REVIEW

by

Prof. Dr. Eng. Rayna Georgieva Bryaskova
University of Chemical Technology and Metallurgy - Member of the Academic Jury

set to render a decision in a procedure for the acquisition of the Academic Degree Doctor of Philosophy (PhD)

in the Professional Field 4.2. Chemical Sciences according to the Classifier of the Areas of Higher Education and Professional Fields (Scientific Specialty: Polymers and Polymer Materials)

Author of the dissertation work: assist. Prof. Erik Vasilev Dimitrov

Title of the dissertation work: Macromolecular design and synthetic strategies for the preparation of polymers with applications in transport and delivery of biologically active substances and oligonucleotides

Scientific supervisors: prof. DSc Stanislav Rangelov, assoc. prof. Natalia Toncheva-Momcheva, PhD

This review has been prepared in accordance with Order No. RD-09-54 of 07.04.2026 issued by the Director of the Institute of Polymers, Bulgarian Academy of Sciences, following the decision of the Academic Jury held on 22.04.2026, and in compliance with the Development of Academic Staff in the Republic of Bulgaria Act (DASRB), the Rules for the Application of the Development of Academic Staff in the Republic of Bulgaria Act, the Rules for the Acquisition of Academic Degrees and Occupation of Academic Positions at the Bulgarian Academy of Sciences, and the Rules for the Acquisition of Academic Degrees and Occupation of Academic Positions at the Institute of Polymers, Bulgarian Academy of Sciences.

1. Biographical information about the candidate

Erik Vasilev Dimitrov was born in 1999 in Kyustendil, Bulgaria. He graduated from the Medical University - Sofia in 2024. In 2019, he started working as a laboratory technician at the Institute of Polymers, Bulgarian Academy of Sciences, where his activities focused primarily on the synthesis and characterization of polymers and the investigation of their supramolecular organization. In 2024, he was appointed as assistant professor at the Institute of Polymers and continued his research activity in the field of polymers and polymer materials.

During this period, Erik Dimitrov participated in a number of national competitions and received several prestigious awards. Among the most notable are: the BAS Award “Ivan Evstratiev Geshov” for young scientists under the age of 30 in the scientific field “Nanoscience, New Materials and Technologies” (2025); the “Prof. Ivan Shopov” Award of the Union of Chemists in Bulgaria for Outstanding Young Scientist in the Field of Polymers (2024); and the Academician Ivan Yuhnovski Award for Outstanding Young Scientist in the Field of Organic Chemistry (2024). He

has also received multiple first prizes for poster presentations at national and international scientific conferences.

2. Assessment of the Scientific and Research Accomplishments of the Candidate

2.1. Assessment to meet the minimal criteria in accordance with Specific the Rules for Granting Academic Degrees set at the Institute of Polymers, Bulgarian Academy of Sciences, Application 1.

The minimum requirements defined in the Rules for the Application of the Development of Academic Staff in the Republic of Bulgaria Act and in the regulations of BAS and the Institute of Polymers for obtaining the educational and scientific degree "Doctor of Philosophy" include two groups of indicators: Indicator A – submitted doctoral thesis (50 points) and Indicator G – scientific publications (30 points). Under Indicator A, assist. prof. Dimitrov submits the present doctoral thesis. Under Indicator G, the candidate presents a total of five publications, all published in prestigious international journals indexed in SCOPUS/Web of Science and ranked in Q1 quartile journals, corresponding to a total of 125 points, significantly exceeding the required minimum of 30 points. Additionally, assist. prof. Dimitrov reports achievements under Indicator D with a total of 30 points and Indicator E with a total of 120 points, although these indicators are not mandatory for obtaining the PhD degree. The overall score therefore amounts to 325 points, substantially exceeding the minimum requirements for acquisition of the educational and scientific degree "Doctor of Philosophy".

2.2. Assessment of the Requirement that the dissertation work contains valuable theoretical or applied scientific results corresponding to contemporary scientific achievements and representing an original contribution to polymer science

The dissertation work is structured in a classical format and consists of nine chapters: Introduction, Literature Review (42 pages), Aim and Objectives of the Study, Experimental Part (35 pages), Results and Discussion (59 pages), Conclusions, Contributions, Appendix, and References, comprising a total of 170 pages. The thesis contains 78 figures and 13 tables. The bibliography includes 153 references, the majority of which have been published after 2010, indicating the relevance and up-to-date character of the scientific background. **The literature review** is comprehensive and appropriately developed in relation to the scope and objectives of the doctoral work. It follows a logical sequence beginning with general aspects of polymers and approaches to their synthesis and subsequently focusing on contemporary methods for the preparation of block copolymers. Particular attention is devoted to so-called "click chemistry" reactions, including azide-alkyne, thiol-ene and Diels-Alder reactions, which subsequently form the methodological basis of the experimental investigations. The review further discusses representative biocompatible polymers such as polyethylene glycol (PEG), polyglycidol (PG), poly(ε-caprolactone) (PCL), as well as various pH- and temperature-responsive systems and their potential application in drug delivery. The final section addresses phospholipids and their synthetic analogues, providing the theoretical basis for the subsequent development of polymer-lipid conjugates. The literature review concludes with a summary that clearly demonstrates the need for developing new platforms for the delivery of therapeutic agents and genetic material through combining different synthetic

