R E V I E W

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Member of the Academic Jury set to render a decision on a procedure for the acquisition of Academic Degree "Doctor of Science" (DSc)
in the Professional Field 4.2. Chemical Sciences according to the Classifier of the Areas of Higher Education and the Professional Fields (Scientific Specialty "Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances", 01.05.10)

Author**:** ***Assoc. Prof. Dr. Vanya Mantareva***, Institute of Organic Chemistry with Centre for Phytochemistry, BAS

Dissertation topic: **Phthalocyanine photosensitizers for photodynamic method towards drug resistance**

1. Subject of review

The set of materials presented by Assoc. Prof. Dr. Vanya Mantareva is in accordance with the Regulations for development of the academic staff of Institute of organic chemistry with sentre of photochemistry (IOCCP), BAS and meets the criteria for obtaining the degree "Doctor of Science" in accordance with the specific rules of IOC in the scientific field 4 Natural sciences, mathematics and computer science; professional field 4.2. Chemical sciences, determined according to art. 1a, para. 2 of the Regulations for application of the Law on the Development of Academic Staff in the Republic of Bulgaria Act (DASRB).

The realisation of the required criteria is based on:

Indicator A: To have the academic degree "Doctor" - 50 points,

Indicator B: presented dissertation for the National Assembly "Doctor of Sciences" (100 points),

Indicator D: A list of 21 scientific papers, 18 (№ 2-16, 18, 20, 21), of which are publications in journals with impact factor, one chapter of a monograph (№ 1) of Nova Science Publishers as well as two publications (№ 17 and № 19) that are not referenced and indexed in Web of Science or Scopus. The total number of points on this indicator is (371 points) with a required 150 points. No information is provided on the use of these publications in other dissertations for the degree of Doctor of Science.

Indicator E: A list of citations in scientific journals indexed in world-famous databases is presented.

128 citations (without auto-citations from all authors) were noted on the publications included in the dissertation, which are equal to 256 points.

According to these indicators, Assoc. Prof. Dr. Vanya Mantareva exceeds the minimum requirements, including in her dissertation scientific papers and citations of these papers, which give a total of 777 points, with a required 500 points.

2. Brief biographical data

Assoc. Prof. Dr. Vanya Nikolova Mantareva was born in Sofia. In 1990 he graduated as a chemical engineer at UCTM-Sofia, then in 1991 he started working as a specialist chemist at IOHCF. During the period 1995-1998 she prepared and defended a dissertation for the acquisition of ONS-doctor. During the period 1991-2014 he worked consecutively as a chemist and researcher. III-I st., And in 2014 he was habilitated as an associate professor at the same institute. After defending her doctoral dissertation, Assoc. Prof. Mantareva specialized several times at the Institute of Macromolecular Chemistry, University of Bremen, Germany (under Prof. Dieter Vörle); University of Luifil, Luifil, Kentucky, USA (under Prof. Alan Morgan) and the Autonomous University of Madrid, Spain in the group of Prof. Thomas Torres.

3. Relevance of the topic and expediency of the set goals and objectives

The dissertation developed by Assoc. Prof. Mantareva is in an extremely interesting and topical scientific field, developing intensively in recent years, such as antibacterial photodynamic therapy. This type of therapy has established itself as a clinical method with non-specific and local effects, with a rapid effect after its application and without the development of resistance to pathogenic microorganisms, due to the peculiarities of the mechanism of photodynamic action. Therefore, finding suitable effective compounds that can be used as photosensitizers is a current scientific field, not only purely fundamental, but also fully applied. On the other hand, more and more research is aimed at optimizing the structure of already known photosensitizers, in order to find the best physicochemical and photobiological properties that contribute to the effect of photodynamic action.

Assoc. Prof. Mantareva has formulated the main goal of her dissertation as a summary of what has been achieved so far in order to establish new scientific knowledge in the development and study of phthalocyanine metal complexes as photosensitizers with photodynamic activity. In connection with this, in the development of the dissertation, the goals are formulated in 4 main directions:

1. Development and study of new phthalocyanine derivatives obtained as complexes of Lu (III), Sn (IV), Pd (II), Zn (II), Si (IV) and Ni (II) and with substituents of 1) chromophore groups; 2) biologically active compounds and 3) inhibitors for pathogenic microorganisms.
2. Finding new synthetic and reproducible approaches in order to obtain other compounds outside the group of phthalocyanine.
3. Investigation of basic chemical, physicochemical and photobiological properties of phthalocyanine derivatives with known and developed new specific approaches for photoactive compounds with optical properties such as phthalocyanines.
4. Finding the connection between molecular structure and photodynamic activity in resistant pathogenic microorganisms.

