



UNIVERSITAS SCIENTIARUM SZEGEDIENSIS
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REVIEW
of the dissertation submitted by

Neha Venkatesh Rangam

entitled

UNRAVELING THE CHEMISTRY BEHIND THE BIOLOGICAL ACTIVITY OF GREEN SILVER NANOCOMPOSITES

To be granted the degree of PhD, Neha Venkatesh Rangam submitted her dissertation under the supervision of Dr. hab Beata Orłowska of the Institute of Physical Chemistry of the Polish Academy of Sciences (Warsaw, Poland) in collaboration with auxiliary supervisor Dr. László Kövér of the HUN-REN Institute for Nuclear Research (Debrecen, Hungary). The topic selection of not only measuring the biological activity of silver nanocomposites but investigating the underlying effects as a function of surface chemistry resulting from various biological extract-based synthesis routes is increasingly relevant, as manufactured nanomaterials are more and more involved in our day-to-day lives, with biomedicine being one of the fields most impacted. Green synthesis methods are also a topic of substantial interest as the sustainable and environmentally benign formulation of functional materials is an ever-present goal to be achieved. The candidate has various high-quality peer-reviewed publications and conference participations highlighting her research merit; the dissertation is comprised of three such publications plus a fourth one that is currently under peer-review.

The main body of the dissertation consists of 132 numbered pages divided into five main chapters that adequately structure the manuscript into a pleasant and logical read. The quality of the writing is good; the low number of structuring errors and typos never disturb the comprehension of key findings. The first main chapter is the Introduction, where the candidate describes the main types of cancer, their diagnosis and conventional treatment, and connects the topic with nanomaterials and silver nanoparticles in particular as a new potential way of therapy. The chapter then circles in on the green synthesis methods used throughout the research, namely brewery wastes and leaf extracts, describing their formation, use and main biomolecular components, although the chapter tackling brewery wastes feels a bit redundant and unnecessarily repeats some concepts described only a few pages prior. The penultimate subchapter is a general list of common characterization methods that in my opinion could have been removed and the space could have been used to describe the actually utilized methods in more detail during subchapter 2.5.

Chapter 2 collects the experimental methods conducted during the doctoral research. The synthesis methods are clearly described and illustrated, although some clarifications would have made the experiments easier to replicate, such as including the pore size of the filter paper instead of its outer diameter or providing centrifugation power in *g* instead of *rpm*. As for the characterization methods,

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their use is well selected and justified, although, in my opinion, a broader explanation for each would have been a useful notion, as multidisciplinary research such as this may leave readers unfamiliar with certain fields at a disadvantage.

Chapters 3 and 4 collect the key findings from research conducted with brewery waste and leaf extract synthesized silver nanocomposites respectively, while chapter 5 provides some overall conclusions and future prospects of the work. While I have some questions about specific aspects further on, some general remarks can be made about the chapters. The breakdown of the topics is clear and makes the results easy to follow. The candidate is clearly familiar with the utilized methods and is generally providing plausible explanations for her observations, although despite the definitive statements that can be read throughout the manuscript, I feel various observations are not sufficiently confirmed. Justifications either in the form of complementary analytical results or even affirming scientific references are sparse throughout the work. Out of the 194 total references, 164 are cited by the end of the Methods, generally in the form of exposition. Since the candidate is clearly well-versed in the surrounding literature, utilizing it to place the work in its scientific context would have highlighted the importance of the results substantially.

The following are some topics I feel could benefit from some more clarification. As the issues are similar in both BW and LE experiments, I will formulate my questions grouped by analysis methods rather than green synthesis components.

