



## STATEMENT

by prof. Maria Georgieva Marudova-Zsivanovits, PhD,  
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about a dissertation for awarding of the academic degree “Doctor of philosophy” (PhD)  
Professional field 7.3 Pharmacy, Doctoral program “Technology of dosage forms and  
biopharmacy”

**Author: Nikolay Stanchev Zahariev**

**Form of doctoral studies:** full-time

**Department:** Pharmaceutical Sciences

**Topic: NANOSIZED CARRIERS FOR TARGETED DRUG DELIVERY FOR  
ANTITUMOR THERAPY**

**Scientific supervisor: Assoc. Prof. Bissera Pilicheva, PhD**

### **1. General presentation of the procedure and the PhD student**

The presented set of materials on electronic media is in accordance with Art. 70 (1) of I. Sec. Acquisition of educational and scientific degree "DOCTOR" and scientific degree "DOCTOR OF SCIENCES" at MU-Plovdiv; Regulations of MU-Plovdiv dated 28.01.2021 and includes all necessary documents.

The PhD student has submitted 3 publications and 1 registered utility model.

Nikolay Zahariev graduated as a Master of Pharmacy in 2017, and from 2018 he has worked as an assistant professor at the Medical University - Plovdiv, Faculty of Pharmacy, Department of Pharmaceutical Sciences. He was enrolled as a full-time PhD student in December 2021. In the period 2019–2024, he participated in the implementation of three scientific projects, financed by the National Scientific Research Fund and MU-Plovdiv. He is a leading researcher in project No. HO-15/2020 at the MU-Plovdiv in the field of biopolymer nano-systems for targeted delivery of drugs for antitumor therapy.

### **2. Relevance of the topic**

In recent decades, modern oncology has been striving to develop innovative drug systems that provide targeted delivery of chemotherapeutics, reduce adverse drug reactions and ensure maximum antitumor effect. In this regard, suitable structures that find increasing application are nanoscale drug delivery systems. To be effective, these nanocarriers must have suitable structure, size, biocompatibility, low toxicity and immunogenicity, as well as pass through biological membranes and avoid clearance from the reticuloendothelial system. They must be biocompatible, biodegradable and non-toxic, therefore often the materials used are natural polymers such as proteins and polysaccharides. Despite progress, the development of effective therapies in oncology

remains a challenge. The design of biopolymer nanocarriers with optimal physicochemical and structural-morphological characteristics and a suitable drug release profile is key to the creation of innovative systems for the targeted delivery of chemotherapeutics that are safer and more effective.

The dissertation presented by Nikolay Zahariev examines the preparation and optimization of daunorubicin nanocarriers and the establishment of the biopharmaceutical behavior, the cytotoxic potential and the influence of the included daunorubicin on the spontaneous contractile activity of smooth muscle preparations. The topic of the presented dissertation is relevant, as it establishes the applicability of casein nanoparticles and casein/fucoidan composite structures to be applied as drug-delivery systems. I believe that the purpose of the research is clearly defined, and the specific tasks are fully correlated with its achievement.

### **3. Knowing the problem**

Nikolay Zahariev's PhD thesis contains an excellently structured and comprehensive literature review, divided into 5 main parts. In the first part, it is discussed in detail the role of nano-sized structures as drug-delivery systems, with special attention paid to systems used in antitumor therapy. Their most important properties that make them suitable for such applications are described and their main disadvantages are highlighted. The second part is dedicated to pH-dependent polymer nanocarriers, paying attention to separate classes of natural polymers whose hydrogels and polyelectrolyte complexes possess pH-sensitive behavior. The role of pH in affecting tumor cells is highlighted. The third part examines the structure and properties of casein and its polyelectrolyte complexes in the context of its application as a carrier of medicinal substances. The last two parts are dedicated to the spray drying method, main characteristics of the medicinal substance daunorubicin and the currently existing nanosystems for its inclusion.

The 232 literary sources used show the good literary awareness of the doctoral student. Over 40% of them are after 2015, which is an indication of knowledge of contemporary scientific research on the topic of the dissertation. The conclusions drawn from the literature review allowed the doctoral student to formulate correctly and clearly the goal and the resulting from it tasks that correspond to the topic of the dissertation.

