

## **REVIEW**

**by Prof. Milen Ventsislavov Dimitrov, Ph.D.**

**Vice Dean for Academic Affairs for students studying in Bulgarian and English, Medical University – Sofia, Faculty of Pharmacy**

**External member of THE SCIENTIFIC JURY**, appointed according to Order No. P - 109-476 / 18. 12.2024 of the Rector of the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna, in the procedure for acquiring the educational-qualification degree "PhD" at the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna, in the field of higher education 7. "Healthcare and Sports", in the professional field 7.3. "Pharmacy" and PhD program "Pharmacology (incl. pharmacokinetics and chemotherapy)",

**On the dissertation work of:** Assist. prof. Ioana Nikolova Sotirova - PhD student in full-time study at the Department of Pharmacology, Toxicology and Pharmacotherapy

**On Topic: "Nanostructured lipid carriers with included Hypericum extract perforatum L. for application to the skin and accelerated wound healing"**

Scientific supervisors: Prof. Kaloyan Georgiev, D.Sc. and Assoc. Prof. Velichka Andonova, Ph.D.

### **GENERAL PRESENTATION OF THE RECEIVED DATA**

The set of materials presented by assistant prof. Ioana Nikolova Sotirova, is in accordance with Art. 69 for Regulations on the Academic Staff development at Medical University "Prof. Dr. Paraskev Stoyanov" - Varna, regulating the terms and conditions for holding academic positions- Varna/08.07.2024.

The dissertation contains 123 pages, illustrated with 17 tables and 38 figures. The bibliography includes 387 literary sources. I have not found any gaps in the documentation submitted by Joanna Sotirova regarding the procedure. I declare that I have no joint scientific work with the doctoral student.

### **BRIEF BIOGRAPHICAL DATA AND CAREER DEVELOPMENT**

Yoana Nikolova Sotirova graduated from the Mathematical High School "Atanas Radev", Yambol, Profile: Mathematics and Informatics in 2015. She obtained a specialty "Pharmacy" - educational and qualification degree "Master", at the Faculty of Pharmacy of the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna in 2020. The career development of MSc. Pharm. Yoana Nikolova Sotirova, as an assistant professor, began in

2021 after winning a competition at the Department of Pharmaceutical Technologies, Faculty of Pharmacy, Medical University "Prof. Dr. Paraskev Stoyanov" - Varna, with teaching and research work in the field of "Pharmaceutical Technology and Biopharmacy". In 2024, she acquired a specialty in "Pharmaceutical technology and Biopharmacy". MSc. Pharm. Yoana Nikolova Sotirova was discharged with the right to defense by Order R-109-476 of 18.12.2024.

## **DISSERTATION EVALUATION**

### ***Relevance of the topic***

The topic of the dissertation is extremely relevant and in accordance with the priority scientific areas developed at the Medical University " Prof. Dr. Paraskev Stoyanov " - Varna, and the doctoral work is multifaceted and multidisciplinary, with a main emphasis on pharmaceutical technologies and pharmacology - *in vivo*. study of the wound healing potential of the developed semi-solid dosage form on an excision wound model in experimental animals, integrating elements of pharmacognosy and pharmaceutical analysis.

The dissertation examines the development of nanostructured lipid carriers containing St. John's wort extract, intended for dermal application and accelerated wound healing.

### ***Structure of the dissertation***

#### **Volume and sections**

The dissertation of Msc Pharm. Yoana Nikolova Sotirova is written on 123 pages and is illustrated with 38 figures and 17 tables. The dissertation is structured in accordance with the adopted European and national standards. It contains the following main sections: Introduction – 1 p.; Literature review – 37 p.; Aims and objectives – 2 p.; Materials and methods – 12 p.; Results and discussion – 33 p.; Conclusions – 2 p.; Scientific contributions – 1 p.; Bibliography – 26 p. The bibliography covers an impressive 387 publications. References for publications in scientific journals, participation in scientific forums and scientific projects related to the dissertation are also attached.

### **Literature review**

The literature review covers an in-depth analysis of the skin with its anatomical structure and physiological protective functions, focusing on wounds and outlining the stages of wound healing and the main factors influencing this process. Further, it presents a summary of the characteristics of St. John's wort, focusing on its phytochemical composition and pharmacological effects, along with the historical information and botanical characteristics



presented. Special attention in the literature review is paid to nanoscale drug delivery systems, which in my opinion are the fundamental center of the conducted research and the achieved positive results.

The information regarding semi-solid dosage forms for application to the skin, in their diversity, possibilities and challenges during application, is also adequately and completely reflected.

