O P I N I O N

by Dr. Olya Stoilova, Professor at the Institute of Polymers – BAS
on the PhD Thesis for awarding the educational and scientific degree "Doctor" in the professional direction 4.2 Chemical Sciences (Polymers)

Author: Denitsa Valerieva Nikolova, PhD-student in the Laboratory on Structure and Properties of Polymers, Faculty of Chemistry and Pharmacy, Sofia University St. Kl. Ohridski
Topic: Smart polymeric materials for modified ocular timolol maleate release

This Report is prepared in response to Order №RD 38-83/14.02.2023 issued by the Rector of the Sofia University (SU), following the decision made by the Academic Jury that was held on 01.03.2023. The Report is in compliance with Development of Academic Staff in the Republic of Bulgaria Act (DASRBA), the Rules for the Application of the DASRBA, the Rules of SU and with the Rules set at the Faculty of Chemistry and Pharmacy (FCP), for applying the Act aforementioned.

Ms. Denitsa Nikolova graduated as a Bachelor in ecochemistry in 2017 from the Faculty of Chemistry and Pharmacy (FCP), Sofia University "St. Kl. Ohridski". In 2018 she obtained her Master's degree in Polymers in the same Faculty, and in 2019 she was enrolled as a full-time PhD-student in the Department of Pharmaceutical and Applied Organic Chemistry, professional direction 4.2 Chemical Sciences (Polymers) headed by Assoc. Prof. Elena Vasileva. On February 1, 2022, she was dismissed with the right of defense.

The PhD Thesis is laid out on 114 pages, contains 20 schemes, 24 figures, 24 tables and 163 references are cited. It is structured as follows: Introduction, Literature review (38 pages), Experimental part (13 pages), Results and Discussion (41 pages), Conclusions and References. The Thesis also includes a list of abbreviations used, a list of publications included in the work and outside the topic of the Thesis, a list of participations in scientific forums and a list of specializations abroad and participation in scientific projects. A declaration of authorship is also presented separately.

The PhD Thesis summarized results published in two international journals with an impact factors (*Polymer International*, IF2021 = 3.213, Q2 and *Gels*, IF2021 = 4.432, Q1). In both publications, Denitsa Nikolova is the first author, which shows that she has a major contribution to the obtained results. In accordance with the national minimum requirements, by group of indicators (A and D), Denitsa Nikolova collects a total of 95 points, which exceeds the minimum required 80 points for the educational and scientific degree "Doctor" in the professional direction 4.2 Chemical Sciences (Polymers). Part of the research results were reported by the dissertation student at 9 scientific forums, 4 of which were oral and 5 were poster presentations.

The PhD Thesis is devoted to the preparation of two types of polymer carriers as a potential drug delivery system for the non-selective beta-blocker timolol maleate (TM), widely administered to eyes as eye drops. In order to achieve prolonged delivery and increase bioavailability of TM in the eye, poly(sulfobetaine methacrylate) (PSBM) particles and hydrogels of PSBM and its copolymers with vinyl pyrrolidone are obtained as polymeric carriers. PSBM particles were synthesized by reversible addition-fragmentation chain transfer polymerization (so-called RAFT), and PSBM hydrogels and its copolymers with vinyl pyrrolidone (with different molar ratios) were obtained by thermally initiated free radical polymerization.

With appropriately selected analytical methods, the main characteristics of both types of particles were determined, such as molecular mass, hydrodynamic diameter, ζ-potential, salt sensitivity, etc. In my opinion the use of SEM analysis to characterize the particles (morphology and size) is not the most suitable method, as can be seen from the obtained results. TEM analysis would be much more informative and useful, but it was not used.

It has been proven that due to the zwitterionic nature of PSBM, the hydrodynamic diameter of both types of particles (uncrosslinked and crosslinked) depends on the temperature change, i.e. exhibit intelligent properties. The loading efficiency and release profile of TM from both types of particles were investigated in detail. The results show that the selected particle loading conditions lead to a relatively low loading efficiency (~30%), and under conditions mimicking those of the eye, cross-linked PSBM particles have a more suitable TM release profile.

The resulting hydrogels were characterized by appropriate methods, determining their equilibrium swelling ratio, elastic moduli, swelling kinetics, transparency, etc. Given the zwitterionic nature of PSBM, it would be useful to perform dynamic mechanical thermal analysis (DMTA) instead of DSC. Once again, a relatively low loading efficiency (up to 30%) of TM in hydrogels was achieved. The composition of the copolymers was found to influence the TM release profile. Moreover, it was shown that these results can help to solve problems in ophthalmology.

It should be noted that Denitsa Nikolova has performed a large amount of experimental work, presented the results correctly and commented on them competently. She has mastered various methods for polymers characterization. This convincingly demonstrates that the educational goal of the PhD Thesis has been successfully fulfilled. Therefore, within the framework of the dissertation, a sufficient quantity and quality of experimental work has been carried out, which reveals the potential for future development and, above all, for the practical application of the obtained materials.

I have some important remarks and recommendations as follows:

Many linguistic inaccuracies were made in the translation of some terms, such as “light scattering”, “symmetric and asymmetric stretching vibrations”, “bending vibrations”, “backbone chain”, etc. In addition, instead of PSBM/SBM, only PSB/SB is abbreviated. It is not correct to generalize “copolymer” (e.g. point V.2.), because there are also hydrogels from the homopolymer. Having in mind the “burst” effect of the TM release, it would be more insightful to present this dependence by the first hour, rather than only by 24 or 48 hours.

These remarks are mainly of a technical nature and do not reduce the value of the presented dissertation. I have the following questions to the PhD Candidate:

1. Has the degree of crosslinking of PSBM particles been determined and how would it affect their potential application?

2. How is the shift of the absorption maximum of TM from 298 nm (Figure 3) to 294 nm (Figure 5) depending on the carrier used explained?

3. Given the potential application of hydrogels as soft contact lenses for the eyes, the dimensions of the tested discs are of exceptional importance, which are not mentioned anywhere in the thesis. What is the thickness and diameter of the different types of hydrogels before and after swelling?

4. What is the difference in the spectra presented in Figure 20 and in Figure 21?

Conclusion: Regardless of the remarks and recommendations made, the PhD Thesis of Denitsa Nikolova fully meets the requirements of the DASRBA, the regulations for its implementation and the recommended requirements of the FCP. **All this gives me reason to vote positively and I suggest to the members of the scientific Jury to support the awarding of the educational and scientific degree "Doctor" to Ms. Denitsa Valerieva Nikolova in professional direction 4.2. Chemical Sciences (Polymers).**

16.05.2023 г. Reviewer:

/Prof. Olya Stoilova, PhD/