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CRITICAL REVIEW OF THE DOCTORATE DISSERTATION
Entitled: „Unraveling the chemistry behind the biological activity of green silver nanocomposites"
Under the supervision of Dr. hab Beata Orłowska
and auxiliar supervision of Dr. Laszlo Kover,
At the Institute of Physical Chemistry of the Polish Academy of Science

Introduction

The development of human civilization and technology has significantly improved the quality of life, health, and longevity. The results of centuries of efforts made by physicians, chemists, biologists, and engineers are truly remarkable. Over the past hundred years, global life expectancy has approximately doubled (Max Roser, 2018, *Life Expectancy*). However, as human lifespan has increased, cancer has emerged as one of the most frequent causes of death worldwide. The scientific history of the fight against cancer spans thousands of years and reflects the evolving understanding of human biology and chemistry. The mid-20th century brought a new era with the development of chemotherapy. Initial drugs, such as nitrogen mustards and antifolates, demonstrated that chemical compounds could kill cancer cells, though often with severe side effects. Currently numerous medical technologies evolved, some are already applied and some are investigated. Antibodies and other "biologics" are frequently employed in cancer treatment, immunotherapies, including CAR-T cell therapy, have shown unprecedented success in some hematological malignancies. Another area of development is polymer nanoparticles which is the hope for clean targeted delivery of cytostatic drugs or other anticancer therapies. Unfortunately organic – polymeric nanoparticles, despite the initial success and hopes, despite the enormous research done, are still not available in hospitals for cancer treatment. The cause of this phenomena is discussed and remains unclear. This makes us look for other solutions, like inorganic or hybrid: organic-inorganic nanoparticles.

Another research success, which was the development of antibiotics, largely participated in the prolongation of human life expectancy. Initially being the blessed and magic medicine, overused – mostly in animal production, lead to the development of antibiotic resistance in bacteria, including pathogens. The growing resistance of bacteria to antibiotics has emerged as a serious threat to global health, making the search for new antimicrobial agents a top priority. These dual challenges, cancer and antimicrobial resistance, represent some of the most pressing problems faced by contemporary biomedical science.

In this context, the doctoral dissertation entitled "Unraveling the chemistry behind the biological activity of green silver nanocomposites", authored by Neha Venkatesh Rangam, addresses a topic of both scientific and societal importance. The thesis explores the potential of silver-based nanocomposites synthesized through green chemistry approaches, in this case, utilizing brewery industry waste and plant extracts as reducing and stabilizing agents, for their biological activity against cancer cells and bacteria.

The Dissertation

The reviewed doctoral dissertation presents the use of waste products from the brewing industry and leaf extracts as reducing agents for silver compounds in the synthesis of silver nanoparticle complexes with organic substances contained in these organic materials. The dissertation begins with a description of the synthesis process and the resulting products in terms of their chemical composition. In the subsequent stages of the work, the influence of the obtained organic silver nanoparticle complexes on the growth and activity of various human cell lines, both normal and cancerous, as well as their bactericidal properties, is investigated. The research is innovative in its use of organic materials derived from agro-industrial waste to produce biologically active nanostructures. It reflects a growing interest in environmentally sustainable synthesis methods, while at the same time responding to the urgent need for novel therapeutic strategies. The idea of repurposing brewery by-products, a largely underutilized waste stream, as components in the formation of bioactive nanomaterials is particularly intriguing, and combines elements of green chemistry, nanotechnology, and biomedical research. Thus, this dissertation fits within a broader framework of modern scientific efforts aimed at identifying new pharmacologically active compounds, particularly those with potential anticancer and antimicrobial effects.

The dissertation includes an extensive literature review and represents a compilation of reports from several research projects. Unfortunately, as is often the case with such compilations, the work does not take the form of a traditional doctoral thesis and lacks a

clearly stated scientific hypothesis that could be either confirmed or refuted in the course of the research. In this particular case, formulating a scientific hypothesis would have been relatively straightforward, and therefore, I kindly ask the author to propose and include a clear doctoral research hypothesis.

The dissertation is well written in terms of formatting and is graphically well presented. It contains the results of numerous experimental studies conducted with great care, as well as analyses of the synthesized nanoparticles using various advanced analytical techniques. It also presents the results of biological property studies of the obtained nanoparticles. Unfortunately, despite the author's diligence, the dissertation is not entirely free of errors and shortcomings, both editorial and related to the experimental design.

Critical Remarks

The dissertation includes a list of abbreviations and a list of symbols. In my opinion, including elemental symbols as abbreviations, or even listing them in the symbols section of a doctoral thesis in the field of natural sciences seams excessive. Furthermore, the list of symbols contains some peculiar entries, such as "Armstrong unit" (Neil Armstrong's moon landing unit?). The list also refers to absorption measured at 6800 nanometers wavelength, which corresponds to the far-infrared region, is it correct?. Both the symbol list and the abbreviations list appear to have been generated in a largely automated manner with the lack of sufficient care.

