## *Reviewer's Report on PhD Thesis of Mgr. Vladimir Sincari*

## *"Self-assembled polymer systems responsive to external stimuli for biomedicine"*

The research results reported in the submitted PhD thesis is an original contribution to scientific advances in the field of novel polymer nanoparticle drug delivery systems. In this multidisciplinary thesis Mgr. Vladimir Sincari proposed and described preparation and characterization of novel macromolecular carriers for anticancer chemotherapeutic agents. The currently available cancer therapies have several drawbacks. Most of them are associated with the short circulation time of antitumor agents in the bloodstream and their low specificity. Polymersomes can protect the active agent against degradation and clearance during the systemic circulation ideally driving it selectively to the sites of action where the damaged cells are present. This makes them a promising candidate for therapeutic drug delivery in cancer. The design and preparation of polymeric vesicles – polymersomes and giant vesicles with advantageous properties for biomedical uses are the main area of research in this thesis.

This Ph.D. thesis is consisting of Introduction part providing comprehensive and detailed information on polymer drug delivery systems, RAFT polymerization technique, microwave-assisted RAFT polymerization, self-assebly of block copolymers, preparation methods of polymeric nanoparticles by self-assembly and methods for characterization of polymers and their assemblies. The Aims of this thesis are introduced in the second chapter. The third chapter of this thesis deals with kinetic studies of microwave-assisted RAFT polymerization of N-(2-hydroxypropyl) methacrylamide and its copolymers with the aim to prepare well-defined homo- and copolymers. Optimization of several reaction parameters (selection of CTA, molar ratio of [CTA]/[I], [M]/[CTA]/[I], solvent) led to better control (i.e., low Đ, predictable molar mass, faster polymerization times) of polymerization proces and allowed controlled preparation of defined poly[N-(2-hydroxypropyl) methacrylamide] polymers. Subsequently, the livingness and end-group functionality of the synthesized macro-CTA was verified by addition of various monomers and by growing a second block and by preparation of block copolymers. This part also includes description of experimental procedures for synthesis of monomers, synthesis of chain transfer agent, kinetic studies as well as results and discussion. In the chapters fourth and the fifth preparation of pH-responsive and ROS-responsive copolymers and polymersomes is described. The synthesized poly[N-(2-hydroxypropyl) methacrylamide] homopolymer was used as macro CTA for preparation of stimuli responsive diblock copolymers by RAFT polymerization followed by preparation of cargo-delivery self-assemblies in the form of

polymersomes via microfluidic technique. Poly([N-(2-hydroxypropyl)] methacrylamide)-b-poly[2-(diisopropylamino)ethyl methacrylate] copolymers were prepared as pH-responsive diblock. Poly([N-(2-hydroxypropyl)] methacrylamide)-b-poly(pinacol ester-protected methacrylamide) and poly([N-(2-hydroxypropyl)] methacrylamide)-b-poly(pinacol ester-protected methacrylate) were prepared as ROS responsive diblock. Synthesized block copolymers and the subsequently prepared stimuli-responsive supramolecular self-assemblies were fully characterized by variety of physico-chemical techniques and their efficacy was determined *in vitro* and *in vivo*. In the sixth chapter of this thesis the preparation of PDMS microfluidic device for the production of giant polymersomes is demonstrated. The size-tuned giant non-responsive polymersomes from poly(butadiene-b-ethylene oxide) and pH-responsive polymersomes from poly[2-(diisopropylamino) ethyl methacrylate-b- poly(ethylene-glycol)] were sucesfully prepared by w/o/w double emulsion method. The obtained results demonstrate the spatial and temporal pH-controlled polymersomes disruption under simulated relevant physiological conditions. Cytotoxicity studies proved excellent biocompatibility of produced polymersomes. The last chapter Conclusions summarizes results obtained during this work.

Each chapter and the dissertation as a whole are clearly written, logically organised, and fully developed. The author communicates in a concise and coherent manner, obtained results are well summarized and commented.

The work is well written, number of grammatical or spelling errors is negligible. My comments and questions are minor and do not affect the overall very high rating of this thesis.

*Formal typographical or grammatical errors*:

- On page 13, paragraph - a different font is used at the beginning of the paragraph
- On page 46 and on page 47 - in the header of both Table 3 and Table 4 MW-assisted instead of MWI -assisted is used
- On page 50 - in Figure 16 (A) Semilogarithmic plots and (B) molecular weight and dispersity versus conversion of microwave-assisted RAFT polymerization of HPMA in water. A specification of symbols indicating values for conventional heating and microwave-assisted RAFT polymerization should be provided, as it is difficult to distinguish which symbols are used for conventional and MWI-assisted polymerization.

*Comments and questions:*

- On page 49 in Table 5 results of kinetic measurements for MWI-assisted RAFT of HPMA are shown using CTA1
  Polymer prepared at 20% conversion was characterized by $M_n$ = 2 530 and dispersity 1.95. Polymer prepared at 28% conversion was characterized by $M_n$ =10 420 and dispersity 1.08. Do you have any explanation why only an 8% increase in polymerization conversion (from 20% to 28%) resulted in an almost 4-fold increase in molecular weight and a significant improvement in dispersity from 1.95 to 1.08?

- In fifth chapter of your dissertation you are dealing with preparation and characterization of block copolymers and polymersomes that are sensitive to elevated levels of reactive oxygen species (ROS). My question is: Do you have an idea of what the ROS concentrations are in healthy and inflamed (tumor) tissue?

The research reported meets internationally recognized standards for doctoral research in its field. This conclusion can be documented by the fact that Mgr. Vladimir Sincari is author or co-author of 8 articles in the international peer-reviewed journals, 4 of which include the results contained in this dissertation. He also demonstrated knowledge of the relevant literature, and the ability to exercise critical and analytical judgment of that literature. His research is satisfactory in its methodology and in its scholarly presentation and format.

Conclusion: I would like to state that Mgr. Vladimir Sincari met all legal requirements relating to Doctoral degree graduates. I recommend this thesis for his PhD defence and as a base for awarding of the candidate by the degree of Doctor of Philosophy.

Ing. Zdeňka Sedláková, CSc.

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