Review Report on the PhD thesis

submitted to Charles University, Faculty of Science,

Department of Physical and Macromolecular Chemistry

Title: **Design and characterization of advanced polymer-coated upconversion nanoparticles**

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The thesis submitted by Mgr. Uliana Kostiv is devoted to lanthanide-based upconversion nanoparticles that absorb near-infrared radiation and emit radiation in visible or ultraviolet region. The experimental work is focused on their synthesis, surface modification and evaluation of their performance in biological studies. Specifically, the thesis aims to demonstrate the synthesis of $NaYF_4$:Yb^{3+}/Er^{3+} and $NaGdF_4$:Yb^{3+}/Er^{3+} nanoparticles with controllable morphology and size, narrow size distribution and high crystallinity. These particles are then modified by introducing hydrophilic and biologically inert coatings, such as silica or PEG-neridronate, that provide colloidal stability and prevent undesirable interactions between the upconversion nanoparticles and biological system or even provide the so-called „stealth“ character to achieve long circulation time in blood vessels. In addition, the particles are modified with specific targeting moieties based on short peptides and are also conjugated with aluminium carboxyphthalocyanine, which offers a platform for NIR-induced photodynamic therapy. Fundamental characterizations of prepared samples by transmission electron microscopy, X-ray powder diffraction, dynamic light scattering, IR spectroscopy and upconversion luminiscence spectra are supplemented by biological studies both *in vitro* and *in vivo*. For the *in vivo* biodistribution study, radiolabelling of the particles was also carried out. Advanced biological experiments included the photodynamic therapy of a mice with xenotransplanted tumor model.

The thesis is written on 50 pages, is supplemented by six papers and includes all standard sections. From the formal point of view, the text is very clear, well-written and readable. The review of literature is well-balanced with respect to the aims of the work and its extent is appropriate. The English grammar and vocabulary are fine with only minimum mistakes and typing errors (however, the use of dangling participles should be really avoided).

The list of publications included in the PhD thesis contains six papers where Mgr. U. Kostiv is the first author. These papers were published in respected journals with high impact factors, e.g. two papers in Nanoscale, one paper in ACS Applied Materials & Interfaces, another one in RSC Advances, etc. Furthermore, four papers where Mgr. U. Kostiv is a co-author are listed and are relevant for the content of the thesis, two of them were already published (ACS Nano and RSC Advances), whereas the other two have been submitted.

My specific comments and questions are as follows:

1) There are some minor details in the list of abbreviations that should have been corrected or formulated in more precise way:

    a. NHS is not *N*-hydroxysulfosuccinimide (this reagent is usually denoted as sulfo-NHS), but NHS is just the *N*-hydroxysuccinimide.

    b. HeLa [cells] should not be defined as "human cervical carcinoma" since it is not a general term for this type of cancer. HeLa is a specific immortal cell line that is based on cell isolated from a cervical tumor of Henrietta Lacks (therefore, the name HeLa) who died on cancer in fifties.

    c. RGD should not be defined as "arnigylglycylaspartic". It is either arginylglycylaspartic acid or arginylglycylaspartyl [group].

2) The author mentioned in the description of DLS in the main text of the thesis: „The DLS reports a mean nanoparticles size [...]". Further, the symbol D_h used in main text is explained as „hydrodynamic diamater". Actually, there are several different values that are calculated based on DLS data. The most common measure is the so-called Z-average size (usually denoted as D_Z), which is the intensity-based harmonic mean. However, other mean values can be calculated and are also reported by the Malvern Zetasizer software, namely the means based on different distributions, i.e., the intensity distribution, volume distribution and counts of particles („number mean" in the Zetasizer software). Could you be more specific, please? Could you discuss why you have chosen a particular mean, please?

3) Could you present the three different distributions obtained from DLS data for at least one sample of colloidaly stable nanoparticles of either $NaYF_4:Yb^{3+}/Er^{3+}$ or $NaGdF_4:Yb^{3+}/Er^{3+}$ in any type of solvent, preferably with only thin stabilizing surface layer (e.g. nanoparticles caped with oleylamine/oleic acid in any organic solvent)? The author reported mostly the mentioned "hydrodynamic diameter"/"mean nanoparticles size" and the polydispersity. However, different weighting and possible transformation of the distribution are important. For example, the number means will signify the large fraction of small nanoparticles although most of the material, most of its weight/volume, might form larger particles.

