

STATEMENT

by

Prof. Dr. Reni Emil Kalfin, PhD

on the dissertation for the award of the Educational and Scientific Degree '**Doctor of Philosophy**'

Professional Field 7.3 "**Pharmacy**"

Doctoral Program "**Technology of Dosage Forms and Biopharmacy**"

Author: Nikolay Stanchev Zahariev

Form of Doctoral Training: Full-time Doctoral Studies

Department: Pharmaceutical Sciences, Faculty of Pharmacy at Medical University - Plovdiv

Topic: NANOSIZED CARRIERS FOR TARGETED DRUG DELIVERY FOR ANTITUMOR THERAPY

Scientific Supervisor:

Assoc. Prof. Bissera Pilicheva, PhD – Medical University of Plovdiv

1. General presentation of the procedure and PhD student

The author of the dissertation is master pharmacist Nikolay Stanchev Zahariev - a full-time doctoral student in the Department of Pharmaceutical Sciences at the Faculty of Pharmacy of the Medical University of Plovdiv, supervised by Associate Professor Biseria Pilicheva, PhD.

The set of materials presented by the doctoral student includes the following documents:

- curriculum vitae in European format with the doctoral student's signature
- a notarized copy of a higher education diploma
- orders for enrollment in doctoral studies and for deduction with the right of defense
- certificate of completion of studies at the Doctoral School of MU-Plovdiv and received credits
- protocol from the departmental council for the preliminary discussion of the pre-dissertation work and the decisions taken for the disclosure of the procedure and for the composition of the scientific jury
- dissertation work
- abstract
- a list of scientific publications on the topic of the dissertation
- copies of scientific publications
- list of participations in scientific forums
- list of noticed citations

- declaration of originality and authenticity of the attached documents

Zahariev's documents are in full compliance with Art. 70 (1) of section one "Acquisition of Educational and Scientific Degree "DOCTOR" at Medical University - Plovdiv and the Regulations of MU-Plovdiv dated 28.01.2021. During his studies at the Doctoral School, the PhD student Nikolay Zahariev received a total of 141 credits with a mandatory minimum of 129 credits.

In connection with the dissertation thesis, the PhD student has enclosed three publications of research articles and certificate of one utility model to his documents.

I declare that I have no conflict of interest within the meaning of Art. 4 (paragraph 5) of the Law on the Development of the Academic Staff in the Republic of Bulgaria and I have no publications in common with Nikolay Zahariev.

Nikolay Stanchev Zahariev was born on August 12, 1993 in Zlatograd. In 2018, he completed a five-year course of study at the Medical University - Plovdiv and obtained the educational and qualification degree "Master" in the specialty "Pharmacy". Nikolay is a member of the team of six scientific research contracts and is a beneficiary of the National Science Program "Young Scientists and Postdoctoral Fellows". He has a good level of written and spoken English. Nikolay is responsible for scientific activity and international cooperation at the Department of Pharmaceutical Sciences of the Medical University - Plovdiv.

On December 15, 2021, assistant Nikolay Zahariev was enrolled in full-time doctoral studies, and on March 22, 2024, he was dismissed with the right of defense.

2. Actuality of the topic

The topic of the dissertation submitted to me for an opinion is related to one of the most serious challenges facing modern biomedical science - the search for new antitumor drugs with high efficiency and good biological tolerance, which target cancer cells. In a report by the International Cancer Research Agency at the World Health Organization, it was noted that for the last 6 years, cancer patients have increased by 28%. Prognosis, based on various mathematical models predict a high growth (about 60%) of morbidity until 2040, and in poor countries it will be significantly higher - about 81%. The fact that cancer is the second leading cause of death should not be overlooked. All this turns tumor diseases into one of the most

widespread epidemic non-infectious socially significant diseases. Despite the great success in the development and application of a number of operative and conservative methods and the indisputable successes achieved, the problem of cancer remains in the focus of the scientific community. The main problems accompanying the modern chemotherapy of malignant neoplasms are the high toxicity and the phenomenon of multiple drug resistance. All this necessitates the search for new agents with targeted antineoplastic action and good biological tolerance. The development of innovative drug systems to provide targeted delivery of cytostatics to the target tumor cells, thus achieving a maximum antitumor effect on one hand and limiting adverse drug reactions on the other, is of priority importance. Everything that has been said so far shows the actuality of the dissertation work in a scientific and scientific-applied sense and justifies the need for the conducted research.

