REVIEW

From

Prof. Krum Stefanov Kafedjiiski, PhD

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(External Member of the Scientific jury based on order No. R-109-469/03 Jul 2025 of the Rector of Medical University-Varna

REGARDING: Procedure for the defense of thesis for awarding the educational and scientific degree "doctor", Area of higher education 7. Healthcare and sports, PD 7.3. Pharmacy, in the doctoral program "Pharmacology (incl. Pharmacokinetics and chemotherapy"

Thesis title:

"Self-emulsifying drug delivery systems as a method to enhance the intestinal permeability of alendronate sodium"

Author of thesis:

Mag. Pharm. Ivaylo Konstantinov Pehlivanov- PhD student in regular form of education in Medical University- Varna

Scientific supervisors:

Prof. Kaloyan Georgiev, PhD, DSc, Assoc. Prof. Velichka Andonova, PhD

GENERAL OVERVIEW OF THE PROCEDURE

The set of documents presented by Mag. Pharm. Ivaylo Konstantinov Pehlivanov is in accordance with the requirements of the Regulations for the Development of the Academic Staff at the Medical University "Prof. Dr. P. Stoyanov" - Varna, Chapter II, Section IV - Conditions and Procedure for Acquiring ONS "Doctor". He is a full-time PhD student enrolled with Order No. R-109-547/03 Dec 2021 in the Department of Pharmacology, Toxicology and Pharmacotherapy, Faculty of Pharmacy, with scientific supervisors Prof. Kaloyan Georgiev, Ph.D, DSc and Assoc. Prof. Velichka Andonova, Ph.D. Ivaylo Pehlivanov has fulfilled all the requirements regarding full-time thesis, which is evident from the submitted documents. He was discharged with the right to defense by Order No. R-109-269/18 June 2025.

BRIEF BIOGRAPHICAL DATA OF THE CANDIDATE

Ivaylo Konstantinov Pehlivanov was born on August 24, 1979 in Varna. He graduated from secondary school No. 8 "Al. S. Pushkin". In 2001 he graduated from the Medical College of the Medical University - Varna and acquired the specialty "assistant pharmacist" - professional bachelor. In 2009, he obtained the title of "Master in Pharmaceutical Chemistry and Technology" from the Faculty of Pharmacy at the State University of Milan of Sciences. In 2020, he acquired a specialty in "Technology of drugs with Biopharmacy" at the Faculty of Pharmacy of the Medical University, Sofia. Ivaylo Konstantinov Pehlivanov's career development as an assistant professor began in 2016 at the Department of Pharmaceutical Technologies of the Medical University of Varna, where he was engaged in teaching and research work in the field of "Technology of Dosage Forms with Biopharmacy". In 2021, he was enrolled as a full-time PhD student in the doctoral program "Pharmacology (incl. pharmacokinetics and chemotherapy)", Department of "Pharmacology, Toxicology and Pharmacotherapy" of the Faculty of Pharmacy at MU-Varna with order No. R-109-547/03.12.2021.

Significance of the topic

The topic of the PhD thesis of Mag. Pharm. Ivaylo Konstantinov Pehlivanov is relevant and in line with the scientific directions at the Medical University - Varna, being in a multidisciplinary field with a main emphasis on pharmaceutical technologies and pharmacology. The PhD thesis itself examines the development of a dual self-emulsifying drug delivery system, providing increased bioavailability of alendronate sodium during its oral administration. Extensive research has been conducted to optimize the composition of the dual self-emulsifying drug delivery system of sodium alendronate (SDEDDS-NaALD). Technological and biopharmaceutical characterization of SDEDDS-NaALD formulated in hard gelatin capsules was also performed. *In vivo* studies were also conducted to determine the amount of NaALD excreted in the urine of laboratory animals after oral administration of optimized model formulations of SDEDDS-NaALD.

Structure of the PhD thesis

The PhD thesis of Mag. Pharm. Ivaylo Konstantinov Pehlivanov covers 140 pages with 34 figures and 33 tables included. It contains the following main sections: Introduction - 1 page; Literature review - 41 pages; Aims and objectives - 2 pages; Materials and methods - 17 pages; Results and discussion - 40 pages; Conclusions - 1 page; Scientific contributions - 1 page; Bibliography - 23 pages, covering 302 publications.

