Review of the doctoral thesis of MSc. Anastasiia-Bohdana Shatan "Magnetic nanoparticles with antibacterial properties: Synthesis, characterization and biological applications"

Thesis of Anastasiia-Bohdana Shatan deals with the synthesis and basic characterization of superparamagnetic nanoparticles followed by their modification in order to obtain the stable and well-defined nano-systems suitable as carriers of antimicrobial agents (silver NPs, polycationic brushes, conventional antibiotics). The topic is timely and extremely important for medical applications, where the role of nanoparticles as antimicrobial agents is still not sufficiently explored and exploited.

Thesis is a result of the 6 years-long study at Charles University under the supervision of Ing. Daniel Horák, CSc. (IMC). It is based on 3 papers in journals with IF (the first author in all three cases; 1 Springer and 2 MDPI; current citations: 22, 8, 0). All the papers are attached. Thesis is written in English, it has 59 pages and 82 references, and the text is divided to 5 chapters: Introduction into the field of interest, Aims, Experimental section followed by the Results and Discussion chapter presenting in a slightly modified way the published papers. Thesis is summarized in Conclusions.

The anti-plagiarism program (Turnitin) detected relatively high 53 % of similarity, which is mostly given by re-using and re-phrasing of published parts in Experimental and Results-Discussions sections. Thus, this situation can be acceptable. Nevertheless, it is a pity that the candidate did not take the opportunity to generalize the results published over 5 years (publications from 2019, 2021, and 2024), and presented only the concise versions of the attached papers.

The introductory parts of the Thesis are informative and provides a very good overview to the field of superparamagnetic nanoparticles and their modification in respect to antimicrobial applications. Results and Discussions section has been already commented above. Further, this part unfortunately contains some introductory and general comments (originated from the corresponding papers), which would be more suitable for the Introduction section. What I also find problematic was fluctuating quality of figures. Their origin or copyright are not mentioned at al. For example, Figure 11 is of rather poor quality, it lacks further comments, signal assignment and it is only the combination of figures taken from SI of the paper No.3.

The work is rather multidisciplinary. Thus, the attached papers have a high number of co-authors. Unfortunately, the candidate´s contribution to the results is not clearly stated in Thesis, and it should be clarified during the defense.

Despite my criticism and problems expressed above and by my questions listed below, Thesis represents the valuable contribution to the field of polymeric drug-delivery systems with antimicrobial action, and I would like to state that MSc. Anastasiia-Bohdana Shatan met all legal requirements relating to Doctoral degree graduates. I recommend this thesis for her Ph.D. defense.

I have several questions and comments to general discussion:

1) Why NPs used for the decoration by silver nanoparticles and polymers were prepared by thermal decomposition, while the particles for encapsulation of Ag-SMT were prepared by coprecipitation, which provided the system of bigger nonuniformity?

2) TEM provided sizes of MNPs in order of 10 nm but DLS around 200 nm. It indicates that there are probably a very small fraction of very large aggregates consisting of roughly hundreds or thousands of nanoparticles, and their LS-signal is seemingly so big that information about the original and relatively small MNPs is hidden, especially if it is measured by the zeta-sizer and not by the LS spectrometer with a goniometer. In other words, most of the arguments based on DLS data are irrelevant to original MNPs, and some of the arguments used by the candidate in Thesis as well as in the attached papers are simply incorrect (changes in Dh by hydration, electric double layer, etc.). Further, it is highly probable that also the ZETA-potential values are related to the large aggregates and not to small MNPs. Moreover, there are micrographs of a few MNPs glued by a silica layer (Figure 2 in the publication No.1), is this situation real, it means relevant for NMNPs in solution, or is it an artifact of TEM? As the effective surface area is very important for antimicrobial applications, I would like to ask the candidate for clarification how the thickness of stabilizing layers on the MNP core was determined.

3) It seems that silver NPs are invisible in the corresponding TEM micrographs and other characterization (such as by UV/Vis spectroscopy) is not provided. As the size and shape of Ag-NP might be important for the antimicrobial action, the issue should be properly addressed. It was stated in the publication No.1 that the mechanism of antimicrobial action of silver nanoparticles was insufficiently understood. Is there any progress after 5 years?

4) MNPs decorated by polymer brushes were prepared by the “grafting-to” approach. Is it possible to produce them by the “grafting-from” procedure?

5) The antimicrobial agent Ag-SMT is a complex of silver with a classical antibiotic SMT. It is more active but less soluble in water that the parent agent. I am wondering about the stability of Ag-SMT in solution and about solubility increase in ammonia solution and by the inclusion to beta-CD. In the literature, only the complex of parent SMT with CD is described. How does it work with Ag-SMT? Further, the inclusion of the agent by CD can cause its lower effective concentration and it can deteriorate its biomedical action. What is the association constant, Ka, of Ag-SMT/CD complex, and how it can be determined?

In Prague, 19th April 2024 Prof. RNDr. Pavel Matějíček, Ph.D.