”**

**performed under the supervision of the supervisor**

**Dr hab. Beata Lesiak-Orłowska, Ph.D., D.Sc.**

**and the auxiliary supervisor**

**Dr hab. Laszlo Kover, Ph.D.**

carried out at the Institute of Physical Chemistry of the Polish Academy of Sciences

**General information**

• *The review of the work presented to me for evaluation was prepared on the basis of a letter from Assistant Profesor Jacek Gregorowicz, professor of the institute - deputy for scientific affairs of the Institute of Physical Chemistry, dated 14.04.2025*

The doctoral dissertation is a compact work, prepared in English, consisting of 147 pages. The work is divided into chapters: 1. Introduction, 2. Experimental methods, 3. Brewery waste synthesized silver nanocomposites, 4. Leaf extracts synthesized silver nanocomposites, 5. Conclusion and future research. The work also includes: Declaration of originality, Acknowledgements, Funding, List of scientific accomplishments, List of abbreviations, List of symbols, Abstract, Summary and References. In the reviewer's opinion, the work should include a chapter Review of literature, because the Introduction is usually a short introduction to the subject of the monograph. In addition, the traditionally adopted division into main chapters, such as Results, Discussion also allows for better organization and clarity of the text. List of scientific accomplishments contains a list of publications that were included in the doctoral dissertation and a list of other publications by the Ms. Neha Venkatesh Rangam. In 4 multi-author publications that were included in the doctoral thesis, the Author is the first author (3 works) or second author (1 work). The works were published in good journals (indexed in Web of Sci.), two in Nanomaterials and one in the International Journal of Biological Macromolecules.

The author also included a declaration of originality.

### **Substantive evaluation of the work**

The statistics on the number of cancer cases are alarming. According to data from the National Cancer Registry, the number of cancer patients in Poland is about 1.17 million people. Cancers cause 25% of all deaths in the country, including 30% of premature deaths in the age group 20-44 years and 50% in the age group 45-64 years. In 2024, about 20 million new cancer cases were recorded worldwide, and nearly 10 million people died from them. Therefore, all activities aimed at searching for new methods and improving the applied therapeutic methods are extremely important and necessary. The work of Mrs. Neha Venkatesh Rangam is dedicated to this problem, hence I consider its purposefulness as current and justified.

The key factor reducing the effectiveness of treatment is multidrug resistance of cancer cells. The mechanisms responsible for the response of cancer cells to chemotherapy are still insufficiently understood. The Author,s preliminary research, conducted using the in vitro method, taking into account the HeLa, A549, MCF-7, MDA-MB-231 cancer cell lines, is part of the search for innovative methods of treating cancer. The second element that determines the significance of the work presented to me for assessment is the search for waste management methods, including the pursuit of a circular economy model (GOZ) and the search for innovative green synthesis technologies. To sum up, I assess the choice of the topic, the aim and the scope of the work very positively.

This review will be discussed in relation to individual chapters.

**Introduction.** In this part of the work, Ms. Neha Venkatesh Rangam described the basic state of knowledge on cancers and their biology, origin, division into types. According to the reviewer, the Author introduced too much encyclopedic and quite general knowledge, which is not necessary in a scientific work. It seems that it would be more beneficial to cite and discuss the physiological mechanisms and causes of imperfections, including critical points, of currently used types of therapy. Although a subsection "Cancer diagnosis and treatment" has been introduced, the cited studies are very general, and the cited literature belongs mostly to review papers, not research papers. Even the subsection "Nanotechnology in cancer diagnosis and therapy" does not cite the results of the latest studies, and especially the observed mechanisms, what is more, the Author refers mainly to review literature, not research papers. The chapter "Metal nanoparticles for cancer treatment" also contains very general information. It is a pity that the cited literature gives such a general view of the state of the art of the problem,

because it does not indicate the mechanisms that justify the therapy proposed by the Author, and it is also difficult to formulate a hypothesis and the objectives of the doctoral thesis on this basis.

The subchapters dedicated to presenting the latest literature on "Mechanism of cytotoxicity of AgNPs" and "Mechanisms of antibacterial activity of silver nanoparticles (agNPs)" are much better prepared. The Author presented in great detail the current state of knowledge on the mechanisms of silver toxicity, including Ag nanoparticles, so I consider this part of the Introduction interesting and comprehensive. The next part of the Introduction is dedicated to the green synthesis of nanoparticles, including Ag nanoparticles. The Author presented basic information on the principles of conducting green synthesis technology, the phases of this process, and factors influencing its optimization. The next chapter, "Brewery wastes for green synthesis of AgNPs", presents a diagram and description of the brewing process, and characterizes the constituents and applications of different brewery wastes and beer. However, in my opinion, the "Introduction" chapter lacks the characteristics of the leaves used to prepare the extracts used for green synthesis. These plants contain many very specific, bioactive chemical compounds, such as terpenes and polyphenols, which may be important in the process of green synthesis.