approaches, including controlled radical polymerization, ionic polymerization and click chemistry, aimed at creating hybrid systems with novel functional characteristics.

The aim of the dissertation work is clearly derived from the literature review and focuses on the development and application of new synthetic strategies for the preparation and modifying of various macromolecular systems with potential biomedical applications as nanocarriers of biologically active substances and/or vectors for nucleic acid delivery. The objectives of the dissertation work logically follow from the stated aim. The experimental section is developed clearly and systematically, incorporating advanced synthetic and analytical methods for the preparation and characterization of novel polymer and polymer-lipid systems. The chosen experimental approaches align with the stated aims and objectives, enabling the verification of the structure and properties of the obtained materials. The Results and Discussion section represents the primary scientific contribution of the dissertation and integrates the findings from the synthesis, characterization, and investigation of the self-association processes and supramolecular structure formation of the prepared materials. The first chapter presents the results related to the preparation of polymer-lipid conjugates using click chemistry based on azide-alkyne cycloaddition reactions. Initially, the synthesis and appropriate functionalization of the lipid analogue 1,3-dihexadecyloxypropan-2-ol (DHP) were carried out, followed by the synthesis and functionalization of a series of biocompatible linear polymers (PG, PEG, PiPOx, PTBA, and PETEGA). The obtained functionalized products were utilized for the synthesis of amphiphilic macromolecules consisting of a hydrophilic polymer block and a hydrophobic lipid-like moiety. The structure and composition of the synthesized products were confirmed by NMR and IR spectroscopy, as well as by gel permeation chromatography (GPC). Additionally, the behavior of the resulting DHP-polymer conjugates in an aqueous medium was investigated to evaluate their self-assembly capacity and formation of nanostructured systems, analyzing the influence of the chemical structure on the resulting supramolecular characteristics. The second chapter of this section discusses the preparation of a novel type of liposome-based spherical nucleic acids (SNAs) by incorporating synthetic nucleolipids obtained through click chemistry reactions between appropriately functionalized single-stranded DNA oligonucleotides and DHP. Two distinct synthetic approaches (azide-alkyne cycloaddition and photoinduced thiol-ene click reaction) are presented for the preparation of the nucleolipids, which enable direct covalent coupling between the two structural components without the use of metal catalysts. The resulting liposomal SNAs were characterized and evaluated as a new class of functional nanostructures combining the properties of lipid systems and nucleic acids. The obtained results demonstrate the feasibility of forming nanostructures with potential applications as nucleic acid delivery systems. The third chapter of this section addresses the preparation of amphiphilic polymer-oligonucleotide conjugates capable of self-associating in an aqueous solution to form SNAs with a polymer core. This was achieved by grafting appropriately functionalized nucleotides onto polystyrene copolymers of varying topologies. The self-associating behavior of the resulting conjugates was investigated, revealing that they form particles composed of a dense polymer core with a corona of radially oriented oligonucleotide chains, which defines them as SNAs. Using appropriate methods, the behavior of the obtained aggregates was monitored, demonstrating the feasibility of tuning their properties by altering the polymer architecture. The final chapter examines the preparation of linear and star-shaped copolymers of polyglycidol (PG) and poly(ε-caprolactone) (PCL); for this purpose,

a series of copolymers with varying topologies, compositions, and molecular weights was synthesized and characterized by applying distinct synthetic strategies. The influence of the resulting copolymers on the properties of niosomal systems was investigated by incorporating them into the structure of niosomal membranes, alongside evaluating the loading capacity and release profiles of hydrophobic substances. The effect of the molecular architecture on the stability and functional characteristics of the obtained nanosystems was also analyzed. Overall, the section is well-structured and demonstrates a consistent link between the synthetic approach, the analytical techniques used, and the observed properties of the resulting systems, while the conclusions drawn are well-reasoned and follow logically from the presented experimental findings.