Literature review

The literature review is reasoned, detailed, up-to-date and directly related to the topic of the PhD thesis and includes an analysis of the oral route of drug administration, the available gastrointestinal barriers, as well as the mechanisms of drug transport across cell membranes. Bisphosphonate derivatives as the first

choice for initiating anti-osteoporosis therapy are reviewed in detail. Routes of administration of sodium alendronate are also discussed. Further on, individual formulation approaches are discussed to increase oral absorption and limit the undesirable gastrointestinal effects of alendronate. Special attention is paid to Self-emulsifying systems, examining their structure, individual excipients, their classification and the mechanism of self-emulsification, preparation methods and compatibility studies between drugs and excipients. *In vitro* drug release, *in vitro* membrane permeation, as well as *in vivo* studies on rats are described in detail.

Goals and objectives

The goal of the PhD thesis is to develop w/o/w dual self-emulsifying drug delivery system, providing increased bioavailability of alendronate sodium during oral administration of the dosage form. It is clearly formulated, and the tasks set for its implementation correspond to the goal.

Materials and methods

A wide range of research methods was used, which shows that the PhD student demonstrates in-depth theoretical knowledge of their essence. The PhD student successfully handles with modern experimental methods such as UV-Vis spectrophotometric method for quantitative determination of alendronate sodium including its validation, determination of water/oil partition coefficient (Kdistr), two-step emulsification technique for preparation of model compositions of SDEDDS-NaALD. The droplet size of SDEDDS-NaALD after dispersion and their size distribution were also evaluated using ZetaSizer, the compatibility between the components of the selected model formulations was evaluated using FT-IR analysis, as well as their rheological properties using a viscometer. Pharmacopoeial tests were also conducted to control hard gelatin capsules containing SDEDDS-NaALD, as well as their in-vitro characterization. The permeation of SDEDDS-NaALD through the Franz diffusion cell (type 3) was also

studied. The oral bioavailability of NaALD from SDEDDS was also determined by determining the quantity of drug excreted in the urine of male white rats. An HPLC-UV-Vis method was also used for the qualitative and quantitative determination of NaALD in biological matrices.

Results and discussion

The conducted own research is presented on 40 pages and is illustrated with 33 Tables and 34 Figures. The research is based on the logically set objectives, as follows:

1. Adaptation of a UV-Vis spectrophotometric method for the quantitative determination of NaALD, included in the studied SDEDDS models, which has been validated according to four main indicators: linearity, accuracy, precision and sensitivity. The influence of polysorbate 80 on the absorption spectra of the ALD/Fe complex was further investigated. The results show that the presence of polysorbate 80 in the medium may compromise the application of the methodology for the determination of NaALD in SDEDDS models. A solution to the problem is the removal of polysorbate 80 from the medium before the analysis.
2. Optimization of the composition of SDEDDS with included NaALD was conducted, determining the solubility of NaALD in lipids, as well as the water/oil partition coefficient (Kdistr). Further in the course of the work, the critical HLB of the primary W/O emulsion was determined, as well as the composition of the emulsifier pairs based on an assessment of their physical stability after centrifugation. In addition, the PhD student skillfully established, using pseudo-three-phase diagrams, the optimal amount of hydrophilic emulsifier to the primary emulsion PE1 in the composition of SDEDDS, and additionally studied the influence of excipients on the stability of the primary emulsion PE1. In addition, the ratios of the secondary emulsifier to the primary emulsion PE2 were also determined.

3. Model formulations of SDEDDS-NaALD were also determined, as well as their physical and thermodynamic stability was assessed. The compatibility of sodium alendronate with selected excipients in the composition of SDEDDS-NaALD was also studied.
4. The time for self-emulsification of SDEDDS-NaALD was determined, as well as the size of the droplets of the dispersed phase after self-emulsification of SDEDDS-NaALD.
5. The rheological parameters of SDEDDS-NaALD were determined.
6. Technological and biopharmaceutical characterization of the SDEDDS-NaALD formulated in hard gelatin capsules was performed, determining the uniformity of the mass and uniformity of dosage units of the hard gelatin capsules, as well as the self-emulsification time and dispersibility of the incorporated SDEDDS-NaALD in biomimetic media.
7. *In vitro* prediction of the permeation of NaALD from hard gelatin capsules containing SDEDDS-NaALD was also performed. For this purpose, diffusion through a dialysis membrane and through a PermeaPad® biomimetic membrane was investigated.
8. Studies on the oral bioavailability of NaALD from SDEDDS were also performed by determining the amount of drug excreted in the urine of male Wistar rats. In addition, the HPLC-UV/Vis analytical method for the detection of NaALD in biological matrices was validated.
9. Studies have also been conducted to monitor the oral bioavailability of NaALD from SDEDDS by determining the amount of drug excreted in the urine of male white Wistar rats.