4. Knowledge of the problem

The literature review shows that Assoc. Prof. Mantareva knows very well the state of research in the field of photodynamic therapy and the use of phthalocyanines as photosensitizers in pathogens with pronounced drug resistance. The literature review focuses on the use of phthalocyanides as photosensitizers in biomedicine from the first studies in the literature to the present day. The application of the method of photodynamic therapy is considered as a new effective approach used in clinical practice and the role of light for the generation of active reactive particles. Particular attention is paid to the fight against resistant microorganisms to antibiotics used in clinical practice, which in recent years has become a major global problem. It is safe to say that the materials cited in the dissertation are correctly and correctly interpreted.

5. Research methodology

The research methods in the dissertation can be grouped in three main directions: synthesis of new phthalocyanine derivatives and their metal complexes, photophysical and photochemical characterization of the obtained compounds, and in vitro microbiological studies against gram-positive and gram-negative bacteria and fungal strain of the genus Candida.

The phthalocyanine compounds used as photosensitizers are photoactive and their functional characteristics are determined by spectral methods of analysis such as absorption and fluorescence spectroscopy, life in the excited state with which well-interpreted results are obtained in solving the goal in the dissertation.

Model pathogenic microorganisms such as gram-positive and gram-negative bacteria and Candida fungi were selected to demonstrate the photodynamic activity of the test compounds.

6. Characteristics and evaluation of the dissertation

The dissertation contains 203 pages and it is illustrated with 63 figures, 27 diagrams and 14 tables. 367 literature sources were used. It reflects the research of Assoc. Prof. Mantareva on

the synthesis, functional properties and photodynamic activity of new phthalocyanines and their metal complexes in the period 2015-2020.

Assoc. Prof. Mantareva's research is innovative and has both fundamental and scientifically applied character.

In the description of the dissertation the classical scheme is used, which in my opinion is more suitable for ONS "Doctor", including three chapters:

The first chapter is a Literary Review (31 pages with cited 157 references), which briefly shows the main structures of phthalocyanines used in the study of photodynamic therapy, the nature of the method and its application in the treatment of resistant pathogens to antibiotics used in clinical practice.

The second chapter, which has a monographic character, is the main and most important in the dissertation is Results and discussion. It covers 108 pages and cites 205 references. This chapter describes the main schemes and methods for the preparation of cationic phthalocyanine complexes with substituents in non-peripheral and peripheral groups, silicon complexes with axial groups, Zn (II) phthalocyanines with biologically active groups based on amino acids, carbohydrates, sterols and parabens. . The preparation of hybrid structures containing hydrophobic phthalocyanines and titanium dioxide or polymer brushes has also been described. The main photophysical characteristics of the synthesized compounds, including the lifetime in the excited singlet state, have been studied in detail, as an important indicator determining the photodynamic processes. The third part covers studies related to proving the photodynamic activity of the synthesized compounds against gram-positive (*Staphylococcus aureus, Staphylococcus mutans, Enterococcus faecalis*) and gram-negative (*Pseudomonas aeruginosa*) bacterial and fungal strain (conducted by *Candida albicans*, colleagues from the Institute of Microbiology, BAS.

The third chapter is entitled Experimental Part. It gives the materials used, equipment, technique, formulations for the preparation of the compounds, their characterization. In my opinion, this part is absolutely unnecessary for such a dissertation, as it is described in detail in the published scientific papers and has passed through the relevant competent reviewers of the respective journals.

7. Contributions and significance of the development for science and practice

The dissertation of Assoc. Prof. Mantareva reflects the research over the past five years on the synthesis and functional properties of new phthalocyanine compounds and their metal complexes for use as photosensitizers in antibacterial photodynamic therapy with potential biomedical applications. That is why I evaluate the results obtained in the dissertation, not only

as significant theoretical and experimental achievements, but also with high potential for practical application.

The main scientific achievements obtained during the development of the dissertation can be grouped as:

I. 40 new phthalocyanine derivatives and their metal complexes have been obtained, and along with traditional zinc and silicon ions lutetium, tin, palladium and nickel ions have been used for the first time in order to obtain highly effective photosensitizers. In 16 of the metal complexes, pyridyloxy or quaternized methylpyridyloxy groups are located in peripheral and non-peripheral positions of the ring molecule. In 24 of the obtained new compounds there are various substituents such as: amino acids (tyrosine, phenylalanine, arginine and lysine), carbohydrates (galactopyranose), sterol (estradiol), methyl-, ethyl-, propyl- and butyl parabens. The preparation is described and the properties of hybrid structures with titanium dioxide and with polymers are investigated.

II. Based on known chemical reactions, original synthetic schemes have been developed, proposing new reaction conditions for the production of new metal complexes, as well as bioconjugates of zinc phthalocyanine complexes with the following molecular groups as substituents:

a) with amino acids linked through an aminophenoxy group by an amide bond;

b) with carbohydrates and parabens, by direct coupling with an ether bond;

c) with carbohydrates and sterols linked through the azidoethoxy group of the triazole ring.