2.3. Assessment of the Scientific Contributions and Personal Contribution of the Candidate

The presented results are logically structured and systematically reflect the fulfillment of the goals and objectives of the dissertation. The scientific work integrates various synthetic approaches for the preparation of well-defined hybrid amphiphilic macromolecules and investigates the properties of the resulting systems. A distinct correlation has been established between the molecular design, the polymer architecture, and the behavior of the obtained nanostructures.

The scientific contributions of the dissertation can be summarized in the following main directions:

1. The first significant contribution is the development of original synthetic approaches for the preparation of polymer-lipid conjugates using azide-alkyne click chemistry, enabling the combination of a phospholipid-mimetic product (DHP) with various types of functionalized polymers. The obtained results broaden the scope of existing synthetic strategies for preparing amphiphilic macromolecules and contribute to the advancement of polymer-lipid nanosystems.
2. A substantial contribution represents the design and synthesis of DHP-functionalized oligonucleotides and the preparation of a novel type of nucleolipids. Through efficient covalent coupling reactions, amphiphilic structures combining the properties of lipid and nucleic components were obtained. The resulting systems pave the way for expanding the methodologies used to prepare and investigate spherical nucleic acids.
3. The third major contribution consists in obtaining linear and star-shaped block copolymers of polyglycidol and poly(ε-caprolactone) and establishing the influence of molecular architecture on self-assembly processes and the properties of the obtained nanosystems. The results demonstrate that variations in copolymer architecture influence parameters such as particle size, colloidal stability and release behavior of incorporated compounds.

These contributions combine fundamental science and practical applications, advancing contemporary approaches in functional polymer materials and drug delivery systems

2.4. Assessment of the Scientific Output and Visibility of the Results

The results of the dissertation are summarized in 5 scientific articles published in high-impact journals within the highest quartile tier (all 5 are ranked Q1). Two of these papers appeared in prestigious American Chemical Society (ACS) journals (Biomacromolecules with a JIF of 5.5 and Macromolecules with a JIF of 5.2), one in Nanoscale Advances (Royal Society of Chemistry, JIF

4.7), and two in MDPI journals (Nanomaterials with a JIF of 5.3 and Pharmaceutics with a JIF of 4.9). In four of the publications (No. 1, 2, 3, and 5), E. Dimitrov is the first author, which unequivocally demonstrates his leading role and high research potential. According to the international Scopus database, the total number of registered citations to date is 20, serving as a strong quantitative and qualitative indicator of the relevance, scientific value, and substantial interest from the international scientific community in the achieved results.

3. Assessment of the qualities of the extended abstract of the doctoral thesis, whether it correctly reflects the contributions of the doctoral thesis

The abstract has been prepared in accordance with the regulatory requirements and accurately reflects all the essential findings of the dissertation. It clearly presents the relevance of the conducted research, the formulated aims and objectives of the work, the primary results obtained, and the conclusions drawn. Furthermore, the achieved scientific contributions are duly noted, and prospective directions for future research are outlined

4. Opinions, notes and recommendations

I have the following remarks and questions:

1. In several sections of the dissertation, foreign terms and transliterated terminology are used (e.g. termination, terminal, abstraction, integration, incorporation), although equivalent Bulgarian scientific terminology is available. Greater consistency in the use of established Bulgarian scientific terminology would further improve the style and readability of the dissertation.
2. In some sections, additional discussion connecting the observed supramolecular behavior with the molecular characteristics of the synthesized systems would strengthen the interpretation of the obtained results.
3. Two different approaches were applied for the preparation of DHP-functionalized nucleic acids: azide-alkyne cycloaddition and photoinduced thiol-ene click reaction. From the presented discussion, the factors determining the selection of the specific synthetic approach are not entirely clear. Could you comment on whether the selection is determined solely by the functional groups employed, or whether additional factors should also be considered, such as reaction conditions, oligonucleotide stability and characteristics of the final products? Could a brief comparison be made regarding the applicability of the two approaches?

5. Conclusion

The presented dissertation for the acquisition of the educational and scientific degree “Doctor” represents an original scientific study and contains a sufficient volume and quality of scientific results. The dissertation and the accompanying materials fulfill the minimum national requirements in accordance with the Act on the Development of the Academic Staff in the Republic of Bulgaria and the respective regulations for its implementation. No indicators of plagiarism have been detected in the dissertation. The critical remarks and recommendations are primarily of an editorial and discussable nature and do not alter my positive assessment of the merits of the work. Based on the foregoing, I give a positive evaluation of the presented dissertation and recommend that the

esteemed members of the Scientific Jury award Erik Dimitrov the educational and scientific degree “Doctor” in Professional Field 4.2. Chemical Sciences, scientific specialty “Polymers and Polymer Materials”.

Date: 20.06.2026

Reviewer:

/prof. dr. eng. Rayna Bryaskova/

Member of the Academic Jury