

Ph.D. Examiner's report on the Ph.D. thesis
BIOPOLYMER-BASED NANOCOMPOSITE AS DRUG DELIVERY
SYSTEM

Submitted by:

Zahra Yadollahi, M.Sc.

to Tomas Bata University in Zlín for the defence in partial fulfilment of the requirements for the Ph.D. graduation in Doctoral study program P3924 / Material Sciences and Engineering in doctoral study branch 3911V040 / Biomaterials and Biocomposites.

Ph.D. Thesis examiner **prof. Ing. et Ing. Ivo Kuřitka, Ph.D. et Ph.D.**

The Thesis submitted by **Zahra Yadollahi** consists of 90 pages of text in total, including 14 pages of obligatory formal parts, such as the table of contents, acknowledgements, abstract, lists of figures, tables, etc.

The main text begins with about 40 pages of introductory chapters, which briefly define the current state of the issue. The first part highlights the importance of protein-based drug nano-delivery systems and describes the properties of whey proteins. The second part of the introduction continues with the specific topic of polyelectrolytes and biopolymer-based polyelectrolyte delivery systems, namely polysaccharides – represented by chitosan – and hybrid protein/chitosan poly electrolyte nanoparticles, culminating in a focused chapter on the main hypothesis concerning whey protein and composite nanoparticles and drug delivery systems based on this material. Then, the aim of the doctoral thesis is defined as investigating the development of a novel polyelectrolyte nanoparticle system, which is based on whey protein isolate and chitosan and possesses suitable structure and characteristics for potential drug delivery application. The achievement of this goal is broken into four stepwise objectives, starting from material fabrication up to the loading of doxorubicin as a model active pharmaceutical ingredient and the immobilization of negatively charged molecules in the system. The literature references are properly selected, and their number is more than sufficient.

The goal of the dissertation has been achieved, as described in the fifth chapter. Although very short, the dissertation still represents progress in the field of study, implementing new ideas in preparing a hybrid protein-polysaccharide composite particulate system based on whey protein isolate and chitosan. However, I would consider it more appropriate to divide Chapter 5 into two separate chapters. First, a description of the experiments and methods, followed by the research outline. Only then would come a chapter titled Results and Discussion, starting approximately from Section 5.3.1. This and subsequent sections present the core results, such as

the effect of pH and concentration on hydrodynamic size and morphology of prepared particles, where I would recommend using AFM techniques for analysis rather than SEM. The identification of chemical group presence and interactions of the functional groups were studied using FTIR, and TG analysis was also performed. Besides the preparation of the materials, the contribution of the work lies in the analysis of colloidal stability and studies of drug loading and in vitro release assays of nano- (or submicron) particles using doxorubicin as a model drug substance. However, I would expect an analysis of the doxorubicin drug release profile with a theoretical model. I also missed a discussion of “Table 6: Comparison of obtained results of current PENs with other studies.” There is no reference to the table in the text of the thesis, even though it provides an important perspective on how the achieved results fit into the context of studies published by other teams, which is an issue certainly worth discussing.

The conclusion chapter is only very brief, but this probably is because it is intended to be a concise summary. It would be beneficial to expand this section by including a commentary on the contribution of the work to science and practice, perhaps using a discussion of Table 6. On the other hand, the future work outlook is set out and consistent with what has been achieved.

I also have some comments on the formal arrangement and clarity of the dissertation. Some corrections would be necessary to improve the quality of the thesis, such as proper rounding of values according to the estimated standard deviations. Some chemical structural formalisms should be in better resolution. The style of tables should be unified, and the readability of graphs in Figures should be improved. The list of acronyms should be alphabetically ordered. Instead PH, a symbol pH, should be used. The chemical structure in the first row of Table 2 in page 50 does not represent a whey protein isolate, but it is 19-fluoro-11-hydroxy-8-(2-hydroxyacetyl)-6,6,9,13-tetramethyl-5,7-dioxapentacyclo[10.8.0.0.2,9.0.4,8.0.13,18]icosa-14,17-dien-16-one, called flunisolide (a glucocorticoid, see Florea, Bogdan I.; Van Der Sandt, Inez C. J.; Schrier, S. Mariette; Kooiman, Klazina; Deryckere, Koen; De Boer, Albertus G.; Junginger, Hans E.; Borchard, Gerrit: Evidence of P-glycoprotein mediated apical to basolateral transport of flunisolide in human broncho-tracheal epithelial cells (Calu-3), *British Journal of Pharmacology*, 2001, vol. 134, # 7, p. 1555 – 1563.)

To summarize, despite the aforementioned comments on some identified shortcomings, this work has demonstrated a result that, although not yet directly applicable in medicine, represents a step toward such application and serves as a starting point for the development of a new type of drug delivery system. Furthermore, if this system proves unsuitable for medical use in the future, it could still have other applications. Additionally, the developed preparation method for a protein-chitosan particle system is universally applicable in other areas.

Finally, it must not be overlooked that the student has already published two publications in impacted journals recorded in the WoS database, which fulfils the number required by the internal regulations of TBU. Both articles are in above-average Q2-ranked journals, according to AIS. The core of the dissertation is published in the article of which the student is the first author. Besides that, the student has experience in presenting at an international conference and

from stays abroad, namely four months in Canada at the University of Waterloo, being also awarded the Mitacs Globalink Research Award - supporting research collaboration between Canada and partner organizations from other countries.

Verdict of the examiner:

In my opinion, the submitted Thesis fulfils the requirements imposed on a PhD thesis. Therefore, I recommend the Thesis for defence, and upon successful defence Zahra Yadollahi, M.Sc., to be awarded a doctoral degree, i.e. the title “Doctor of Philosophy – Ph.D.”.

In Zlín, 19th November 2024

Ivo Kuřitka

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