The introduction ends with a statement of the aim of the work, although the second part of this fragment constitutes the scope of the work, so it would be better to call this subsection Introduction and scope of the work.

**The Experimental methods** chapter is preceded by a list of reagents and cell lines used in the experiment. I assess the selection of cell lines very positively: cancer and normal. The Author described the method of preparing extracts of brewery waste and leaves, as well as the scope of their analysis. The subsequent subsections, concerning the preparation of extracts and carrying out the green synthesis process, as well as the physicochemical characteristics of the nanocomposites obtained as a result of green synthesis, have been described in great detail, so I assess this part of the work as distinctive. The part of the methodology concerning biological studies of the obtained nanocomposites (studies on cell lines and bacteria) has also been presented in great detail, and proves Ms. Neha Venkatesh Rangam knowledge and commitment to the implementation of bio-analytical studies.

In general, I consider the description devoted to experimental methods to be well-prepared, allowing for an exact repetition of the conducted studies. Perhaps detailed

descriptions of the principles of operation of some commonly used tests (LDH, MTT) could be omitted, but they do not diminish the positive assessment of this chapter of the work. According to the reviewer, this chapter should include a note regarding the number of repetitions in individual experiments, the methods used and statistical programs for the interpretation of results. I consider the lack of a detailed description of the method of administering BW and LE nanocomposites to the culture medium in studies on cell lines (ensuring sterility of BW and LE, method of administration - to the medium before its administration to the wells or directly to the wells, the moment of administration, etc.) due to the tendency of Ag nanoparticles to form agglomerates and a protein corona is a certain shortcoming of the work.

Moving on to the evaluation of the results of the work and the methods of their presentation, as a reviewer I must point out several formal shortcomings, namely; very poor presentation of images and some graphs (fig. 4.11; 3.12; 3.14). In particular, the images from the confocal microscope are partially illegible, while increasing the volume of the work by a few pages and increasing the size of the figures would not be a problem. In addition, most of the images, tables and graphs are described in too general a manner. The principle of preparing scientific papers states that a table "taken out of the text" should be completely understandable, in the case of most of the ones presented in this work, this principle does not apply. Interestingly, several images that were also presented in publications were correctly signed by the Author. Also, the values given in the tables should always have the same number of characters after the dot, even if it is "0". Moving on to the substantive evaluation of the results, one may wonder why the traditional division into: results, discussion of results, etc. was not made, but the work was divided thematically, and within two topics the results were presented and a discussion and summary were conducted. In my opinion, this reduces the readability of the work, and presenting conclusions in three versions does not help to make a final synthesis of the results of the work. However, I respect this choice of the Author and it does not diminish the overall evaluation of the work. Therefore, the next chapter is "Brewery waste synthesized silver nanocomposites".

**"Brewery waste synthesized silver nanocomposites"**. In the subchapter "Characterization of BW nanocomposites", the Author presented the results of many analyses in great detail, paying particular attention to the dependence of the obtained results on the applied reduction reaction temperature. Interesting results also concern the type of chemical compounds obtained in the form of nanoparticles (Ag<sub>3</sub>PO<sub>4</sub> vs. AgCl vs. Ag met). Undoubtedly, these results are innovative

and indicate interesting relationships between the reduction reaction environment and the type of obtained Ag nanoparticles. Very interesting results also concern the surface characteristics of the tested nanocomposites, which undoubtedly proves the knowledge and research inquisitiveness of the Author. It is a pity that the nanocomposites were not analyzed using DLS methods and the Zeta potential was not determined, although this methodology was described in the chapter "Experimental methods", especially in the context of the planned biological analyses. Moreover, it seems that the assessment of the morphology / size and shape of nanoparticles performed using TEM is not very reliable, considering the quality of the obtained images, where the nanoparticles are agglomerated and poorly visible due to the presence of other compounds present in the solution. In addition, the Author should provide the method of observing the nanoparticles - the number of repetitions and the number of preparations, which would determine the reliability of the result. The studies included in the subsection "Anticancer activities of BW nanocomposites" presented interesting results of biological analyses, although the question arises as to how the size of the fluorescent signal was determined, which allowed for the statement of "significant decrease". As is known, the statement significant should be justified in statistical analysis.

To sum up, it should be stated that the biological studies were conducted with great care, and considering the large number of variables, they certainly required considerable commitment and diligence to master this wide range of analyses.