In the abstract, the use of brewery waste for the chemical reduction of silver is justified as a method of managing excessive production of brewing by-products. I find it doubtful that the development of a potential anticancer or antibiotic drug would ever consider such waste management as a meaningful objective. In modern pharmaceutical development, we aim to use materials that are highly defined and reproducible, regardless of cost. The production of silver nanoparticles is also unlikely to consume meaningful part of the brewery waste generated worldwide. I also believe that the use of poorly defined natural substances as therapeutic agents represents a step backward in the development of pharmaceutical science. I would like to remind the author that as early as the 16th century, Paracelsus postulated that the medicinal properties of natural substances result from specific chemical compounds contained within them. This ultimately led to the isolation of the first pure active pharmaceutical ingredient (API)—morphine from opium—in 1806 by Friedrich Sertürner. The research presented in the dissertation should rather focus on finding and identifying new therapeutic compounds or their composites, preferably through the targeted search for specific

substances, rather than as a means of waste management. However such waists are also an interesting research area where new drugs could be found, what justify this research.

The description of the synthesis methods for the organic-silver nanocomposites does not include information regarding light exposure during drying or compound formation. It is well known that silver compounds degrade rapidly under light, a property that laid the foundation for photography. In this context, both during the mixing of organic substances in solution and during drying, light undoubtedly affected the decomposition of residual precipitated silver chlorides or phosphates. I am not requesting a precise quantification of this effect, but rather emphasizing the importance for experimental reproducibility. On one day the sun may shine brightly, providing strong illumination, while on another day the light levels may be significantly lower. Some samples may be positioned on top during drying, while others are beneath them, leading to variability in their chemical composition. Ideally, such procedures should be carried out under constant low-light conditions or, preferably, in darkness under red light.

I also regret that the precipitates obtained after centrifugation, washing, and drying were not weighed, or at least, I found no reference to such measurements in the dissertation. The lack of data on the dry mass of the final product significantly hinders any discussion of its chemical composition and amount of added silver in it. The composition of such a medicinal product, specifically the ratio of organic and inorganic matter should be presented. Moreover, no negative control of biological experiments were performed, that is, experiments conducted identically but without the addition of silver nitrate, and also silver precipitates but by other means. It is well known that both beer and brewery waste contain significant amounts of protein, which could denature under elevated temperatures and be recovered as a precipitate. Such denatured proteins would form sediment regardless of whether silver ions are present. Without these control experiments, it is impossible to determine whether the observed anticancer properties stem solely from the presence of silver or from other compounds present in brewery waste that are precipitated through thermal treatment or interaction - oxidation with silver ions.

It would also be beneficial to determine the water content of the obtained precipitates, as drying at 37 °C does not ensure complete water removal. The precipitate should be weighed after drying at 37 °C, then subjected to higher-temperature drying, calcination, until constant weight is achieved, followed by re-weighing of mineralized sample as a final weighing step. This would allow for the determination of the mineral content. Table 3.2, which presents the composition of the obtained precipitates, incorrectly suggests that these contain only inorganic compounds. The same issue applies to the compositions listed in Table 3.3. Table 3.4,

which presents XPS analysis results, clearly indicates the presence of carbon, oxygen, and nitrogen. Table 3.1 presents the approximate chemical composition of the brewery waste used in the experiments, but due to the simultaneous use of decimal points and two different comma styles as separators, the data are difficult to interpret. Overall, the presented results demonstrate that the obtained precipitates have a highly complex chemical composition.

The biological testing of the obtained silver nanocomposites was conducted with care and the results are clearly presented and discussed. However, also in this case, the research lacks a properly biological approach, specifically, an adequate number of negative controls. I believe that for comparison, silver nanoparticles synthesized using a standard and widely used method should have been included. For instance, the earliest described method by Michael Faraday, which involves the reduction of silver using citric acid. Additionally, the effect of brewery waste alone on cell cultures was not evaluated. It is possible that some of the antibacterial or anticancer activity is due to compounds naturally present in the waste. Such comparisons would help determine whether the proposed method offers any advantage over existing approaches, or whether drinking beer might be just as effective.

It should also be noted that the viability assays used in the dissertation are only indirect measurement methods. It is known that certain substances under test can influence the outcome of such standardized assays, have fluorescent, adsorbent or enzymatic activity. Therefore, it is always advisable to carry out a full series of control measurements under identical conditions but without cells, to examine how the nanocomposite itself might affect the test results.

Final Remarks

The doctorate dissertation “Unraveling the chemistry behind the biological activity of green silver nanocomposites”, authored by Neha Venkatesh Rangam, is an original scientific work. The Author of this work proved to be an independent researcher who could plan, conduct, and describe the results of scientific research. I think the results are scientifically sound, and minor errors or omissions in methodology do not detract from the positive reception of the research work. The Author of the dissertation has already published some results. I found three publications directly from this current thesis, and the Author published together seven scientific publications.

I hereby consider that the doctoral dissertation “Unraveling the chemistry behind the biological activity of green silver nanocomposites”, prepared by Neha Venkatesh Rangam meets the conditions specified in Article 187 of the Act of 20 July 2018 - The Law on Higher

Education and Science (Journal of Laws of 2023, item 742, as amended). Due to the positive review, I propose to forward the dissertation to the following stages of the doctoral procedure.

(Uważam, że rozprawa doktorska: “Unraveling the chemistry behind the biological activity of green silver nanocomposites”, przygotowana przez Neha Venkatesh Rangam odpowiada warunkom określonym w art. 187 ustawy z dnia 20 lipca 2018 r. Prawo o szkolnictwie wyższym i nauce (Dz.U. z 2023 r., poz. 742 ze zm.). W związku z pozytywną recenzją proponuję skierowanie rozprawy do dalszych etapów przewodu doktorskiego.)

Tomasz Adam Ciach

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