3. Knowing the problem

The question of the doctoral student's knowledge on the problem finds its answer in the literature review presented by him. At the beginning of the literature review, Nikolay presents the main characteristics of nanoparticles as drug delivery systems and summarizes in a table which of them are approved for clinical application in cancer. The PhD student emphasizes that the goal in developing nano-sized drug delivery systems is to optimize their pharmacokinetic profile and stability by controlling the rate and extent of drug release, as well as limiting the influence of environmental factors.

Due to the presence of ionizable functional groups, polymer nanocarriers can undergo reversible or irreversible structural changes depending on the environment pH. Unlike other stimulus-sensitive carriers, the activity of pH-sensitive nanocarriers does not depend on external factors, but is determined solely from variations in body pH. The PhD student has well summarized in a table the pH variations both along the gastrointestinal tract and also in endosomes and lysosomes in healthy and cancer cells, respectively.

Among the most commonly used pH-sensitive polypeptides are the milk proteins lactoglobulin and casein. Therefore, the third section of the literature review is dedicated to the family of calcium-phosphate binding phosphoproteins (casein) as a carrier for drug delivery systems. The effects of pH on the structure of the casein micelle and the incorporation of the dosage form into this micelle are graphically

presented. The PhD student focuses on protein cross-linking strategies and types of cross-linking agents.

In the fourth chapter of the literature review, Nicolay summarizes the methods for the preparation of polymeric nanoparticles and discusses extensively spray drying as a fast, easy and reproducible method for the preparation of micro- and nano-sized carriers.

The fifth section of the literature reference is logically devoted to daunorubicin and its application in antitumor chemotherapy, since the aim of the dissertation work is to develop a model of a drug delivery system for the targeted delivery and controlled release of daunorubicin from the carrier, which will lead to the limitation of unwanted drugs reactions during therapy.

In general, the literature review is written with experience and knowledge. In conclusion, I positively evaluate the analytical approach of Nikolay Zahariev, his knowledge on the investigated problem and the creative evaluation of the literary material.

4. Research methodology

The casein particles were obtained by a coacervation method followed by spray drying. On the basis of the optimal conditions for the preparation of casein nanoparticles, four models of nanoparticles loaded with daunorubicin were developed by varying the polymer:drug ratio. The PhD student obtained the casein/fucoidan composite nanoparticles by polyelectrolyte complexation, cross-linking of the complex with glutaraldehyde and subsequent spray drying. Based on predetermined optimal conditions for the preparation of casein/fucoidan composite nanoparticles, three models of daunorubicin-loaded nanoparticles were developed at a casein:fucoidan ratio of 1:1 and varying the concentration of the crosslinker 1% (w/v), 2% (w/v), 3% (w/v) and the sputtering intensity (30%, 40%, 50%). The size, distribution and zeta potential of the obtained particles were determined by dynamic light scattering. A scanning microscope was used to visualize the surface morphology of the particles, while the shape of the particles was examined using a transmission electron microscope. The size of the nanocomposite microparticles and their size distribution were determined by laser diffraction with a dry and wet dispersion analyzer. The effect of various substances on mitochondrial function was reported using the MTT test. The phase state of the medicinal substance was investigated by powder X-ray diffractometry, thermogravimetric and differential thermal analysis and differential scanning calorimetry. Changes in the spontaneous

contractile activity of smooth muscle preparations after exposure to daunorubicin and daunorubicin-loaded nanoparticles were also investigated.

In conclusion, the PhD student uses a very wide range of experimental methods that allow to arrive at an answer to the tasks solved in his dissertation work.

