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REVIEW

PhD thesis MSc. Eng. **César Hernández** entitled: *Assessment of the effects of various carboxylic acids as solvents, characterization and enhancement of mechanical and antibacterial properties of wet-spun chitosan fibers.*

The review was prepared in a response to the letter from Prof. Barbara Błażejczyk-Okolewska, Chair of the Council for Academic Degrees of the Technical University of Łódź in the disciplines of mechanical engineering, materials engineering, dated July 11, 2023.

1. The subject of the dissertation and definition of the research problem

The dissertation consists in the preparation of chitosan fibers by wet spinning, using various carboxylic solvents' acids, in particular acetic acid, citric acid and lactic acid, and further evaluation of the effects of the above-mentioned solvents on improving the fibers' mechanical properties. Another research aspect was to evaluate the impact of ursolic acid on antibacterial properties and tripolyphosphate (TPP) as a cross-linker to enhance the mechanical properties of investigated fibers. Numerous applications of chitosan in medicine, agriculture, food processing, cosmetics and wastewater and water treatment, due its beneficial properties such as biodegradability, biocompatibility, low toxicity, antimicrobial activity presented in the dissertation, make the dissertation thesis is a current one. The topic is relevant to the research on new methods of polymer fibers' modification.

2. Substantive assessment of the dissertation

The submitted dissertation counts 124 pages and is divided into 5 chapters. The work contains 41 figures and 27 tables, listed at the beginning of the dissertation. The study begins with abstracts in Polish and English, and chapters presenting the literature review. The literature review is described in two chapters. The third chapter presents the experimental part, while the fourth chapter contains the results of the research. The dissertation ends with conclusions, contained in chapter five. In the experimental part, the Author describes the applied research methods and the materials, and also discusses the method of manufacturing chitosan fibers. At the end of the dissertation there is a list of scientific literature of 74 items, which is not impressive for a doctoral study.

The dissertation entitled *Assessment of the effects of various carboxylic acids as solvents, characterization and enhancement of mechanical and antibacterial properties of wet-spun chitosan fibers* is very precise and clearly defines the subject area.

The literature review begins with an introduction describing biopolymer materials, their production, properties and applications, focusing on the use of biopolymers in the medical and pharmaceutical industries. The particular attention is paid to materials, such as polyhydroxyalkanoate (PHA), polylactic acid (PLA), polyglycolic acid (PGA) and chitosan. Based on a brief introduction, the Author describes the motivation and defines the purpose of the work, which was to produce chitosan fibers using solvents in the form of three carboxylic acids by wet spinning, and to modify the fibers with an ursolic acid to improve their antibacterial properties and enhance the mechanical properties, using tripolyphosphate as a cross-linking agent.

In the second chapter, the Author describes the division of biopolymers into degradable and non-degradable ones. Further on, the production of biopolymers is described, taking into account the influence of factors such as feedstock selection, extraction/isolation, purification, modification, polymerization, formulation or processing. In the following subsections, the Author describes the properties of biopolymers regarding biodegradability, biocompatibility, versatility, sustainability. He then describes chitin and chitosan, their production methods, properties, chemical structure, as well as the effect of chemical and physical cross-linking on mechanical properties, chemical stability, solubility and aqueous permeability. The Author also presents commonly used cross-linkers agents. In the penultimate subsection of the literature review, the Author explains the recesses of the wet spinning technique and the features affecting the parameters of the manufacturing process, e.g. the choice of polymer,

solvent, their concentration, process conditions: temperature, spinning speed, coagulation, bath composition, rinsing, neutralization and drying. In the last section, the Author describes carboxylic acids, oleanolic and ursolic acid, presenting their structure and physicochemical properties.

This part of the dissertation is an introduction to the implementation of the planned research work and is based on 52 scientific articles.

The experimental part of the thesis begins with a description of the materials and chemical reagents used in the study, as well as listing the measurement apparatus. Next, the Author describes and justifies the selection of chitosan solutions in acetic, citric and lactic acid solvents, their concentration and the conditions of the homogenization process, and checks the rheological properties of the solutions by determining their viscosity and shear stress at different temperatures. On the basis of these tests, the doctoral student determines the stability of the various solutions and thus selects a solution with a chitosan concentration of 7 wt% as the solution best suited for fiber production. The studies further described in this work proved the choice to be correct and allowed the Author to definitely narrow down the research area. In the following subsections, the Author describes in details the method of producing chitosan fibers in solutions of particular acids, taking into account the specified parameters of spinning process.

Moving on to the most interesting part of the dissertation, i.e. the presentation of the experimental results (Chapter 4), I would like to emphasize that I am impressed by the presentation of the research results: very clear drawings, clear tables and great aesthetics of the work. This part of the work begins with a description regarding the SEM method as a useful tool in the study of the microstructure and morphology of various materials. I find this information completely superfluous, and I would rather encourage the Author to describe the measurement conditions the observations were performed under, such as the detector type, the accelerating voltage, the microscope operation mode, the sample preparation method, etc., instead of general information about the method. In the following subsections, the Author describes the fibers linear density of determining it for all the types of fibers. The density values are achieved by measuring the mass of string using the direct count system formula. What is definitely missing in this section is a description of the obtained results and the Author's commentary on whether and how the density values affect the mechanical properties of the fibers. The next subsection of the dissertation deals with the fibers mechanical properties. In the study, such parameters as the specific strength at maximum force and the relative elongation at maximum force were determined. Based on the results, the Author came

to the valuable conclusion that fibers obtained in the presence of acetic acid show the best mechanical properties.