### **4. Research methodology**

Spray drying was chosen as a method for obtaining placebo and daunorubicin-loaded nano-sized structures from casein and casein/fucoidan polyelectrolyte complex, which was previously optimized in terms of optimal dimensions and high production yields. The nanostructures are characterized by state-of-the-art and reliable research methods such as dynamic light scattering, scanning electron microscopy, high-performance liquid chromatography, X-ray powder diffractometry, differential scanning calorimetry, thermogravimetric analysis, FT-IR, etc. A number of tests were conducted to establish the interaction of the developed nanostructures with cells. The chosen methods allow achieving the goal and completing the set tasks. During the research work, the PhD student has mastered various methods of analysis, familiar with modern

analytical methods, which is why I consider that the educational task in the development of the dissertation has been fulfilled.

## **5. Characterization and evaluation of the dissertation work and contributions**

The dissertation is very well laid out and structured, with the individual chapters presented in chronological order on 156 standard pages. In the first 2 chapters of the dissertation, 13 figures and 3 tables are included, and in the results and discussion section own results are presented in 17 tables and illustrated in 37 figures. The studies were conducted with modern scientific equipment, which guarantees reliable results. The obtained data are discussed correctly, which shows that the PhD student has acquired skills in presenting results.

The doctoral student made five contributions to the dissertation work, which I define as original and of high scientific value.

- For the first time, casein-based nanoparticles were developed in which daunorubicin was successfully incorporated.
- For the first time, a polyelectrolyte complex between casein and fucoidan was developed, which was obtained in the form of a nanostructure.
- For the first time, relaxation of smooth muscle was found after administration of casein in micromolar concentrations.
- For the first time, the effect of daunorubicin on the spontaneous contractile activity of smooth muscles was investigated. This study is proposed as an alternative method for evaluating the biological effect of a medicinal substance in real time.

Along with the mentioned scientific contributions, a utility model was registered on the basis of the developed dissertation work, which proves the existence of interest in the application and implementation of the achieved results.

## **6. Evaluation of the publications and personal contribution of the doctoral student**

In the list of publications of Nikolay Zahariev in connection with the dissertation work, three articles are presented, all of which are referenced and indexed in WoS and Scopus. Two of the publications are in the journal *Polymers* (MDPI) with IF = 5.0 and quartile Q1, and the third is in the journal *Pharmaceutics* (MDPI) with IF = 5.4 and quartile Q1. The fact that in all publications the PhD student is the first author, unequivocally illustrates his personal contribution to the scientific developments. Evidence of the high scientific value of the achieved results is the interest of the international scientific community in them, expressed in 14 citations found in the Scopus database. Included in the dissertation materials is a registered utility model "Casein/fucoidan composite nanoparticles as a delivery system for daunorubicin hydrochloride", which confirms the significance of the research and its practical relevance. The results of the conducted research have been reported at six scientific forums, two of which are international.

The publication activity of Nikolay Zahariev meets the requirements for the acquisition of the academic degree "Doctor" and in some respects exceeds them - the minimum national requirements and the minimum requirements of the Regulations of MU-Plovdiv are met, since the

doctoral student has submitted 3 publications in refereed and indexed journals in WoS and Scopus (42 points out of required 30).

I have no critical remarks and recommendations to the conducted research and presented materials. From the point of view of the great applied potential of the subject, I would recommend to continue research in this area with other biopolymeric materials and medicinal substances.

### **7. Abstract**

The abstract (70 pages) corresponds to the content of the dissertation and represents a shortened version of its essence, fully and faithfully reflects the results achieved in the dissertation, as well as their analysis.

### **CONCLUSION**

The dissertation contains scientific, scientific-applied and applied results, which represent an original contribution to science and meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for the Implementation of Law and the Regulations of the Medical University - Plovdiv. The presented materials and dissertation results fully correspond to the specific requirements adopted in connection with the Regulations of the Medical University - Plovdiv for the application of the law.

The dissertation shows that the doctoral student Nikolay Stanchev Zahariev possesses in-depth theoretical knowledge and professional skills in the scientific specialty "Technology of dosage forms and biopharmacy", demonstrating qualities and skills for independent conduct of scientific research.

Due to the above, I confidently give my positive assessment of the conducted research, presented by the above-reviewed dissertation work, abstract, achieved results and contributions, and I propose to the honorable scientific jury to award the academic degree "PhD" to Nikolay Stanchev Zahariev in a doctoral program "Technology of dosage forms and biopharmacy".

21.05.2024

Залчено на основание  
Чл.5 §1, 6, "В" Регламент (ЕС)2016/679

Prepared the opinion: .....  
Prof. Maria Marudova-Zhivanovich, Ph.D