- I. Throughout chapters 3.2.1 and 3.2.5 where the candidate is discussing the morphology of silver nanocomposites synthesized using brewery wastes using XRD and TEM respectively, the term “nanoparticle” sparingly appears throughout the text, however in different contexts. During the XRD discussion, it seems to be used as a synonym for crystallite, while in the TEM analysis, the term seems to refer to the spherical inorganic components of the composites, which may or –more likely– may not be single crystals, therefore the use of the word is inconsistent. Furthermore, certain samples like BW5Ag3 and BW7Ag3 do possess spherical morphologies even though their size may exceed the limits of conventional nanoparticles. As the dissertation does not include size distribution histograms from electron microscopy, could the candidate compare these results and explain what she considers nanoparticles in her samples?
- II. Looking at the leaf extract-based XRD results in chapter 4.2.1 there are two observations that seem to conflict with the patterns presented. Throughout the manuscript, XRD peaks at 2θ values of 36.3° , 55.1° and 57.4° are denoted to AgPO_4 and are used to identify the presence of the phosphate phase within the samples. On page 103 discussing the diffraction patterns of LE-based composites, the candidate writes that AgPO_4 was only detected in LE2 samples, specifically the ones synthesized at 25°C and 50°C . Considering Figure 4.1 however, the highlighted peaks marked by (*) cannot be seen in the sample synthesized at 25°C , but do seem to be present in LE3Ag3 prepared at 80°C . There is a conclusion drawn from this regarding the temperature dependence of the formation of the phosphate phase, that therefore appears to conflict with the data. Furthermore, the $55.1^\circ - 57.4^\circ$ double peaks appear to be present in LE3 composites, which is excluded from the evaluation of the results in Table 4.2.

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- III. Several conclusions are drawn based on the XPS analysis of the samples regarding various oxygen-containing silver phases from polymer- and hydroxide-, to oxide variations that are not identified with other methods. Their presence is calculated from the deconvolution of the XPS spectra between 360 and 380 eV BE, where a double peak is present from which 7 different materials are identified in various permutations in figures 3.5 and 4.5. In these spectra, some Ag species are missing their doublet pairs and their area seems relatively small. Does the "best mathematical fit" verify the detected quantities?
- IV. Regarding the *in vitro* experiments in general, there is a framing in the manuscript that despite performing identical experiments, healthy cells were used to test the biocompatibility, while cancer cell lines were used to assess the cytotoxicity of the various nanocomposites. This framing is ultimately extended into the notion by the end that the "*nanocomposites were biocompatible to normal [...] cell lines in the concentration range where they were cytotoxic to cancerous cells...*", but I am not sure this is a conclusion that can be confidently drawn from the results provided by the manuscript. Based on the MTT and LDH data, broadly speaking there were 2 cell lines (HeLa and A549) that were more susceptible towards nanocomposite exposure, while the other 2 (MCF-7 and MDA-MB-231) were generally more resistant than the healthy HEK293 embryonic kidney cells. If the labels were removed from the figures, I don't think one could select the healthy cells based on their superior viability, and if the live/dead assays were also performed on more resistant cells as well, the conclusions from the fluorescent imaging might have also been quite different. Could the candidate elaborate on her thought process regarding this issue?
- V. In chapter 4.3.2 (page 118) the second paragraph says: "*The observed LDH activity of cells [which is proportional to cell damage and therefore cell death] was highest at a lower concentration of 0.1 $\mu\text{g ml}^{-1}$ and the activity decreased with increasing concentration. This suggests that as the concentration of nanocomposites increases, cell proliferation is reduced...*". The next paragraph then seemingly contradicts this with "*... LE nanocomposites inhibit cell growth at lower concentrations, while at higher concentrations, they induce both cell growth inhibition and cell death*", a statement that is reiterated at the end of page 126. Could the candidate resolve this apparent contradiction?
- VI. Table 5.1 is a bit peculiar. Prior to it, the candidate identified a biphasic toxicity curve from the MTT data of the nanocomposites and defined their corresponding concentrations of 50% response as 1st and 2nd phase EC₅₀ values in tables 3.7 and 4.8. Later in Table 5.1 these are renamed IC₅₀ and LC₅₀ respectively but are now compared to values from the literature collected from other silver-containing nanomaterials which are denoted as EC₅₀ values, which makes direct comparisons confusing. Furthermore, in my experience, IC/EC values collected from viability tests depend on many additional factors; it is common to not only use cell lines from the same source during a piece of research, but people also usually try to use the same thawed batch and consider the number of cell passages performed etc., as even identical cell types can provide widely different results. All things considered, identifying the best nanosilver-based options depending solely on these numbers may lead to inaccurate

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conclusions. Is there a better way of discussing the results in order to get a more accurate picture of its impact?

The above-mentioned inquiries and propositions do not diminish the merit and importance of the research. The work clearly indicates that the candidate acquired sufficient and valuable knowledge in her field and is able to draw her own conclusions from multidisciplinary data. It is my conviction that Neha Venkatesh Rangam should be awarded the degree of PhD following a successful public defense.

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