*The literature review is comprehensive, well-argued, and includes 387 literary sources that are contemporary, related to the topic of the dissertation, and accurately reflect the current state of the problem, which also demonstrates the good theoretical preparation of the doctoral student.*

### **Purpose and objectives**

The goal of the dissertation is clearly and precisely formulated – development of nanostructured lipid carriers containing St. John's wort extract rich in HP, intended for dermal application and accelerated wound healing.

The tasks set for its implementation are formulated precisely and clearly and correspond to the set goal, which shows that the doctoral student has a thorough knowledge of the research problem..

### **Materials and methods**

A wide range of research methods have been mastered and used. The doctoral student skillfully handles and successfully applies numerous, modern experimental methods, such as high-performance liquid chromatography, DLS and ELS methods, X-ray diffraction analysis, ATR - FTIR analysis, transmission electron microscopy, texture analysis, viscometry. The antiviral effect (cytotoxicity, infectious viral titer, effect on viral replication, virucidal action, influence on viral adsorption) and antimicrobial (antibacterial and antifungal) activity of NLC and HP - NLC was evaluated. It was carried out in “in vivo study” of the wound healing potential of a gel containing St. John's wort extract rich in HP and a gel containing HP - NLC and determined antioxidant status (antioxidant capacity and degree of oxidative stress).

### **Results and discussion**

The results obtained are presented concisely, clearly and well-illustrated with 21 figures and 11 tables, included sequentially in 4 sections, covering a total of 13 subsections.

To obtain an extract of St. John's wort rich in hyperforin ( HP ), the doctoral student turns to the classical maceration approach, which is further modified in order to limit the influence of light and atmospheric oxygen in the extraction process. The emphasis in this part of the doctoral work is the use of a validated HPLC - UV protocol with excellent linearity and high sensitivity (limit of quantification of HP  $1.0 \mu\text{g} / \text{ml}$  ) for qualitative and quantitative analysis of HP in the obtained extracts, as well as the traced influence of the solvation module on the extraction yield.

Further in the course of the work, the doctoral student developed and characterized twenty formulations of unloaded (placebo) nanostructured lipid carriers, using high-speed homogenization and subsequent ultra sonification, in order to evaluate the influence of the composition and experimental conditions on their characteristics.

A study was conducted to determine the average size, polydispersity index and zeta potential, which are important parameters in terms of the selection of optimal formulations for further work. In addition, the doctoral student established the influence of experimental conditions (homogenization rate, duration of ultrasonication and ultrasonication temperature) on the stability and physicochemical characteristics of the proposed NLC formulations, in order to select the optimal nanocarriers in which to load the HP-rich St. John's wort extract.

Additional analyses were performed on the selected systems loaded with HP-rich St. John's wort extract to determine shape and internal morphology (TEM), degree of crystallinity (XRD ), loading efficiency. and extract-lipid interactions (IR).

The studied lipid nanosuspensions loaded with HP-rich St. John's wort extract were also evaluated for antiviral activity, initially determining the non-toxic concentration range, and then sequentially evaluating the effect on viral replication, virucidal activity, influence on viral adsorption and their antimicrobial efficacy. It has been proven that the established antimicrobial activity of HP- NLC nanosuspensions is due entirely to the extract loaded in them, and not to any of their structural components.

In addition, loading of HP-rich St. John's wort extract into nanocarriers leads to an increase in the stability of HP, a compound characterized by extremely low chemical stability, which is further evidence of the practical contribution of the developed dissertation work.

The development of a semi-solid dosage form (biphasic hydrogel) as a carrier of the lipid nanosuspension HP- NLC 4 loaded with St. John's wort extract, rich in HP, determined as optimal as a result of the conducted studies, and its characterization is a natural continuation and upgrade of the studies already conducted, providing an opportunity to complete the cycle for developing a medicinal product for cutaneous application, while at the same time allowing



to overcome some technological difficulties related to the properties of the nanosuspension. The prepared model bigels were evaluated according to the visual appearance, homogeneity of the distribution of the two phases, pH, physical stability, mechanical properties - spreadability, hardness, cohesiveness, adhesiveness, rheological properties.

Held in live studies, using an excisional wound model, of the wound healing potential of the finished dosage form (HP- NLC - BG 2 ) determined to be optimal as a result of the conducted studies are a natural conclusion to the developed dissertation work and pave the way for potential application of the developed form in clinical practice.

Wound healing was also monitored in dynamics, through visual observation and photography of the wound areas. Plasma antioxidant status, antioxidant capacity and degree of oxidative stress were assessed, and from the results obtained it can be reasonably assumed that the antioxidant effect of the developed dosage form is mainly achieved by binding free radicals.