For the preparation of the new compounds, original approaches were used, such as:

a) coupling the bioactive molecule as a functional group to phthalonitrile at the 3- or 4-position and subsequent cyclotetramerization to phthalocyanine;

b) binding to four or eight of the possible peripheral and non-peripheral positions of the ring phthalocyanine molecule;

III. An original experimental setup for conducting experimental studies in the light spectrum has been created and the main optical physicochemical characteristics of the new phthalocyanine derivatives have been found. The obtained results of the main photophysical (absorption and fluorescence) and photochemical (singlet oxygen generation and photostability) properties prove the potential of the developed new photosensitizers for biomedical applications with photodynamic method.

IV. A method for pharmacokinetic studies based on chemical extraction and measurement of the fluorescence signal intensity with quantification of the accumulation, retention and clearance of phthalocyanine compounds due to long-wavelength fluorescence has been developed by fluorescence spectroscopy. overlaps with cellular fluorescence. By this method, a relatively high accumulation was found in resistant Gram (+) and Gram (-) pathogenic bacteria, and for a fungal strain of Candida albicans, as well as in formed pathogenic biofilms. In addition, accumulation selectivity has been demonstrated, with a quantitative increase without redistribution in cell membranes at an irradiation spectrum of 365 nm and 635 nm.

V. In vitro photobiological studies with pathogenic microorganisms were performed with the synthesized compounds, and a permissible concentration range for the application of phthalocyanine leads (0.1 - 20 μM), permissible radiation doses (12 - 60 J.cm -2) and optimal irradiation energy (50 - 100 mW.cm -2) without thermal or other side effect as a result of light irradiation. The developed methodology is also applied to study the photodynamic efficiency of other photosensitizers and other pathogenic microorganisms, to determine the effectiveness of inactivation.

8. Evaluation of the publications on the dissertation

The overall scientific production of Assoc. Prof. Vanya Mantareva includes 61 scientific articles. According to Scopus, these papers are cited a total of 699 times (excluding self-citations from all authors), with h index = 14. In the dissertation for the degree of "Doctor of Science" included 21 scientific papers published between 2015 and 2020, 18 of them are publications in scientific journals, referenced and indexed in the world-famous Web of Science and Scopus databases, one paper is a chapter in a book by Nova Science Publishers, and two papers are publications in SRJ publications. The publications of Assoc. Prof. Mantareva do not repeat those published in connection with the "Doctor" degree and her habilitation as an associate professor. According to the attached reference, the articles participating in the description of the dissertation are cited 128 times. This shows the relevance of the research topic and the very good international response of the research, given the relatively short five-year period.

All 21 scientific papers included in the dissertation are interdisciplinary in nature, which is related to the participation of teams of specialists in various scientific fields, and in most of them involve its foreign partners. In 6 of the publications Assoc. Prof. Mantareva is the first, and in 13 she is a corresponding author, including in the chapter of a book. This shows her

significant contribution in the presented works. The distribution of scientific papers by quartiles is as follows: Q1 - 6, Q2 - 3, Q3 - 6, Q4 - 3 and 2 are in SJR journals.

Also attached is a list of participanion in 12 international scientific conferences with reports presented personally by Assoc. prof. Mantareva.

9. Abstract

The abstract accurately and clearly reflects the results obtained during the development of the dissertation and is made according to the requirements of the rules of the Institute of Organic Chemistry with the Center for Phytochemistry and BAS.

10. Critical remarks and recommendations

The new compounds and their metal complexes were identified by IR, Mas and ^{1}H NMR spectroscopy. Why they are not also characterized by ^{13}C NMR spectroscopy, given that their solutions have been made?

Some scientific terms have used incorrectly.

11. Personal impressions

I have no specific personal impressions of Assoc. prof. Dr. Vanya Mantareva.

12. Recommendations for future use of dissertation contributions and results

Given the relevance and importance of the research conducted by Assoc. prof. Dr. Mantareva, I recommend continuing research in this field in the search for new chromophore systems.

Conclusion

The disertation contains scientific, scientific-applied and applied results, which represent an original contribution to science and meet the requirements of the Law for Development of the Academic Staff in the Republic of Bulgaria. The presented materials and dissertation results fully comply with the specific requirements of the Regulations of IOCCF-BAS. The dissertation thesis shows that Assoc. Prof. Dr. Vanya Mantareva has theoretical knowledge and professional skills in the scientific specialty 4.2 Chemical Sciences, "Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances" demonstrating qualities and skills for conducting research with obtaining of original and significant scientific contributions. That is why I do recommend on the members of the scientific jury to render a positive decision for the acquisition of the **Academic Degree "Doctor of Sciences"** on **Assoc. Prof. Dr. Vanya Mantareva** by professional field 4.2. Chemical sciences, "Bioorganic chemistry, chemistry of natural and physiologically active substances".

02.08.2021 Reviewer:

prof. Ivo Grabchev, DSc