

REVIEW

by Prof. Denitsa Bogomilova Momekova, DSc.

Faculty of Pharmacy, Medical University of Sofia

on

a dissertation for awarding the educational and scientific degree

"Doctor of Philosophy" in the Field of higher education 7. Health care and sports

Professional field 7.3 Pharmacy and Doctoral program "Technology of drug dosage

forms and biopharmacy"

Author: Nikolay Stanchev Zahariev – full time doctoral student

Department: Pharmaceutical Sciences

Topic of the dissertation: *"Nanosized carriers for targeted drug delivery for anti-tumor therapy"*

Scientific supervisor: Assoc. Prof. Bissera Pilicheva, PhD – Faculty of Pharmacy, Medical University of Plovdiv

This Review is prepared in response to Order № P 1175/ 16.04.2024 issued by the Rector of University of Plovdiv and the decision of the first meeting of the scientific jury, held on 19th April 2024. The Review is in compliance with the Act of Development of Academic Staff in the Republic of Bulgaria (DASRB), the General regulation on its implementation and the Institutional regulations for the application of the aforementioned law at Medical University of Plovdiv.

1. General presentation of the procedure and the doctoral student

The set of materials presented by doctoral student Nikolay Stanchev Zahariev is in full compliance with Art. 70 (1) of Section I. 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 the following documents:

- Application to the Rector of MU-Plovdiv for disclosure of the procedure for the defense of a dissertation work
- 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 credits received

- a protocol of the departmental council for the preliminary discussion on 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 of the dissertation
- a list of scientific publications on the topic of the dissertation
- copies of scientific publications
- list of participations in scientific forums
- declaration of originality and authenticity of the documents attached

In connection with the dissertation, the doctoral student has presented three scientific publications. All documents submitted by the doctoral student are in full compliance with the Regulations for the procedure for acquiring scientific degrees and holding academic positions in the Republic of Bulgaria, as well as in accordance with the procedure for acquiring the Doctorate of MU-Plovdiv.

2. Brief biographical data of the PhD student

Nikolay Stanchev Zahariev was born on August 12, 1993. In 2017, he completed his higher education at the Faculty of Pharmacy of the Medical University of Plovdiv, obtaining a master's degree in pharmacy. In 2018, he joined the Department of Pharmaceutical Technologies at the Faculty of Pharmacy of the Medical University of Plovdiv as an Assistant Professor. Since 2022 and currently, Nikolay Zahariev specializes "Drug Technology and Biopharmacy" at the Medical University - Plovdiv. In parallel, Nikolay Zahariev attended several additional classification courses on "Protection and humane treatment of experimental animals used for scientific or educational purposes" at the Faculty of Veterinary Medicine at Thrace University - St. Zagora as well as a training course in Electron Microscopy, organized by the Bulgarian Crystallographic Society. The PhD candidate has a very good command of written and spoken English.

By order of the Rector of MU-Plovdiv dated 17th of December 2021 Nikolay Zahariev is enrolled in full-time doctoral studies at the Department of Pharmaceutical Technologies at the Faculty of Pharmacy, MU-Plovdiv, and on 01.04. 2024 has been deducted with the right of defense.

3. Actuality of the topic and appropriateness of the set goals and tasks

The concept aimed at providing optimal concentrations of the drugs to its respective targets is called drug targeting. One of the most promising approaches for the practical realization of the concept of targeted drug delivery and, respectively, overcoming the disadvantages associated with drugs of a small therapeutic width and/or unfavorable pharmacokinetic properties, is based on their encapsulation in nanosized carriers. An important advantage of the nanocarriers are the possibilities they offer for surface modification and functionalization, which allows optimization of their colloidal stability, pharmacokinetics and tissue distribution. In the recent years, the development of nanoparticles has expanded to a wide range of clinical applications. However, despite their intensive research, a serious problem in the technological and therapeutic aspect is their unsatisfactory efficiency of delivering drug molecules in therapeutically adequate concentrations to the target compartments. This necessitates the development of a new generation of nanosized vehicles, the

so-called intelligent nanosystems allowing targeted release of the encapsulated drugs as a result of a stimulus by the body (e.g. change in pH, enzyme composition, etc.).

In this regard, the idea of optimizing the delivery of the anthracycline antibiotic daunorubicin by incorporating it into nanoparticles based on natural, biocompatible and biodegradable polymers - casein and fucoidan, owing to the potential for pH-dependent targeted drug release is of great scientific and practical importance, which undoubtedly demonstrates the relevance of the topic of the dissertation work.

