REVIEW REPORT

by Prof. Petar Dimitrov Petrov, Institute of Polymers - BAS

on PhD Thesis in Professional Field 4.2. Chemical Sciences, scientific specialty Polymers

Title: Smart polymeric materials for modified release of timolol maleate in the eye

PhD student: Denitsa Valerieva Nikolova

Supervisors: Assoc. Prof. Dr. Elena Vassileva and Assoc. Prof. Dr. Lachezar Christov

Documents of candidate: Denitsa Nikolova is enrolled as a full-time doctoral student in Professional Field 4.2. Chemical Sciences, doctoral program Polymers at the Department of Pharmaceutical and Applied Organic Chemistry on 01.02.2019 by the Rector of Sofia University "St. Kliment Ohridski", order No. RD 20-242/28.01.2019. Since 01.02.2022 she has been granted the right to defend her PhD dissertation with a decision of the Faculty Council of the Faculty of Chemistry and Pharmacy from a meeting held on 11.01.2022 and the order of the Rector of Sofia University "St. Kliment Ohridski" No. RD 20-50/14.01.2022. The PhD student has passed all the exams, according to her individual study plan, and the materials submitted by her in the procedure for earning the scientific degree "doctor of philosophy" meet the requirements of the Act on Development of the Academic Staff in the Republic of Bulgaria and the regulations for its application.

Education and professional development: Denitsa Nikolova completed her higher education in 2017 at the Sofia University "St. Kliment Ohridski", Faculty of Chemistry and Pharmacy, where she obtained a bachelor's degree in Ecochemistry. One year later, she graduated with a master's degree in Polymers from the same faculty. She then worked as a chemist at the Institute of Polymers of the Bulgarian Academy of Sciences before being enrolled as a full-time PhD student. In the period 2017 – 2022, she did several short-term specializations at the Leibniz Institute for Polymer Research, Dresden, Germany and the University of Barcelona, Spain. She was a team member of numerous research projects and disseminated scientific results by attending various scientific forums in Bulgaria. In 2021, she was awarded the prize for the best poster presentation at the 12th scientific session "Young scientists in the world of polymers", and in 2023 Denitsa

Nikolova won the national prize "Prof. Ivan Shopov" of the Union of Chemists in Bulgaria for "Outstanding young scientists in the field of polymers".

Topicality of doctoral thesis: Currently, the polymeric carriers of biologically active substances are of great interest in materials science, medicine, and pharmacy due to their potential to increase the efficiency of a given therapy, and thereby to improve the quality and life expectancy of patients. The design and fabrication of highly efficient carriers, which can overcome a number of barriers in the human body, is still a challenge for scientists, due to the need to combine a number of specific properties and functionalities in one system. The "smart" carriers, which can react to various external or internal stimuli, have attracted much attention because they can ensure more precise control in the delivery and release of drugs. In this regard, polyzwitterionic carriers are a very promising class of materials, with proven sensitivity to changes in the salt concentration, temperature, and pH of the medium. The focus of the dissertation is on the synthesis and evaluation of "smart" polymeric materials based on the zwitterionic polymer poly(sulfobetaine methacrylate) (PSBMA) as systems for the controlled delivery of timolol maleate (TM). To my opinion, the research topic is at the forefront of the investigations in the field of polymers, and the results are of interest from both a fundamental and an applied point of view.

Scientometric indicators relevant to the dissertation: The results obtained are published in two research articles in international journals with an impact factor - Polymer International (IF^{2021} - 3.213; Q2) and Gels (IF^{2021} - 4.432; Q1). The first paper is already cited twice, according to SCOPUS. Thus, the minimum requirements of the abovementioned Act and the Specific rules and conditions for acquiring scientific degrees in Sofia University (30 points by group of indicators G) are fulfilled. Denitsa Nikolova is the first co-author in the two articles, which is indicative for essential contribution to the research carried out.

Structure and content of the dissertation: The dissertation follows the generally accepted rules for structure and is composed of the sections Introduction, Literature Review, Aim and Objectives, Experimental, Results and Discussion, Conclusions and References. Lists of publications, attendance of scientific forums and specializations, and participation in research projects are included. The dissertation is written in Bulgarian on 118 pages and includes 24 figures, 24 tables and 19 diagrams. 163 literary sources were used.

In the Introduction, the author emphasizes the application of the beta blocker timolol maleate for the treatment of glaucoma and the existing problems related to its relatively low bioavailability. At the same time, the potential of certain polymer systems to improve this characteristic has been noted. The Literature Review begins with basic classification, synthesis methods, and key properties of polyzwitterions. The focus is on their sensitivity to changes in environmental parameters, antibacterial activity, and biocompatibility. A brief overview of various drug delivery systems – nano- and microparticles and hydrogels – is made. In the next step, polysulfobetaines as drug delivery systems are discussed in more detail, with an emphasis on the latest forms of application in medicine. Special attention is paid to the ophthalmic drug delivery route, and to timolol maleate as a widely used drug for the treatment of various diseases. At the end of the Literature Review, it is concluded what should be the ideal carrier of timolol maleate in ophthalmic application and it is suggested that the zwitterionic polymer poly(sulfobetaine methacrylate) possesses characteristics approaching the ideal carrier. The Aim and Objectives of the dissertation are formulated precisely and clearly. The Experimental part describes in detail the procedures for the synthesis of nanoparticles and hydrogels from poly(sulfobetaine methacrylate), as well as the methods used for their characterization. The protocols for loading the carriers with TM and studying the release profile of the active substance are presented as well. The most essential part of the dissertation is the Results and Discussion section. It is divided into two subparts - nanoparticles of PSBM and copolymer hydrogels of sulfobetaine methacrylate (SBM) and 2-vinylpyrrolidone (VP). Nanoparticles of linear and cross-linked PSBM synthesized by RAFT polymerization were obtained. Both types of particles are of nanoscopic size at a temperature corresponding to the temperature of the surface of the human eye, while at room temperature the particle size is a few micrometers. This phenomenon is explained by the tendency of PSBM macromolecules/particles to self-associate under certain conditions. At room temperature, associates are formed as a result of physical bonds through dipole-dipole interactions, characteristic for zwitterionic polymers. As the temperature increases, these physical interactions begin to break, the associations between PSBM particles/macromolecules are destroyed and the hydrodynamic diameter decreases. The salt sensitivity of nanoparticles of cross-linked PSBM was investigated. It was proven that, in addition to the destruction of the associates, the presence of salt in the aqueous solution also leads to swelling of the cross-linked PSBM nanoparticles. The loading efficiency and loading capacity of TM in PSBM particles was determined, and the values are

