

Review Report on the PhD thesis

submitted to the Faculty of Science, Charles University

Department of Physical and Macromolecular Chemistry

Title: **Magnetic nanoparticles with antibacterial properties: Synthesis, characterization and biological application**

Author: **MSc. Anastasiia-Bohdana Shatan**

Supervisor: **Ing. Daniel Horák, CSc.**

The submitted thesis is focused on development of magnetic nanoparticles with complex coatings that provide antibacterial and antifungal properties, thereby aiming for new and promising nanomaterials for applications in medicine and water purification. The magnetic cores of the developed particles are based on common iron oxides with the spinel structure, whereas different antimicrobial coatings are synthesized by combining various approaches and materials, such as the encapsulation into silica, its modification with suitable alkoxysilanes, decoration of particle surface with ultrafine silver colloids, covalent anchoring and coating with cationic polymers, and complex organic functionalization. The corresponding products are subjected to conventional characterizations and are employed for treatment of several model pathogens *in vitro*, including both prokaryotic and eukaryotic cells, with the former being represented by both Gram-positive and Gram-negative bacteria.

The main text of the thesis spans 43 pages (including the English and Czech abstracts and list of abbreviations) and is followed by an 8-page list of references with 82 items. The appendix comprises three publications with lengths of 12, 15 and 17 pages. The text follows the traditional structure with an introduction, experimental part, results and discussion, and conclusions. However, the results and discussion section includes some introductory paragraphs, which would fit much better into the introduction. The introduction itself could be expanded to provide more detail as it currently only offers a basic insight into the studied topic.

From the formal point of view, the text is written well and clearly, the English grammar is quite fine, while typing errors are rather scarce. While some minor formal problems and imperfections will be addressed at the end of this report, one point will be highlighted right here. I really lack a clear summary what work was done by the candidate alone and which experiments, measurements or data analyses were carried out by other colleagues or on the service basis. Although such an acknowledgement may not be compulsory for this kind of work, it would be appropriate to include this information, specifying all names and respective affiliations.

The PhD thesis is accompanied by three original papers in impacted journals, and the candidate is the first author for all of them. One paper was published in the well-established

journal *Pharmaceutical Research* (Q2) issued by Springer, whereas the other two papers were published in MDPI journals.

Before going to specific comments, I would like to state that I truly appreciate the experimental work done by the candidate and believe the thesis is ready to be defended. At the same, I have to be critical with respect to several important issues, which relate both the scientific work and the submitted thesis.

My main comments regarding the scientific and factual content are as follow.

- 1) The text of the theses is not free of factual mistakes and problematic statements, which is a burden especially for introduction. Specifically, introductory sections 1.1 “Magnetic nanoparticles (MNPs)” and 1.2 “Properties of MNPs” include several incorrect or misleading comments regarding magnetic materials and their properties. Selected examples follow.
 - a. On p. 9, it is written: “*Maghemite ($\gamma\text{-Fe}_2\text{O}_3$) has a cubic structure; its each unit contains 32 O^{2-} ions, $21\frac{1}{2}$ Fe^{3+} ions and $2\frac{1}{2}$ vacancies.*” First, the actual number of vacancies in the spinel unit cell of maghemite is 8/3. Second, the maghemite structure, which can be easily derived from the magnetite structure by introducing the vacancies on the octahedral sublattice, can be either cubic or tetragonal. If the vacancies are introduced randomly, the resulting structure can be described by the $Fd\bar{3}m$ space group similarly to magnetite. Some ordering of these vacancies is also possible, and at least partial ordering often occurs, e.g., in samples prepared by the thermal decomposition method. This may lead to a cubic system whose symmetry is described by the enantiomorphous pair of $P4_132/P4_332$ space groups. However, tetragonal phase may arise with the symmetry of $P4_12_12/P4_32_12$, exhibiting clear tetragonal super-reflections in powder XRD.
 - b. The following comment on p. 9 explaining the absence of hysteresis in superparamagnetic state is not correct: “*Unlike ferromagnetic materials that exhibit hysteresis and retain magnetization after an external field is applied and removed, superparamagnetic materials do not exhibit hysteresis loops. This is because their magnetization can rapidly switch direction in response to temperature changes.*”
 - c. Classification of magnetic materials is provided on p. 10, where three main groups are distinguished: (i) diamagnetic materials, (ii) paramagnetic materials, and (iii) ferromagnetic and ferrimagnetic materials. Surprisingly, the diamagnetic materials are classified as “magnetic materials”, while antiferromagnetism and antiferromagnetic materials are not mentioned in the text at all. Moreover, Fig. 2 is not properly explained and is quite misleading. I wonder if the candidate can explain what the circles and arrows symbolize and if the candidate can provide a brief comment to the depicted materials, please.
 - d. I also wonder why hematite is described in section 1.1 titled “Magnetic nanoparticles (MNPs)”. This section describes three members of the iron oxide

