

## REVIEW

by Prof. Petar Dimitrov Petrov, DSc.  
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Member of the Academic Jury set to render a decision on a procedure for the acquisition of the degree "Doctor of Philosophy" (PhD) in the Professional Field 4.2. Chemical Sciences according to the Classifier of the Areas of Higher Education and the Professional Fields, Scientific Specialty "Polymers and Polymer Materials"

Author of the dissertation: Ina Borislavova Anastasova

Topic: Electrospun hybrid materials of poly(L-lactide-with-D,L-lactide) and chitosan derivatives with directed patterned design for potential applications in biomedicine and for photocatalytic water purification

Supervisors: Prof. Dr. Olya Stoilova and Prof. Dr. Milena Ignatova

This Peer Review is prepared in response to Order № ПД-09-182 of 02.12.2025, issued by the Director of the Institute of Polymers, Bulgarian Academy of Sciences, following the decision made by the Academic Jury that was held on 15.12.2025.

The Review is in compliance with the *Development of Academic Staff in the Republic of Bulgaria Act (DASRB)*, the *Rules for the Application of the Development of Academic Staff in the Republic of Bulgaria Act*, the *Rules of BAS* and with the *Rules set at the Institute of Polymers, Bulgarian Academy of Sciences (IP-BAS)*, for applying the Act aforementioned.

### 1. Biographical information about the candidate

Ina Borislavova Anastasova was born in 1992 in Sofia. She is a graduate of the Faculty of Chemistry and Pharmacy, Sofia University "St. Kliment Ohridski", where she successively obtained the "bachelor" degree (specialty: Ecochemistry; professional qualification - chemist) in 2019 and the "master" degree (specialty: Chemical Engineering and Modern Materials; professional qualification - modern spectral and chromatographic methods of analysis) in 2021. Next, she was enrolled as a PhD student at the Institute of Polymers-BAS in 2021, and in 2024 she was appointed to the academic position of "assistant" at the same institute. In the period 2021 - 2024, she was awarded several awards from scientific forums, including the award for oral presentation at the 4-th Interdisciplinary Doctoral Forum with International Participation, held in Sandanski in 2023. Asst. Anastasova was also awarded by the Union of Chemists in Bulgaria "Professor Ivan Shopov" award for outstanding young scientist in the field of polymers for 2024.

### 2. Assessment of the scientific and research accomplishments of the candidate

The minimum requirements specified in the abovementioned act and rules for the scientific activity of candidates for the acquisition of the scientific and educational degree "doctor of philosophy" include two groups of indicators: Indicator A - dissertation thesis (50 points) and Indicator D - scientific publications (30 points). Ina Anastasova is the author of the present dissertation and co-author of 2 research articles in the journal Polymers, which

belongs to the highest category (Q1, 25 points) of scientific publications, referenced and indexed in world-known databases of scientific information (Web of Science and Scopus). Thus, the sum of the candidate's points under both indicators is 100 (50 + 50), with a required minimum of 80, which formally covers the minimum requirements for the PhD degree.

The dissertation is structured according to the generally accepted rules in the field of chemical sciences and contains the following sections: introduction, literature review, goal and objectives, results and discussion (two separate chapters), experimental part, conclusions, scientific contributions and future work, list of publications and contributions to conferences, and references. The dissertation is written in Bulgarian and is 123 pages long, of which the share of: the literature review is 41 pages; the experimental part – 10 pages; discussion of the results – 36 pages, etc. The systematization and visualization of the results is done with the aid of 35 figures and 1 scheme. In total 323 literary sources are cited, arranged in alphabetical order. The wide time range of the mentioned sources makes a good impression – from the middle of the 20-th century to the present, with sources from the last 15 years prevailing. The literature review provides an overview and critical analysis of the current state of scientific developments in some areas of polymer science, thematically related to the dissertation. The focus is on chitosan and its derivatives and homo- and copolymers of lactic acid, as well as their hybrid fibrous materials obtained by electrospinning and electrospraying. Various potential applications in medicine and environmental protection are also considered. At the end of the literature review, conclusions are drawn regarding various possibilities for enriching and expanding the existing knowledge in the field of fibrous hybrid materials from the above-mentioned polymers and methods. The goal of the dissertation is formulated precisely and clearly. To achieve it, 4 main tasks have been defined. The most essential part of the dissertation is the Results and Discussion section, which is divided into two chapters. The first chapter is devoted to the preparation and characterization of fibrous materials from poly(L-lactide-co-D,L-lactide) and the Schiff base of chitosan (synthesized after the reaction of chitosan with 8-hydroxyquinoline-2-carboxaldehyde). At the next stage, complexes of the fibrous materials with  $\text{Cu}_{2+}$  and  $\text{Fe}^{3+}$  ions were obtained and their antibacterial and anticancer activity was studied. The second chapter presents the results of a study on hybrid fibrous materials containing poly(L-lactide-co-D,L-lactide), quaternized chitosan oligosaccharide and ZnO and  $\text{Fe}_3\text{O}_4$  nanoparticles with different designs, obtained by combining electrospinning and electrospraying. The antioxidant and photocatalytic activity of the systems was studied as well. In the experimental part, data on the materials used, the procedures for the synthesis of chitosan derivatives and various methods for preparation and physicochemical and biological characterization of the obtained systems are presented. The dissertation is completed with six main conclusions and two original contributions.

