REVIEW

of a doctoral thesis for obtaining the educational and scientific degree "DOCTOR", in the specialty "Polymers", direction 4.2 - Chemical Sciences

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Form of PhD: full-time PhD student

Department: Department of Chemistry and Pharmaceutical Chemistry, Faculty of Chemistry and Pharmacy, Sofia University "St. Kliment Ohridski"., Sofia University.

Topic: *' Intelligent polymeric materials for modified release of timolol maleate in eyes'*

Scientific Supervisors: Assoc. Prof. d-r Elena Vassileva and Assoc. Prof. d-r Lachezar Christov

The procedure for the doctoral thesis defense was determined by the order № РД-38-83/14.02.2023 of the Rector of Sofia University "St. Kliment Ohridski".

Reviewer: Prof. d-r Bistra Dimitrova Kostova, Faculty of Pharmacy, Medical University-Sofia; member of a scientific jury assigned by order № РД-38-83/14.02.2023 of the Rector of Sofia University "St. Kliment Ohridski".

Overall presentation of the procedure

The presented set of materials is in accordance with the requirements specified in the "Regulations on the terms and conditions for acquiring scientific degrees and occupying academic positions" at SU "St. Kliment Ohridski", art. 67 (5), according to Art. 30(1) of "Rules for implementation of the law on the development of academic staff in the Republic of Bulgaria" and includes:

- Rector's order for enrollment in full-time doctoral studies;
- Rector's order for dismissal with right of defense;

• dissertation work and abstract to it;

• diploma for previous educational degree – master's;

• certificate of passed exams

• curriculum vitae;

• declaration of authorship

• certificate of compliance with the national minimum requirements for the educational and scientific degree "doctor";

• copies of scientific publications in connection with the dissertation work.

Brief biographical data about the author of the thesis

Denitsa Valeriyeva Nikolova was born on 29/03/1994. In 2013 she graduated from the Sofia Mathematical High School "Paisii Hilendarski". In 2017 she graduated with a Bachelor's degree in "Ecochemistry" at the Faculty of Chemistry and Pharmacy, Sofia University "St. Kliment Ohridski". In 2018 she received the degree of Master in Polymers from the Faculty of Chemistry and Pharmacy, Sofia University "Sv. Kliment Ohridski". She conducted a number of specializations, some of which - at the Leibniz Institute for Polymer Research in Germany and at the University of Barcelona in Spain. She currently works as a Young Researcher for the "National Program of Young Scientists and Postdoctoral 2" project, MES. She speaks English, German and Russian.

Relevance of the topic

Efficient delivery of drug substances in the eye is a complex task from a technological, biopharmaceutical and therapeutic point of view. The problems of very low drug bioavailability, difficulties in ensuring the basic requirements in terms of sterility, stability and tolerance significantly complicate the development of dosage forms for ophthalmic administration. For this reason, the possibility to optimize therapy by creating functional, intelligent carriers for active molecules is undoubtedly significant, which undeniably demonstrates the relevance of the thesis topic.

Description and evaluation of the thesis

The dissertation submitted to me for review is written on 118 pages, illustrated with 20 schemes, 24 tables and 24 figures. The thesis includes the following sections: introduction, literature review, aim and objectives, experimental part, results and discussion, conclusions and references.

Introduction

The introduction demonstrates a valid reason why the dissertation researcher set out to work on this topic. One of the most useful medications for the treatment of glaucoma is timolol maleate. The main dosage form used for its delivery is liquid - eye drops. With this type of administration, a major problem appears to be low bioavailability, a consequence of the short retention time. In this regard, the development of systems to prolong the contact time of the drug substance and improve the efficiency of targeting appears as an opportunity to master the main problems from a technological and therapeutic point of view.

Literature review

The literature review comprises 36 pages, with a fully sufficient amount of references - 143, included to summarise the research on the topic. A substantial part of the literature sources are from the last 5 years, which shows knowledge of the current state of the problem as well as illustrates its contemporary relevance.