*The doctoral student's ability to analyze and interpret the obtained results and complex dependencies makes an excellent impression, which gives me reason to believe that she is already an established researcher.*

*The discussion of the obtained results in the "Results and Discussion" section is done with understanding, precision and thoroughness, which demonstrates the excellent theoretical preparation of the doctoral student, as well as her ability to think multifacetedly, analytically and critically.*

### **Conclusions and scientific contributions**

The conclusions /seven in number/ and the contributions /seven in number - five of a scientific-theoretical nature and two of a scientific-applied nature/ are adequately formulated and are a logical aggregation of the in-depth analysis and interpretation of the data obtained.

### **Evaluation of the contributions of the dissertation work**

The contributions of the dissertation work clearly formulated and correctly presented, of a predominantly scientific-theoretical nature, but also including those of a scientific-applied nature.

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

- ✓ An optimal lipid composition was derived and experimental conditions established for obtaining stable nanostructured lipid carriers ( NLCs )
- ✓ For the first time, NLCs, including glyceryl- based ones behenate and almond oil/borage oil, are loaded with HP-rich St. John's wort extract (> 40%).
- ✓ It has been developed to be optimal in terms of average particle size and size distribution, zeta potential, degree of crystallinity, loading efficiency, physical stability, in vitro antimicrobial efficacy and antiviral activity of NLC carrier of HP-rich St. John's wort extract
- ✓ For the first time, bigel has been used as a semi-solid carrier for dermal application of NLCs, including those loaded with HP-rich St. John's wort extract.
- ✓ A bigel has been formulated based on a poloxamer 407 HP-NLC4 nanodispersion gelled with borage oil and sorbitan monostearate, with mechanical and rheological characteristics optimal for application to the skin.
- ✓ An original protocol has been developed to obtain a St. John's wort extract with a high HP content (> 40%)
- ✓ An innovative semi-solid dosage form has been prepared - a combination of biphasic gel and NLCs (gelled with poloxamer 407 lipid nanodispersion and borage oil structured with sorbitan monostearate), for dermal application of HP-rich St. John's wort extract.

#### **Publications and participation in scientific forums related to the dissertation work**

The results of the dissertation work of MSc Pharm. Yoana Sotirova are reflected in 2 full-text, real articles in English, one of which is in the journal Gels with IF 5.0 (2023), according to JCR on Clarivate analytics, and the other in a magazine Scripta Scientifica Pharmaceutica - official scientific journal of the Medical University - Varna. Evidence of 3 participations in scientific forums, 2 national and 1 international (Malmö, Sweden) is presented. The list of publications and scientific activity of the doctoral student is in accordance with the requirements and reflects the application of the methodologies, as well as the results of his own research and proves the dissemination of scientific information obtained in connection with it.

#### **Author's abstract**



The author's abstract is written in accordance with the requirements of the regulations, in a concise form, with an adequate volume and aptly summarizes the content of the dissertation.

### **Project financing**

The fact that the research on which the dissertation is based has received financial support from the Science Fund of the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna makes an excellent impression.

### **CONCLUSION**

The dissertation on the topic: " Nanostructural lipid carriers with included Hypericum extract perforatum L. for application to the skin and accelerated wound healing ", authored by Assist. prof. Msc Pharm. Ioana Nikolova Sotirova, is up-to-date, original and meets the requirements for awarding the educational and scientific degree "PhD". The dissertation contains scientific-theoretical and scientific-applied results that represent an original contribution to science and meet the on the requirements of the of the of the Act on the Development of the Academic Staff of the Republic of Bulgaria, the Regulations for its implementation and the Regulations on the Academic Staff development at Medical University "Prof. Dr. Paraskev Stoyanov" - Varna.

The dissertation shows that the doctoral student, Assist. prof. Ioana Nikolova Sotirova, possesses theoretical knowledge and professional skills in the scientific specialty "Pharmacology (incl. pharmacokinetics and chemotherapy)", by demonstrating qualities and skills for independently conducting scientific research.

The presented dissertation and the attached publications confirm the personal contribution of the doctoral student and demonstrate his excellent knowledge of scientific issues. The doctoral student has also mastered a large number of modern research methods, which allows her to convincingly present the obtained results, skillfully analyzing and interpreting them in order to formulate the relevant conclusions and contributions.

In view of the above, I give my **POSITIVE ASSESSMENT** of the research conducted, presented in the above-reviewed dissertation, Author's abstract, achieved results and contributions, and **I PROPOSE** to the esteemed scientific jury to award the educational and scientific degree of 'PhD' to Assist. Prof. Ioana Nikolova Sotirova in the scientific specialty "Pharmacology (incl. pharmacokinetics and chemotherapy)".

Sofia,  
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/ ..... /  
Prof. Milen Dimitrov, Ph.D.