The preliminary check with specialized software, carried out by the PhD student, shows a very low degree of similarity of the text of the dissertation work, which is proof that it has not been plagiarized.

The topic of the dissertation is modern, and the presented results are original and authentic. They contribute to enriching the existing knowledge in the field of fibrous materials from biodegradable and biocompatible polymers containing (bio)active substances

or inorganic nanoparticles. The current research is a continuation of the long-term work of the team from LBAP at the Institute of Polymers of the Bulgarian Academy of Sciences in this field, while at the same time being characterized by novelty and distinctive elements.

## Chapter 1

A Schiff base of chitosan (Ch-8Q) has been synthesized by reacting the polymer (average viscometric molar mass 380,000 g/mol and degree of deacetylation 80%) with 8-hydroxyquinoline-2-carboxaldehyde (8QCHO). The reaction proceeds under mild conditions, in an acetic-acidic aqueous medium by adding 8QCHO, previously dissolved in ethanol, to a polymer solution. The product was isolated, purified and characterized. Using proton NMR analysis, it was calculated that the degree of substitution was 73%. It was not discussed how the obtained value corresponds to the pre-determined degree of substitution and what is the molar mass of the modified chitosan (higher or lower than the starting chitosan). At the next stage, by using the electrospinning method, new fibrous materials have been obtained from Ch-8Q and poly(L-lactide-co-D,L-lactide) (PLDLLA). The solutions of the two polymers in trifluoroacetic acid were prepared in advance, at a mass ratio of PLDLLA/Ch-8Q of 50:50 and 70:30. It is noteworthy that the measured dynamic viscosity of the solutions decreases in the presence of Ch-8Q, namely: pure PLDLLA - 4200 cP; unmodified chitosan -1900 cP; PLDLLA/Ch-8Q 70:30 - 1700 cP; PLDLLA/Ch-8Q 50:50 - 940 cP. It was noted that the mats obtained from the latter composition are too fragile for biological experiments, and therefore the PLDLLA/Ch-8Q 70:30 composition was chosen for the following studies, in which good processability and mechanical resistance of the fibers is achieved. It is not clear on what basis/analysis this conclusion is made. The analysis of the morphology of fibrous materials with different compositions (pure PLDLLA and its mixtures with unmodified and modified chitosan) clearly shows that the inclusion of Ch-8Q in the composition of the fibers has a negative effect on their uniformity and homogeneity. For example, fibers from pure PLDLLA have an average diameter of  $360 \pm 90$  nm, and those from PLDLLA/Ch-8Q (70:30) have an average diameter of  $187 \pm 128$  nm. The high value of the average standard deviation in the latter is striking. Due to the lack of mechanical test results, it is impossible to assess to what extent the non-uniformity of the fibers has a significant impact on the strength of the new materials. At the next stage of the study, complexes of fibrous materials with  $\text{Cu}^{2+}$  and  $\text{Fe}^{3+}$  ions have been formed. The chemical composition, structure and thermal stability of the complexes and the starting fibrous materials are studied in detail using various methods. Given the fact that the new systems were developed for medical applications, as well as the specificity of DSC analysis, I note that heating above 200 °C is inappropriate. The explanation that the observed broad endothermic peak between 25 and 100 °C is associated with desorption of water or TFA residues is not convincing to me. Biological tests prove high antibacterial (*S. aureus*) and antitumor (MCF-7 and HeLa cells) activity of PLDLLA/Ch-8Q mats and their complexes with  $\text{Cu}^{2+}$  and  $\text{Fe}^{3+}$ . Although such results demonstrate the potential of new systems for the development of biomedical devices, it must be pointed out that experiments with normal (non-cancerous) cells (BALB/c 3T3 mouse fibroblasts), after 72 hours of exposure (incubation), show an worryingly low percentage of cell viability:  $35.3 \pm 4.0\%$  for complexes with  $\text{Fe}^{3+}$  and  $5.4 \pm 3.7\%$  for complexes with  $\text{Cu}^{2+}$ . Only the fibrous materials from LDLLA/Ch-8Q exhibit high cell viability ( $90.0 \pm 12.2\%$ ). It has been

established that when cultivating normal BALB/c 3T3 mouse fibroblasts in the presence of  $\text{Cu}^{2+}$  and  $\text{Fe}^{3+}$  complexes of PLDLLA/Ch-8Q mats, morphological changes of the cells and nuclei characteristic of early and late apoptosis occurred. It is important to note that cytotoxicity is a basic characteristic of any newly developed material intended for use in human and veterinary medicine. In my opinion, despite the high antitumor activity of the complexes, it is necessary to make a more detailed assessment of the conditions and reasonings of their practical application.