A general characterization of the polyzwitterions is presented, showing their structure, their preparation methods and discussing in detail the main properties relevant to their use as drug carriers, namely: solubility, salt, temperature and pH sensitivity. The possibilities of using polysulfobetains in macro and nano versions as potential drug carriers are shown. The two types of drug delivery systems related to the present topic, polymeric micro and nanoparticles, and polymeric hydrogels, are described, respectively. Drug delivery to the eye, the difficulties of efficiently performing ocular resorption, and the complications regarding the therapeutic outcomes of currently used drug-delivery systems, are discussed. The properties of the model drug substance, thymolol maleate, and existing micro- and nanocarriers for it are described in detail, as well as the possibility of modifying its release by contact lenses.

The literature review is well written and very informative, summarizing the scientific advances on the dissertation topic. As a consequence of the literature review, the dissertation formed the thesis to demonstrate the potential of smart polymeric materials based on the zwitterionic polymer poly(sulfobetaine methacrylate) as drug-delivery systems for timolol maleate, in the form of cross-linked and linear nanoparticles, with potential ocular applications, or copolymer hydrogels of poly(sulfobetaine methacrylate-co-vinyl pyrrolidone), with potential application as soft contact lenses for the eyes.

Aim and objectives

The aim of this thesis is defined precisely and clearly, and fully corresponds to the title of the thesis. In order to achieve the stated goal, 7 tasks have been adequately formulated, the solution of which would enable to obtain achievements applicable in practice.

Experimental part

An adapted RAFT polymerization was used to prepare linear and cross-linked nanoparticles of poly(sulfobetaine methacrylate) (PSBM), and the procedure is described and illustrated in detail. The resulting particles were loaded with the drug substance and were very well structurally and morphologically characterized by gel permeation chromatography, scanning electron microscopy, and their size and size distribution and ζ-potential were determined by dynamic laser light scattering. In view of their potential application as drug-delivery systems, their salt sensitivity and loading efficiency (EE %) with timolol maleate were determined. Using paddle method, an in vitro drug release study was conducted from the proposed structures and mathematical models were applied to investigate the kinetics of the process.

Synthesis of copolymer hydrogels of sulfobetaine methacrylate (SBM) and vinyl pyrrolidone (VP) (poly(SBM-co-VP)) by thermally initiated free radical polymerization was also carried out. The resulting platforms were characterized with respect to their swelling degree, the elastic modulus was determined, and differential scanning calorimetry and infrared spectroscopy studies were performed. In vitro studies of drug release rate and extent were also performed, as well as calculations to describe the kinetics of the process.

Results and discussion

The actual research is presented in 40 pages and is very well illustrated with 22 tables and 18 figures. It has been conducted according to the assigned tasks in the following main areas:

Synthesis of linear and cross-linked nanoparticles of poly(sulfobetaine methacrylate) and characterization of the resulting particles.

Due to the crucial importance of particle size in ophthalmic applications, a very significant, in my opinion, study of the hydrodynamic diameter of the particles was carried out using dynamic laser light scattering. The temperature dependence of their hydrodynamic diameter and of their zeta potential has been established, and the data obtained have been explained by the self-association of the PSBM particles at different temperatures.

Loading of polyzwitterionic particles with timolol maleate and investigation of their potential as drug-delivery systems for ocular administration.

The loading efficiencies of the two types of particles are between 23% and 30%, respectively. The in vitro release profiles of timolol maleate from the resulting particles showed significant differences, with the PSBM NP showing a significant "burst" effect and releasing approximately 90% of the drug substance by 9 h, whereas with the PSBM lin, no "burst" effect was observed, but only 30% was released within 9 h. It is quite correct to generalize that of the two formulations, PSBM NP is more suitable for ophthalmic administration. A study of the kinetics of the release process shows that the process is best described by the first-order model.

Synthesis of hydrogels of poly(sulfobetaine methacrylate-co-vinyl pyrrolidone) copolymers with different compositions and characterization of the properties of the resulting copolymers

Newly synthesized copolymer hydrogels of sulfobetaine methacrylate (SBM) and vinyl pyrrolidone (VP) (poly(SBM-co-VP)) were investigated with respect to swelling rate and loading capacity, and these parameters were found to be significantly affected by their composition. Conversely, the results for modulus of elasticity and loading efficiency are not significantly affected by composition. In order to distinguish the differences obtained from the efficiency and loading capacity studies, two statistical methods of data analysis were applied, namely the Overall ANOVA test and the Tukey post hoc test, which I consider to be a particularly positive highlight of the work, because that makes it possible to clarify the situation in detail and to allow the data

obtained to be interpreted more correctly. Studies on the control of drug release have shown that the resulting systems release timolol maleate in direct dependence on their composition. In all samples, a "burst" effect was observed to a more or lesser extent, the compositions with a higher content of monomeric CBM units, which managed to optimally retard drug release, showing the most suitable profiles. Research data indicate that the newly synthesized systems can be successfully used as carriers for timolol maleate for ocular administration.