4. Knowing the problem

Evidence of the doctoral student's knowledge of the problem can be found in the literature review of the dissertation work, which is written on 43 pages with 232 cited references, a volume fully sufficient to review the latest trends in the scientific field of the dissertation. A large part of the cited literature comes from the last 5 years, which illustrates an in-depth look at the current state of the problem. The review is structured in 5 subsections. In the first and second sections, the main advantages of nanoscale carriers, as well as the challenges associated with them, are discussed in detail. The latest trends in the design of nanoscale platforms for drug delivery are also outlined, namely the development of stimulus-sensitive carriers with targeted drug release. The third subsection shows the possibilities of using casein as a natural biodegradable and biocompatible polymer for the preparation of nanoparticles, by means of various cross-linking agents or as a result of electrostatic interactions with suitable polyelectrolytes. A subsequent subsection of the review details the prospects of spray drying as a method for obtaining reproducible populations of nanosized carriers of high drug loading efficiency and optimal physicochemical characteristics. In the last section, the pharmacological properties of daunorubicin and its application in antitumor chemotherapy, as well as approaches to reduce the severity of its unwanted effects, by means of its encapsulation in nano-sized carriers, are discussed.

The literature review is very well and logically structured, and modern trends in the design of nanoscale vehicles are logically summarized, which is proof of the good theoretical knowledge of the problem by the candidate.

5. Research methodology

The Materials and Methods section of the dissertation is very well written and detailed enough to allow easy reproduction of the described methods. The well-chosen experimental design and the use of modern methods for the preparation and characterization of daunorubicin-loaded nanoparticles are impressive. The dissertation has also used the advantages of multifactorial design to evaluate the simultaneous influence of several variables on key characteristics of the obtained carriers, allowing the selection of optimal process parameters to obtain reproducible populations of nanoparticles with high loading efficiency and desired physicochemical characteristics. All this makes it possible to obtain correct results and conduct research at a high scientific level.

6. Characteristics of the dissertation

The dissertation presented to me for review contains 156 pages, illustrated by 20 tables and 50 figures and 232 literary sources cited. The dissertation includes the following sections: introduction, table of contents, list of abbreviations used, literature review, aim and objectives, materials and methods, results and discussion, conclusions, contributions

and bibliography. The structure of each section is appropriate as the individual subsections follow the logic of the experimental work performed and the commented results.

The purpose of the dissertation work is clearly formulated based on the critical analysis of the scientific literature on the subject of the dissertation. For successful completion of the set goal, eight tasks are formulated, the solution of which gives the opportunity to achieve practical results.

Undoubtedly, the most important part of the dissertation work are the results of own research and their discussion, described in 62 pages and richly illustrated with 37 figures and 16 tables. The experimental results logically follow the implementation of the set tasks and can be summarized as follows:

- Design and detailed characterization of empty and daunorubicin-loaded casein nanoparticles. Nikolay Zakhariyev used the coacervation method with subsequent spray drying as the main method for their preparation. To find the optimal conditions for obtaining nanoparticles of high loading and desired physicochemical characteristics, the doctoral student used a full 3^2 factorial design. As a result of the analysis, it was found that the concentration of casein and the crosslinking agent has the greatest influence on the yield of nanoparticles, their sizes and loading efficiency - with optimal concentrations of 1% and 1.5 M, for casein and CaCl_2 , respectively.
- Using the optimal composition of casein and crosslinking agent, nanoparticles loaded with daunorubicin were prepared at varying drug to polymer ratio, and based on the data obtained on the encapsulation efficacy and loading capacity, the doctoral student concluded that the optimal ratio of polymer: daunorubicin was 25:1.
- The prepared drug-loaded nanoparticles were subjected to detailed physicochemical characterization, and it was found that the concentration of the polymer and the crosslinking agent also influence the morphology of the obtained particles. They are dense and spherical at the higher concentrations of casein and crosslinking agent and toroidal in shape at the lowest concentrations tested.
- After the evaluation of the release profiles, a pH-dependent release of daunorubicin was found in some of the developed formulations. This gives the doctoral student reason to continue research in the direction of developing nanocarriers with targeted delivery of daunorubicin to the tumor interstitium, thus a second type of nanocarriers based on casein and fucoidan, with the hypothesis being that the modification of the surface of the casein particles with a fucoidan coating, will result in a stalling of the release of daunorubicin in the systemic circulation, while providing a controlled release at the tumor site. The main contribution of Nikola Zahariyev in this direction is the derivation of the optimal composition of nanoparticles (ratio of casein and fucoidan), as well as optimal technological parameters for obtaining particles with desired physicochemical characteristics and high loading efficiency. As a result of the factorial design, a mathematical model was derived, characterized by a very good correlation between the predicted and obtained experimental data, which is of extreme practical importance and allows accurate prediction of the response at different levels of variation of the technological parameters.
- As a next step in the research, a detailed oncopharmacological evaluation of the elaborated daunorubicin loaded nanoparticles was conducted in a comparative way vs. free drug. The obtained results show that the encapsulation of daunorubicin leads to optimization of its cytotoxic activity. The mechanism by which the developed nanosized systems cause eradication of the treated tumor cells was proven to be by generation of ROS

and disruption of the integrity of mitochondrial membranes and hence, compromise of the normal functioning of these cell organelles.