## Chapter 2

A complex approach has been applied to obtain hybrid materials with a multicomponent structure, consisting of PLDLLA mat, with embedded  $\text{Fe}_3\text{O}_4$  nanoparticles, in which ZnO nanoparticles were fixed to the fiber surface with the aid of quaternized chitosan (QCOS). The hybrid materials obtained (ZnO/QCOS-on-( $\text{Fe}_3\text{O}_4$ -in-PLDLLA)) possess both photocatalytic and magnetic properties. The key element of the preparation method consists in simultaneous electrospinning of a PLDLLA solution with  $\text{Fe}_3\text{O}_4$  nanoparticles dispersed therein and electrospaying of a QCOS solution with dispersed ZnO particles. It is noted that a mixture of DCM/DMF solvents was used to prepare the PLDLLA solution, in a ratio of 3:1 (v/v), and that in the time of the experiment, no published data on the use of this particular solvent system for electrospinning of PLDLLA are known. Another essential element determining the effective fixation of ZnO nanoparticles on the surface of the fibers is the addition of an optimal amount of QCOS to the solution. By appropriate analyses, it was found that the presence of inorganic particles affects the thermal properties and crystal structure of the electrospun materials. For the first time, the antioxidant activity of fibrous materials containing ZnO was evaluated by DPPH free radical scavenging test, and the photocatalytic activity was studied by degradation of methylene blue (a model organic pollutant) upon irradiation with UV light. The properties of ZnO/QCOS-on-( $\text{Fe}_3\text{O}_4$ -in-PLDLLA) materials were evaluated based on comparison with other fibrous materials containing one or more of the components - PLDLLA,  $\text{Fe}_3\text{O}_4$ -in-PLDLLA, ZnO/QCOS-on-PLDLLA. Using TEM and SEM analyses, the distribution of  $\text{Fe}_3\text{O}_4$  particles inside the fibers and of ZnO particles on their surface was shown. It is evident from the pictures that for both types of particles mainly agglomerates are observed. No (preliminary) study has been described on how the starting amounts of the two types of nanoparticles are selected and whether this is the optimal content. It has been shown that when irradiating an aqueous solution of methylene blue with UV light in the presence of fibrous materials of the type ZnO/QCOS-on-PLDLLA and ZnO/QCOS-on-( $\text{Fe}_3\text{O}_4$ -in-PLDLLA) about 90% of the dye is degraded in 3 hours. Also, the high photocatalytic activity of the hybrid materials is maintained after 5 cycles of use. No data is available on the mechanical properties of the materials.

I accept the scientific contributions formulated by the PhD student regarding the synthesis for the first time of: i) a Schiff base of chitosan by modification with 8-hydroxyquinoline-2-carboxaldehyde, and ii) novel hybrid fibrous materials based on PLDLLA and a chitosan derivative via electrospinning and electrospaying.

### **3. Assessment of the qualities of the extended abstract of the doctoral thesis, whether it correctly reflects the contributions of the doctoral thesis**

The extended abstract has been prepared according to the requirements and contains all the essential results of the dissertation work.

### **4. Opinions, notes and recommendations**

Overall, the dissertation is well-structured, written concisely and easy to read. The results are presented clearly, and their interpretation (with small exceptions) is correct. Data from a wide range of analytical methods are included, which undoubtedly contributes to a more complete characterization of the materials. Aiming to consolidate data from different systems, one of the conclusions made is not sufficiently precise. I consider as speculative the claim that complexes of fibrous materials from PLDLLA/Ch-8Q with  $\text{Cu}^{2+}$  and  $\text{Fe}^{3+}$ , in which the viability of normal cells is too low, are suitable candidates for application as wound healing materials and as effective agents for local treatment of cancer. The logical conclusion from the available data is that these materials are not suitable for medical applications. In my opinion, the original elements of the second scientific contribution relate to the composition and structure of the materials and the mixed solvent used, not to the preparation strategy. I would recommend using the terms molar mass instead of molecular mass; mass ratio instead of weight ratio. I also recommend in forthcoming studies to involve tests on the mechanical properties of fibrous materials, such as tensile strength, elastic modulus, and elongation at break.

Regarding some of the experiments, I have the following questions:

- Concerning the synthesis of the Schiff base of chitosan, why is it necessary to add absolute ethanol to the aqueous solution of the polymer before the dropwise addition of the reagent 8-hydroxy-quinoline-2-carboxaldehyde, which is also dissolved in ethanol?

- In the above-described reaction, chitosan with an average viscometric molar mass of 380,000 g/mol and a degree of deacetylation of 80% was used. How were the moles of polymer (2.5 g, 14.8 mmol) calculated?

- From the provided data on dynamic viscosity (p. 50), the solutions of the Schiff base of chitosan exhibited a lower viscosity than the solution of unmodified chitosan. How would you explain this fact, taking into account that theoretically the molar mass of the modified polymer is higher than that of the starting chitosan?

- After 3 hours of UV light irradiation, the model pollutant was degraded by the hybrid materials, containing ZnO up to 90 - 92%. Is it possible to improve this result and how?

### **5. Conclusion**

According to the documentation presented by the candidate, on her publications reviewed and the above assessment, I recommend on the Academic Jury to render a positive decision for the acquisition of the PhD degree on Ina Anastasova.

**Date: 06.02.2026**

**Reviewer: Petar Petrov**

**Member of the Academic Jury**