All results of the conducted research are thoroughly analyzed and discussed. It is particularly impressive that at the end of each subsection of the chapter "Results and Discussion" the relevant conclusions are drawn with precision and thoroughness by the PhD student. In conclusion, the conclusions that arise from the results obtained from each objective set in the thesis are clearly and precisely formulated.

Conclusions

The conclusions that arise from the results obtained from each of the objectives set are formulated clearly and precisely.

Evaluation of the contributions of the PhD thesis

The contributions are clearly formulated and presented, with those of a scientific and applied nature prevailing.

The most significant contributions of the thesis can be summarized as follows:

1. Chemically, physically and thermodynamically stable SDEDDS with NaALD (7%, w/w) were formulated, based on coconut oil, polysorbate 80, sorbitan monooleate, phosphatidylcholine, gelatin and water, which self-emulsify in an aqueous medium (0.1N HCl) to form microemulsions.
2. A UV-Vis spectrophotometric method for the quantitative determination of NaALD incorporated into lipid-based drug delivery systems via complex formation with Fe3+ has been validated.
3. The diffusion model with a biomimetic membrane used to study the permeation of NaALD from SDEDDS is suitable for predicting the behavior of the system in vivo.
4. NaALD is included in SDEDDS with improved permeability of the drug substance through biological membranes
5. The developed new approach for incorporating NaALD into SDEDDS with improved permeability of the drug through biological membranes is suitable for further scaling up in industrial conditions.

Publications and participation in scientific forums on the topic of the PhD thesis

The results of the PhD thesis of Mag. Pharm. Ivaylo Pehlivanov are reflected in 2 full-text articles in English, one of which is in the journal IMAB with SJP 0.115

(2020), as well as in the journal Proceedings of science with SJP 0.115 (2023). An article was also published in the official scientific journal of the Medical University of Varna - Varna Medical Forum. Evidence of 3 participations in scientific forums is presented: 2 national and 1 international (Belgrade, Serbia).

Abstract

The abstract has been prepared in accordance with the requirements and includes an introduction, aim and objectives, materials and methods, results and discussion. The presented research and discussion fully reflect the main results achieved in the PhD thesis. The conclusions coincide with those in the PhD thesis. The scientific contributions are also included, as well as a list of publications and participation in scientific forums in connection with the PhD thesis. The abstract itself includes the essence of the problem, the research conducted and the interpretation of the results obtained, as well as the formulation of the relevant conclusions and generalizations.

Critical remarks and recommendations

I have no critical remarks.

CONCLUSION

The submitted PhD thesis for the acquisition of the ONS "doctor" of Mag. Pharm. Ivaylo Pehlivanov, a full-time PhD student at the Medical University "Prof. Dr. P. Stoyanov" - Varna, meets the requirements, and is focused on a specific interdisciplinary field and will be of interest to the pharmaceutical industry.

After I became acquainted in detail with the presented set of materials and documents on the procedure for defending the PhD thesis of Mag. Pharm. Ivaylo Pehlivanov, I believe that the topic, volume, results obtained, conclusions drawn and contributions formulated fully meet the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for the Implementation of the LDASRB (RILDASRB), and the

Regulations of the Medical University "Prof. Dr. P. Stoyanov" - Varna for a PhD program, and are also the result of the author's own research and development under the expert guidance of the tandem of scientific supervisors.

Considering the above arguments and the presented PhD thesis, I give a **POSITIVE ASSESSMENT** and recommend to the members of the respected Scientific Jury to vote for awarding the **Educational and Scientific degree of "Doctor"** to Mag. Pharm. Ivaylo Pehlivanov in the scientific specialty **"Pharmacology (incl. pharmacokinetics and chemotherapy)", professional direction 7.3. "Pharmacy".**

Заличено на основание чл. 5, §1, б. „В“ от Регламент (ЕС) 2016/679

Sofia,

28.07.2025

/Prof. Krum Stefanov Kafedjiiski, PhD/