In conclusion, from the analysis of the results of the presented research as the main contributions of a scientific and scientifically applied nature of the dissertation work, the following can be summarized: (i) New polymer nanomaterials based on poly(sulfobetaine methacrylate) cross-linked with poly(ethylene glycol diacrylate) were synthesized, which have been characterized and proposed as carriers of timolol maleate in the eye. (ii) Copolymer hydrogels based on sulfobetaine methacrylate and vinyl pyrrolidone cross-linked with poly(ethylene glycol diacrylate) were synthesized and considered as possible systems for ophthalmic delivery of timolol maleate in the form of soft contact lenses.

Conclusions

The conclusions presented here accurately reflect the results obtained according to all the tasks set.

References

Covers 163 sources, formatted precisely and as required.

Characteristics and evaluation of the abstract.

The abstract is 43 pages in length. It has been prepared in accordance with the requirements and is fully consistent with the dissertation submitted to me for review.

Scientific metrics related to the thesis

The PhD student Denitsa Nikolova presents 2 (two) publications on the dissertation work for implementation "Minimum required points by groups of indicators for acquiring the educational and scientific degree "doctor" according to “Rules for implementation of the law on the development of academic staff in the republic of Bulgaria”, in the prestigious journals Polymer International (Q2, IF=3.213) and Gels (Q1, IF=4.432), which correspond to 45 points. The required minimum, for the ESD "doctor" in the field of "Chemical Sciences" is 80 points, and

Denitsa Nikolova collects a total of 95 points, which exceeds it. The PhD student has also submitted two more publications external to the thesis, also in the very prestigious journals European Polymer Journal (Q1, IF=5.546) and Polymer International (Q1, IF=3.213). She has participated in 10 (ten) scientific forums, of which 4 (four) were oral presentations and 6 (six) poster presentations. She has participated in 2 (two) projects on the topic of her thesis.

The importance of Denitsa Nikolova's work is also evidenced by the prestigious, national award of the Union of Chemists in Bulgaria "Outstanding Young Scientist in the Field of Polymers" in the name of Prof. Ivan Shopov, received in 2023.

Questions, recommendations and remarks

The dissertation is written and organized very well. The conducted research is based on the most up-to-date methods of analysis and evaluation. The interpretation of the obtained results is presented logically and clearly. I have no critical remarks, except for some stylistic inaccuracies, which are purely editorial and in no way detract from the quality and contributions of the work. I have the following questions for the PhD student: 1. What is the volume of the acceptor phase, in the conducted research on the in vitro release of timolol maleate from PSBM particles and why did you choose this method for its determination? 2. The conducted research on the swelling kinetics of the gels is at the so-called "physiological conditions". Don't you think it would be good to run it under a wider range of conditions (temperature, other solvents), with a view to potentially including other drug substances with problematic solubility?

CONCLUSION

I express my belief that Denitsa Nikolova's dissertation *"Intelligent polymer materials for modified release of timolol maleate in the eyes"* fully meets the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations on the Terms and Procedures for Acquiring Scientific Degrees and for occupying academic positions at SU "St. Kliment Ohridski" and the minimum national requirements in the relevant professional field. The conducted research contains significant results and contributions of scientific and scientific-practical importance for the optimization of polymeric drug-delivery carriers for ophthalmic application. It also shows that the doctoral student Denitsa Nikolova possesses in-depth theoretical knowledge and professional skills for independent research in the scientific specialty "Polymers".

Based on the above, I confidently give my **POSITIVE** assessment and recommend to the respected members of the Scientific Jury to award Denitsa Valerieva Nikolova the educational and scientific degree "**DOCTOR**", specialty "Polymers", field 4.2 - Chemical Sciences.

17.05.2023 Reviwer:

Sofia / Prof. d-r B. Kostova /