5. Characteristics and evaluation of the dissertation and contributions

Dissertation thesis is written on 156 pages according to the standard scheme and includes all the sections recommended for obtaining the educational and scientific degree "Doctor" at the Medical University - Plovdiv. The dissertation is properly constructed, very well written and richly illustrated with 50 figures and 20 tables. The bibliography consists of 232 correctly described literary sources that meet the tasks and objectives of the dissertation.

The RESULTS AND DISCUSSION chapter is the most important part of the PhD student's work. The experimental results are presented clearly and concretely, following the progress of the set goals and objectives. The obtained results are numerous and their scientific and applied importance will grow. Discussion of the results frames the PhD student as a knowledgeable researcher. Once again, the logic of the research conducted is evident in the discussion, and directions for future experiments can be seen.

CONCLUSIONS AND CONTRIBUTIONS summarize the experimental data obtained by the PhD student. In his dissertation, Nikolay reached original results. One of the most important contributions is that a casein-based nanoparticle model was proposed for the first time to incorporate the chemotherapeutic daunorubicin. The proposed model for the study of the spontaneous contractile activity of smooth muscles is original, in order to be an alternative to the *in vitro* studies on the degree of release, allowing the evaluation of the biological effect in real time.

Of a contributing nature is the developed drug delivery system combining casein and fucoidan in the same composite nanostructure.

It should be noted the practical orientation of the obtained results in the dissertation work, which are of interest to medical practice.

6. Assessment of the PhD student's publications and personal contributions

Nikolay Zahariev personally participated in the conducted dissertation research, in the experimental work and in the statistical processing of the obtained data. Proof of the doctoral student's personal involvement are the publications he

attached in connection to the topic of the PhD thesis, in all of which he is the first author. Dissertation contributions noted by the PhD student are his own work, obtained with the support and assistance of the supervisor.

As it is a tradition among the graduates of Prof. Pilicheva, all articles related to the dissertation thesis have been published in journals with the highest Q1 quartile, and are visible to the international scientific community. In this case the articles have already been cited 14 times. Last year, an utility model with authors Nikolay Zahariev and Bisera Pilicheva was also registered.

The PhD student has presented results obtained during the development of his dissertation work at four conferences in Bulgaria and at two symposiums for young scientists in Szeged, Hungary. In all these messages on scientific forums, Zahariev is the first author.

7. Dissertation abstract

The abstract fully corresponds to the content of the dissertation thesis, and from it one can get a very good idea of the investigations performed. The abstract is well designed. All main results from the dissertation work, their analyses and discussion are included. As in the dissertation itself, the graphic materials and their statistical processing are precisely performed.

CONCLUSION

In conclusion, the dissertation thesis of assistant Nikolay Zahariev is dedicated to an actual problem for pharmacy and medical practice. When performing the tasks, Nikolay shows a very good knowledge of the experimental setups and methods, maturity in the presentation and interpretation of the results, and in-depth professional skills in the scientific specialty "Technology of dosage forms and biopharmacy". During the development of the dissertation, Nikolay Zahariev obtained original experimental data, which have not only scientific value, but are of interest for medical practice.

The presented results and scientific publications in connection with the dissertation fully meet the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its Application and the Regulations of the Medical University - Plovdiv for the application of the Law on the Development of the Academic Staff in the Republic of Bulgaria.

According to the sufficient amount of presented material, the overall layout of the thesis, the demonstrated qualities and skills for independent performance of

research by the PhD student, and the obtained results of an original contribution character, evidence of which, in addition to the three publications with the highest quartile, is also the registered utility model, I give my positive assessment and with conviction I do propose to the honorable members of the Scientific Jury to award the Educational and Scientific degree "Doctor of Philosophy" to Nikolay Stanchev Zahariev in the doctoral program "Technology of dosage forms and biopharmacy" in professional field 7.3. "Pharmacy".

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

21 May 2024

Reviewer: ✓

Prof. Reni Kalfin, PhD