Department of Experimental Physics

Faculty
of Science

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Opponent review of PhD Thesis of Mgr. Nikola Matějková

The present PhD Thesis written by Mgr. Nikola Matějková under the supervision of Prof. Zuzana Bílková is entitled ***Nanomaterials for Drug Delivery and Other Bioanalytical Applications***.

The present PhD Thesis reacts on actual demands and deals with three very hot topics of research, such as: (i) development of drug delivery systems based on hyaluronic acid nanoparticles, (ii) generation of polyion complexes for intracellular mRNA delivery, and (iii) exploitation of a composite nanomaterial for viral RNA extraction with possible *in situ* detection.

The **Dissertation Thesis of Mgr. Nikola Matějková is very clearly written and logically arranged** into classical chapters including Annotation, Abstract, Lists of Figures, Tables, and Abbreviations, Introduction (pages 21-64), Aims and Objectives (page 65-66), Materials and Methods (pages 67-80), Results and discussion (pages 81-137). The main part of the Thesis ends up by Conclusions and future perspectives (page 138), followed by references (on pages 139-183) and supplements (pages 184-188). The list of work published by the PhD candidate, Mgr. Nikola Matějková, in international highly-ranked scientific journals, or presented on international/national conferences is starting on page 189 of the final pdf file. Title pages of five published articles where the PhD candidate is twice in the role of the first-author (publications in Q1 journals) and thrice as a co-author (publications in Q1-Q3 journals), can be found out on pages 192-196. It is obvious that experimental results (around 56 pages) slightly dominate over the introductory part (around 43 pages), which is **in full agreement with standards required for PhD Theses**.

The first part of **Introduction** provides a broad portfolio of nanomaterial types serving as drug delivery systems including stimuli-responsive nanomaterials (pages 21-40). It

is a **very good text with relatively simple, but accurate explanations**, that can be even **recommended as a study book chapter for undergraduate students**. The next part of Introduction introduces nanomaterials used as therapeutical agents, i.e. either in the treatment of cancers, or against microbes, viruses, as anti-inflammatory species, or even in vaccine development. The next subchapter (1.3) of Introduction deals with nanomaterials for diagnostics. Finally, subchapter 1.4 briefly describes principles, advantages and disadvantages of analytical methods for nanomaterials characterization. I highly appreciate the logical order of subsections and appropriateness of introduced methods brief description within 1.4 subchapter, i.e. going from nanomaterial size evaluation, through inner structure investigation and chemical composition to surface analysis.
To this subchapter **(1.4), I would have only a comment** that TEM (transmission electron microscopy) images and their statistical analysis can be also used for particle size distribution assessment (very recently discussed in ACS Nanoscience, doi: 10.1021/acsnanoscienceau.4c00076) and therefore, this technique could be mentioned in 1.4.1 section as well (although, I would maintain its current location in section dealing with structure and morphology analysis). At the same moment, it should be reminded that TEM-determined nanoparticle diameters are generally of smaller values than those determined by DLS (dynamic light scattering), because hydrodynamic diameters (particles and their hydration shell) are measured in the latter case. As the PhD candidate states later in Results and discussion section, it is, however, important to determine hydrodynamic diameters when heading towards *in vivo* applications. It is therefore fully understandable that mostly DLS results are considered when hydrodynamic diameters compared.

Objectives of the present Thesis are very clearly defined and looking at the PhD candidate´s publications as well as reading Results and discussion section of her PhD Thesis, they were absolutely fulfilled.

Methodology and work plan are adequately described in Experimental part as well as in Results and discussion. I would like to express my satisfaction with the flow of the

text and **logical justification of step-by-step nanomaterial characterization that is worth of following by any PhD student.**

There are no obvious errors and/or spelling mistakes in the whole text. I have only a few remarks which do not lower the overall high quality and excellence of this **outstanding PhD Thesis**:

- Page 39, the PhD candidate writes about photo-stimulation of drug delivery systems and specifies the ranges of UV, visible and NIR: "UV (10-400 nm), visible (380-780 nm), or near-infrared (790-1400 nm)". However, there are obvious 20 nm and/or 10 nm gaps.
- Page 84: usual abbreviation for adipic acid dihydrazide is ADH and not AAD.
- Page 76-77: TiO_2NT@Fe_3O_4NPs preparation procedure is referenced, but not specified. It is a usual practice to describe it at least briefly.

I fully agree with the PhD candidate that nowadays many researchers do not perform thorough characterization of the newly developed engineered nanomaterials unfortunately, which leads to a low reproducibility of results and sometimes even incorrect conclusions.

Finally, I have **a few questions**:

1) In section 4.2, reporting about the formation and characterization of a polyplex consisting of selected polymer (alkyne-poly(2-ethyl-2-oxazoline)$_{70}$-poly(2-methoxy-carboxyethyl-2-oxazoline diethylenetriamine)$_{50}$, abbrev. AED2, and a modified mRNA (page 107), it is not clear what type of mRNA modification is considered. In Experimental section, it is written solely (page 72): "The used mRNA was Firefly Luciferase mRNA (luc-mRNA), gifted by Drew Weismann (Perelman School of Medicine, University of Pennsylvania, USA)." Can Mgr. Nikola Matějková comment it?

2) Staying in section 4.2, the percentage of bound mRNA (to AED2, in comparison to the original mRNA concentration) differs according to the

experimental data stemming from absorbance and fluorescence measurements: approx. 50% of unbound mRNA as derived from absorbance measurements vs. 10% of unbound mRNA based on fluorescence data. It is explained by a lower precision of absorbance measurement (page 112). However, rather than a term "precision", it is the difference in sensitivity of these spectrophotometric techniques. Can the PhD candidate compare their sensitivity? How could be explained the fact that no clear difference or trends were observed for various N/P ratios in polyplex fluorescence signals when the percentage of bound mRNA evaluated (page 112)? Based on Figure 44 (page 112), one could have impression that none of the spectroscopic methods (neither absorbance, nor fluorescence) reflects any differences among various N/P ratios in polyplex considering the error bars. Which method would be recommended by Mgr. Nikola Matějková as a reliable one for bound mRNA percentage evaluation? Agarose electrophoresis or any other method?

3) Titanium oxide nanotubes decorated with magnetite nanoparticles were used to extract viral RNA in the third part of the Thesis experimental section. It has been presumed that a similar affinity of this nanomaterial for phosphopeptides (proven by the research group of her supervisor) can be exploited in the case of RNA extraction (page 121). Can the PhD candidate, during her answer, point out and demonstrate the similarity of phosphopeptides and RNA on molecular level? Can she discuss and provide details about types of chemical bonding preferences of the used nanomaterials (TiO_2 and Fe_3O_4)? As far as I understood, in her work she compared the RNA extraction ability reached by means of an engineered composite material (TiO_2NT@Fe_3O_4NPs) with that achieved using commercially available porous TiO_2 microspheres of two different sizes (5 μm and 10 μm). However, surface chemistry and reactivity of porous titanium dioxide microspheres and the composite material can differ due to differences in chemical composition, not speaking about shape (tubes vs. spheres) and size differences. Therefore, is it reasonable to compare these

materials mutually since they differ by more than one factor? Can she explain Table 13 (page 126) since it is unclear to me?

I recommend this PhD Thesis to be defended. Subsequently, I fully support Mgr. Nikola Matějková to become a doctor, i.e. Ph.D. title can be awarded after her oral PhD defence.

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