higher for the particles of linear PSBM. The TM release profiles from the two types of PSBM particles, under conditions mimicking ocular administration, differed significantly. The particles of cross-linked PSBM quickly release about 70% of TM (burst effect), while the nanocarriers of linear PSBM released only about 20% of the active substance for the same time (30 min.). The remaining amount of TM was released slowly within 24 h. Using different kinetic models, a pseudo-Fickian diffusion mechanism of TM release from PSBM particles is predicted, which is characterized by the fact that the release of TM is mainly controlled by the diffusion of molecules. The difference in the release profiles is explained by the more intense electrostatic interactions between the positively charged molecules of the drug substance and the negative charges in the particles of linear PSBM.

Copolymer hydrogels of SBM and VP were synthesized with three different compositions - 1:2, 1:1 and 2:1 mole ratio. The equilibrium degree of swelling of the hydrogels increases proportionally to the content of the more hydrophilic monomer VP. The larger amount of adsorbed water leads to a minimal decrease in the elastic modulus of hydrogels with a higher fraction of VP. In fact, the discussed differences in values are not significant and in general the hydrogels have similar properties. The loading efficiency of TM is identical and within statistical error. On the other hand, the loading capacity of TM decreased with increasing the amount of SBM. Overall, the values are very low and reach up to 2%. The TM release profiles from the copolymer hydrogels are characterized by a burst effect, with from 40 to 70% released TM within the first 30 min. The remaining amount of TM was released slowly, with a pronounced dependence on the composition of the copolymers. The higher the SBM content, the slower the release of TM. An exception is the hydrogel obtained at a molar ratio of SBM/VP of 1:1, which released TM with the slowest rate within the first 9 hours. Using ANOVA analysis and Tukey post hoc test, the authors concluded that the composition of the copolymer (the amount of SBM in the polymer network) plays a determining role on the release profiles, because the SBM units form a physical zwitterionic network that further hinders the release of TM. However, the physical network is gradually destroyed by temperature changes and the available salt (part of the experimental conditions of the TM release experiment), and after given time this effect contributes to the release of a larger amount of TM. 85% transparency (permeability) of the TM-loaded hydrogels was determined, which is sufficient to provide a clear view through the materials. It should be noted that hydrogels absorbed much of the radiation in the UV-B region. These characteristics are highlighted as an

advantage of the developed systems for potential application as soft contact lenses. The dissertation ends with three Conclusions that highlight the obtained results.

Questions, recommendations, and remarks: The dissertation is well designed, the results are presented clearly and comprehensibly. Regarding some terms used and the interpretation of some of the results, I have the following remarks and questions:

- Several times in the text, incl. in the Aim and Objectives section, is mentioned "cross-linked and linear PSBM nanoparticles". In my opinion, the correct statement is "nanoparticles of cross-linked and linear PSBM".

- I consider unreliable the result of the particle size distribution, determined with the aid of scanning electron microscopy. The criterion for selecting only 100 particles is unclear, and there is also the question of why the average diameter of the dried particles (~ 6 μm) is more than twice larger than the hydrodynamic diameter of the hydrated particles, determined by dynamic light scattering (~ 2.5 μm).

- Concerning the so-called burst effect, the percentage of rapidly released active substance should be determined from the shape of the curve, not from the first point measurement. For example, it can be seen from Figure 13 that the rapidly released TM from particles of cross-linked PSBM reached approximately 70%, and not as claimed approximately 50%.

- Your results show that TM-loaded hydrogels exhibit UV-B blocking ability. Do you have an explanation if this is due to the hydrogel itself or the TM embedded in it?

Evaluation of the extended abstract: The extended abstract is prepared according to the requirements and describes all the essential results of the dissertation. In the abstract, the PhD student has added five contributions of the presented work.

Conclusion: The dissertation submitted by Denitsa Nikolova for earning the scientific degree "doctor of philosophy" is written in Bulgarian and is based on published original results. For the first time, two types of PSBM systems have been developed for the controlled delivery of timolol maleate to the eyes. The volume and quality of the dissertation comply with the generally accepted rules, while at the same time it meets the requirements of the Act on Development of the Academic Staff in the Republic of Bulgaria and the regulations for its application. The remarks I have made do not change my positive opinion of the merits of the dissertation. Based on the above,

I recommend the respected members of the Scientific Jury to vote for earning the scientific degree "doctor of philosophy" to Denitsa Nikolova.

Sofia, 17/05/2023 Reviewer: …….

/Prof. Peter Petrov/