In the further part of the dissertation, Mr. Hernández presented and discussed the method of surface modification of fibers with the highest mechanical parameters using tripolyphosphate and ursolic acid. The fibers after TPP modification were subjected to structural properties evaluation (FTiR), morphological evaluation (SEM), their linear density and mechanical properties were studied. The fibers after modification with ursolic acid were examined via infrared and UV spectroscopy, scanning electron microscopy and antimicrobial activity tests against Gram-positive and Gram-negative bacteria. Conducting the above tests made it possible to show that the modification with TPP improved the mechanical properties, while the ursolic acid modification induced the antibacterial activity only against Gram-positive bacteria. However, I cannot agree with the Author that the value of tensile strength increased by 15% and the value of relative elongation by 9.75%, since taking into account the standard deviation the actual value of tensile strength parameter increased by 7.7% and for relative elongation no improvement was observed at all. Thus, we can only speak of a certain upward trend for tensile strength. Unfortunately, the Author did not perform a proper statistical analysis showing significant changes and did not attempt to describe the actually obtained values. Moreover, he did not explain the reason for the increase in tensile strength and the decrease in relative elongation. I would like to point out that a result should be treated as it is and although the true value might be sometimes undesirable, it is more valuable for researchers. In this part of the work I also greatly miss the attempt to study the effect of both TPP and ursolic acid on the mechanical and biological properties of chitosan fibers in parallel. It would have been a great success for the PhD student to obtain fibers not only with higher mechanical parameters but also with antibacterial or even bactericidal functions.

The section on antibacterial research definitely lacks a description about research methodology related to the sample preparation and the bacteria culture itself.

Among the weaknesses of the work I would emphasize the lack of adequate discussion based on the findings of other researchers. The Author did not take proper care to highlight his achievement against the background of other research centers. Consequently, in my opinion, the work has the character of a very good grant report or illustration guide rather than a scientific work. Nevertheless, the Author carried out a number of syntheses of chitosan fibers using the wet-spinning method and realized the work's goal of demonstrating the improved mechanical properties and antimicrobial properties of the obtained fibers.

The research was carried out correctly, and the conclusions, although not very extensive, are posed well enough. Thus, the work meets the requirements for doctoral theses.

3. Specific comments on the editorial and linguistic aspects of the dissertation

The dissertation submitted for evaluation was aesthetically prepared, the figures and tables are very clear and legible, and the layout of its various parts is correct.

In the abstract written in Polish, the Author made a number of stylistic errors, but considering the fact that he is not native in Polish and the entire dissertation is written in English, I do not raise this remark as a complaint affecting the quality of the evaluated dissertation. Simultaneously, as I am not native in English I will not assess the correctness of English grammar and linguistic errors. I can only state that I read the dissertation with great ease. Regarding editorial errors, I would like to point out that the Latin names of bacteria, such as *Escherichia coli* and *Staphylococcus aureus*, should be written in italics, and the terms referring to the bacterial genus "Gram-positive" and "Gram-negative" should be written in capital letters. Volumetric units such as cubic centimeters should be written with a superscript next to the number that appears (instead of "cm3" it should be "cm^3"). According to the rules of editing scientific papers, usually a reference to a figure or table appears in the text and only then can it be inserted in the work. I missed such a reference to most figures and tables. Such spontaneous insertion of drawings without any reference causes chaos and does not meet the standards of a good scientific work. On page 91 of the work, instead of the reference [31] to the literature list, there should be a reference [30, Pati et al.], and next to Nasimento et al.'s entry, there should be a reference [47].

4. Comments and questions for the PhD student

While reading the dissertation, some comments and questions for discussion came to my mind:

1) Basing on the observation of the fiber morphology, the Author concludes about the "smooth surface" of the fibers, Was the Authors' subjective assessment supported by determining the parameters responsible for roughness by other research techniques?
2) How does the "smooth" surface of fibers affect their mechanical properties?
3) Does the linear density of the fibers determined in the work affect the mechanical properties? If so, in what way?

4) Figure 31 on page 93 does not present visible changes in the correlation of the described bands at 1647cm^{-1} and 1587 cm^{-1}, please explain how the Author determined the decreasing absorbance ratio?
5) Did the PhD student confirm the formation of chemical bonds between chitosan and TPP that he wrote about on pages 96 and 99?
6) Why did not the Author perform mechanical tests for chitosan fibers impregnated with an ursolic acid?
7) Based on the way the fibers were developed, does the Author plan to produce a scaffold for tissue regeneration in the future?

5. Final conclusions

Summarizing the evaluation of the doctoral dissertation of César Hernández, MSc. Eng., entitled *Assessment of the effects of various carboxylic acids as solvents, characterization and enhancement of mechanical and antibacterial properties of wet-spun chitosan fibers*, I conclude that the Author, in the course of the conducted research, confirmed the research hypothesis and realized the scientific objective, making proper conclusions.

In view of the above, I conclude that the dissertation presented for review meets the requirements of the Act of July 20, 2018 Law on Higher Education and Science (Journal of Laws 2021, item 478, as amended), and I am applying to the Council for Academic Degrees of the Technical University of Łódź in the disciplines of mechanical engineering, materials engineering for admission of César Hernández, MSc. Eng., to the further stages of the doctoral dissertation.

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