The results obtained by the doctoral student show the undoubted potential of the developed nanocarriers as promising nanoplatforms for targeted delivery of daunorubicin.

During the evaluation of the dissertation, I was impressed by the skillful analysis and discussion on the obtained results made by Nikolay Zahariev, proving the in-depth knowledge of the subject by the doctoral student.

The last section of the dissertation contains eight well-defined conclusions, fully corresponding to the essence of the work, which I fully accept.

7. Contributions and significance of the PhD thesis for science and practice

The contributions of the dissertation summarized by Nikolay Zahariev are scientific and scientifically applied in nature. I would like to draw attention to 3 of them, which I consider particularly important:

- For the first time, nanocarriers for the targeted delivery of daunorubicin based on casein and casein:fucoïdan were developed and characterized in detail. The Nanocarriers obtained are characterized by optimized cytotoxic activity as compared to the free drug and are able to release the drug in controlled manner.
- Casein-induced smooth muscle relaxation has been investigated and proven for the first time.
- A model has been developed for the evaluation of the spontaneous contractile activity of smooth muscles, allowing the evaluation of the biological effect in real time.

I believe that the contributions of the dissertation work, summarized by the doctoral student, are to a significant extent his own work, of course with the assistance of his scientific supervisor - Associate Professor Bisera Pilicheva. For the significance of the results obtained and the prospects for their practical implementation certifies the implemented useful model.

8. Scientific-metric indicators related to the dissertation

The doctoral student, Nikolay Zahariev, presents 3 (three) publications summarizing the most important results of the dissertation work. It should be noted that all three articles are published in journals of a high impact factor and rank Q1 for polymer science and pharmacy, which unequivocally shows the significance and relevance of the conducted research. Nikolay Zahariev has presented reports on the topic of the dissertation at six national and international scientific forums. It should be noted that both in the publications and in the announcements from the scientific forums, the doctoral student is the first author, which once again testifies the significant contribution and personal involvement of Nikolay Zahariev in the development of the presented dissertation work.

9. Summary of the dissertation

The summary is written on 66 pages and includes the set aim and tasks, the description of the methods used, the obtained original results and an adequate discussion, the conclusions and contributions made. It was prepared according to the requirements laid down

in the relevant normative documents and fully corresponds to the content of the dissertation.

10. Recommendations and remarks

The dissertation is written very well and is readable with pleasure. The interpretation of the obtained results is convincing. I have no significant remarks, but only one suggestion for future research of the candidate on the subject. Given the pronounced colloidal instability of nanosized carriers, an integral element of their detailed technological and biopharmaceutical characterization is the evaluation of their stability under storage and prediction of shelf life. In this regard, my recommendation to the PhD student, for his future similar research, is to plan and conduct stability studies in order to complete the cycle of developing new nanoscale carriers.

CONCLUSION

The dissertation of PhD Nikolay Zahariev, entitled "*Nanosized carriers for targeted drug delivery for antitumor therapy*" contains original results with a contribution in the field of the targeted delivery science. The in-depth analysis and indisputable quality of the dissertation work show that the doctoral student has in-depth theoretical knowledge and professional skills in the field of the scientific specialty "Pharmaceutical technology and Biopharmacy" and in particular, in the development and characterization of nanosized drug delivery systems. Nikolay Zahariev shows erudition and professional skills for the independent formulation of scientific hypotheses, conducting research and correctly summarizing and interpreting the obtained results.

I believe that the candidate fully meets the quantitative criteria for awarding educational and scientific degree "Doctor", as defined in the relevant regulatory framework, namely the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the General regulation on its implementation and the Institutional regulations for the application of the aforementioned law at the Medical University of Plovdiv.

Based on the above, I confidently give my positive assessment of the dissertation work and recommend to the honorable members of the scientific jury to vote for awarding the educational and scientific degree "Doctor" to Nikolay Stanchev Zahariev in the professional field 7.3 Pharmacy and the scientific specialty "Technology of dosage forms and biopharmacy".

Заличено на основание
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Sofia

Reviewer

/Prof. Denitsa Momekova, DSc/