4) I wonder what the chemical homogeneity of the $NaYF_4:Yb^{3+}/Er^{3+}$ and $NaGdF_4:Yb^{3+}/Er^{3+}$ systems is? The host compounds, $NaYF_4$ and $NaGdF_4$, were doped with Yb^{3+} and Er^{3+} cations, namely by large quantity of Yb^{3+} and low amounts of Er^{3+}. Well, there are clear evidences that both the dopants are involved in individual nanoparticles (or their majority) of the host compounds. However, no conclusive data on the homogeneity were presented.

    One may suggest that there is a whole distribution of nanoparticles of various compositions or even that the two dopants are unequally distributed into the different phases observed in the prepared samples (see, e.g. the publication no. 2). Provided

that the homogeneity of the samples has not been analysed in detail, could you present some data on comparable systems from literature, please?

Although there might be some literature with HRTEM-EDX studies that show chemical mapping in high resolution and chemical analysis of individual nanoparticles, we can have still concerns about the accuracy of the quantification and corresponding conclusions. There are some disturbing issues related to EDX chemical analysis of such samples. Moreover, the analysis of selected particles on a TEM grid does not provide relevant view on the whole sample.

In my opinion, it would be also useful to carefully analyse XRD patters with respect to line broadening. Naturally, the small size of nanoparticles delimits the XRD coherence length, which leads to line broadening. However, there can be also some strain contribution to the line broadening, in our case the "chemical strain", which is given by certain distribution of lattice parameters that originates in the distribution of chemical composition of individual particles. By performing Rietveld analysis of XRD patters and by applying a suitable profile function, namely the Thompson-Cox-Hastings pseudo-Voigt function, we can resolve the size and strain contributions. Care has to be taken to determine also the instrumental function, e.g. by measuring a suitable strain-free standard (e.g. tungsten, LaB_6) with large grains.

Are there any relevant papers that applied rigorous analysis of the chemical homogeneity of materials studied in the thesis or comparable systems?

5) Thousands of studies have employed the evergreen of the functionalization of nanoparticles, namely the coating of particles with silica combined with application of alkoxysilanes that possess various functional group suitable for coupling. The most popular choice is the application of 3-aminopropyltriethoxysilane (or 3-aminopropyltrimethoxysilane) followed by amide coupling. Then, all possible functional moieties can be attached like targeting molecules, fluorescent dyes, therapeutic agents, etc. The same strategy was used in certain cases also by the author of the thesis.

   However, such silanes cause inherent hydrolytic instability of complex nanoparticles. For example, the targeting moiety, albeit originally covalently anchored to the silica shell, will be released, or an organic corona, originally attached to the particle surface, will be gradually lost [1, 2]. It has to be said that the deep-rooted functionalization procedures and various anchoring techniques based on aminoalkyltrialkoxysilanes are often applied for the preparation of elaborate nanoparticles that are subsequently used in advanced biological studies, but unfortunately, not even the short-term stability of such systems is analysed. This repeatedly occurs despite the clear evidence, published as early as 1980 [1].

   Have you studied the stability of the covalent functionalization in an aqueous suspension?

6) Interestingly, the author studied the "$NaYF_4:Yb^{3+}/Er^{3+}@SiO_2-NH_2$" nanoparticles by DLS in water and reported hydrodynamic size (see p. 29 of the main text). However, these particles do not form a colloidal stable suspension in water, and their hydrodynamic size cannot be determined in water (the data obtained cannot be repeatable).

References

1. Bridger K and Vincent B 1980 The terminal grafting of poly(ethylen oxide) chains to silica surfaces. *Eur. Polym. J.*, **16** 1017-1021.
2. Zhang Z K, Berns A E, Willbold S and Buitenhuis J 2007 Synthesis of poly(ethylene glycol) (PEG)-grafted colloidal silica particles with improved stability in aqueous solvents. *J. Colloid Interface Sci.*, **310** 446-455.

All my comments should be considered only as formal and minor objections and suggestions for the discussion.

I really appreciate the work of Mgr. U. Kostiv, both her experimental work with relevant publication outputs and the PhD thesis, which describes a great deal of work by providing a nice summary. The research is highly competitive on the international level and forms a nice contribution to the field of upconversion nanoparticles. The publications included in the thesis surpass the typical level of a Ph.D. candidate.

In conclusion, the thesis of Mgr. Uliana Kostiv meets all requirements imposed on this kind of work and is ready to be defended. The excellent results presented in the thesis and published in established journals definitely fulfil conditions for the Ph.D. degree.

Prague, 26.6.2018

Ing. Mgr. Ondřej Kaman, Ph.D.

Fyzikální ústav AVČR