In the subsection Discussion, the Author referred to the obtained results and discussed potential dependencies and causes, what is more, she outlined potential mechanisms that could have determined the directions of reactions and observed phenomena, especially regarding the obtained phase Ag nanostructures. The discussion is conducted in a logical and mature manner. However, one may have reservations about the broad consideration of the involvement of ROS in the mechanisms of toxicity using cell lines and bacteria due to the fact, that such results were not presented. The analysis of the biocompatibility/toxicity of nanocomposites was carried out at a basic level, which largely prevents a more in-depth explanation of the biological mechanisms observed in studies on cells and bacteria. However, the dissertation is carried out in the field of chemical sciences, which indicates that biological studies are an interesting complement to chemical studies, what is more, they give this work an additional scientific value. Nevertheless, I believe that even at this level of research on the toxicity of various forms of Ag nanocomposites towards different cell lines, the available

(numerous) literature has not been fully used in the discussion. Conclusion, to a large extent is a repetition of the obtained results. I definitely believe that the work should contain concluding remarks (presented in the form of points), that provide key answers to the questions posed in the aim of the work, including an indication of the scientific and practical implications of the results.

**Leaf extract synthesized silver nanocomposites.** The next chapter, "Leaf extract synthesized silver nanocomposites", is a presentation of another part of the results concerning leaf extracts. The obtained results were presented in a similar way to the results of BW studies. The Author obtained many new, interesting results and relationships concerning the physical characteristics of nanocomposites obtained with the participation of leaf extracts. Interesting results include studies of the types of Ag phases and studies of the surface of nanocomposites. However, in my opinion, similarly to BW, there was no determination of the size of nanoparticles using the DLS (Dynamic Light Scattering) method and zeta potential measurement. The size of nanoparticles and their size distribution, as well as the stability of the colloid/suspension introduced into the in vitro culture are crucial for their toxicity/biocompatibility and should be strongly documented. In my opinion, the use of only the visualization method using TEM in the case of BW, and in the case of LE the lack of even this method is a significant shortcoming of the presented studies in the context of biological applications of BW and LE nanocomposites. The discussion of the results obtained in the studies on LE nanocomposites is unfortunately, to a large extent, a repetition of the results. Although the application of the green synthesis method to obtain Ag nanoparticles is quite widely studied, the Author refers to only three literature items in the discussion. The conclusion of the discussion is another synthesis of the obtained results. According to the reviewer, the lack of concluding remarks (presented in the form of points), also formulated at the end of this chapter, is a shortcoming of the work.

**Chapter 5 "Conclusions and future research"** contains two parts. The first "Major conclusion" is rather a summary of the results. The conclusions part does not usually conduct discussions, especially on such a wide scope (Table 5.1) as presented by the Author. In my opinion, the majority of the Conclusions chapter should be included in the Discussion part. A significant shortcoming of the work is the lack of concluding remarks, which should have been presented at the end of the summary of the chapters on BW and LE, and preferably at the end of the work. The "Future research pathway" part is an interesting ending to the work, and I assess this fragment of the work positively.

**References** is the last part of the work, and unfortunately they were not presented in the manner used in scientific works. Apart from the fact that the cited literature is mainly review papers, not research papers, their presentation is not proper, because it does not contain basic data regarding the publication, especially the name of the journal and the year of publication. This is surprising because in the Author's publications "References" are presented properly.

#### **Review summary**

The research undertaken by Neha Venkatesh Rangam, M.Sc. "Unraveling the chemistry behind the biological activity of green silver nanocomposites" carried out under the supervision of the Dr hab. Beata Lesiak-Orłowska, Ph.D., D.Sc. and the auxiliary supervisor Dr hab. Laszlo Kover, Ph.D., carried out at the Institute of Physical Chemistry of the Polish Academy of Sciences allowed to obtain many valuable results, some of them innovative in nature. Numerous and well-documented results are undoubtedly a significant achievement, contributing new values to the development of chemical and biological sciences. Despite several formal errors and my subjective comments of a substantive nature, I positively evaluate the scientific dissertation of Ms. Neha Venkatesh Rangam, M.Sc. In my opinion, the work meets the requirements for doctoral dissertations and, due to its scientific value, contributes to the development of chemical sciences. The doctoral dissertation of Ms. Neha Venkatesh Rangam, M.Sc. meets the conditions formulated in art. 187 of the Act of July 20, 2018, the Law on Higher Education and Science (Journal of Laws of 2024, item 1571). I hereby submit to the Scientific Council of the Institute of Physical Chemistry an application to admit Ms. Neha Venkatesh Rangam, M.Sc. to further stages of the doctoral procedure.

Ewa Sawosz Chwalibóg, 18.06.2025