family, namely hematite, magnetite and maghemite, allegedly, as the only promising iron oxides for medical applications. First, the role of hematite is not rationalized in the text. Second, the fascinating polymorph of ϵ -Fe₂O₃ cannot be disregarded.

- 2) It is mentioned in section 3.4 “Characterization methods”, see p. 23, that the hydrodynamic diameter, polydispersity, and electrophoretic mobility (converted to ζ -potential) of nanoparticles were obtained by dynamic light scattering (DLS). However, the electrophoretic mobilities and corresponding values of ζ -potential must have been determined by electrophoretic light scattering (ELS), which should be distinguished from DLS.
- 3) The thesis describes the preparation of silica-coated magnetic particles and their subsequent modification with 3-mercaptopropyltrimethoxysilane (MPTMS), which should facilitate their decoration with ultrafine silver nanoparticles. Regarding the decoration with the silver colloid, the candidate wrote on p. 29: *“Following the precipitation of AgNO₃ with sodium borohydride in the presence of Fe₃O₄@SiO₂-SH particles, silver nanoclusters were formed on the particle surface (Figure 8).”* Surprisingly, Fig. 8 shows - instead of some illustrative transmission electron micrographs - just a nice cartoon how such particles are formed. Several controversies are involved here.
 - a. Direct covalent attachment of the silica shell to the iron oxide core is depicted in this cartoon. However, the starting iron oxide particles were originally capped with oleic acid (due to synthesis by the thermal decomposition method), i.e. oleic acid molecules were covalently attached to these particles, forming oleate complexes with iron centres on the particle surface. Actually, this is the reason why the candidate applied the well-known technique of encapsulation in a reverse microemulsion with the IGEPAL CO-520 surfactant (i.e. poly(oxyethylene)nonylphenyl ether with ~5 oxyethylene units) as a suitable stabilizer. We should point out that the thorough removal of oleic acid from the surface of such particles is rather difficult. Is the aforementioned picture silica-coated particles in Fig. 8 really realistic? Is there any evidence for the direct covalent attachment of the silica to the iron oxide?
 - b. According to a transmission electron micrograph of the final product in the corresponding publication (paper no. 1, fig. 2d), the decoration is poor – the degree of coverage of the silica surface with silver nanoparticles is very low. The given micrograph shows only a single large formation of particles glued together with silica. It would be nice to see more micrographs to get a better idea about the morphology of the studied sample and to rule out its heterogeneity. Actually, I would not be surprised if a non-negligible fraction of silver was precipitated separately in form of free particles, while a considerable amount of silica surface remained almost non-decorated. Could the candidate provide more micrographs and comment on these issues, please?

- c. The modification of silica surface with MPTMS will inevitably decrease the colloidal stability of resulting particles in water. The extent of this modification will be probably decisive for their colloidal stability but the situation might be further complicated by the possible heterogeneity of the resulting product. Specifically, precipitation of almost pure MPTMS-based silica or uneven distribution of MPTMS may occur, leading to different populations of particles in the corresponding product. In such a scenario, the MPTMS-rich fraction would be characterized by strong aggregation and fast sedimentation, while only particles with a low degree of functionalization with MTMPS would remain dispersed and would provide a signal during DLS measurements. The thesis does not address the issue of colloidal stability of the MTMPS-functionalized product as well as of the final silver-decorated product properly. I wonder if the candidate can comment on the colloidal stability of these samples, discuss any observation of sedimentation and provide a better insight into DLS measurements of hydrodynamic size (repeatability of hydrodynamic size, fitting procedure). I also wonder why zeta potential was not measured for the MPTMS-functionalized and Ag-decorated samples (according to the Table 1, p. 31).
- 4) According to the interesting (and somewhat idealized) scheme in Fig. 10, an elaborate organic functionalization, including covalent attachment of β -CD, was applied to maghemite particles prepared by coprecipitation and subsequent oxidation. This functionalization was designed to attach a cargo of silver sulfmethazine (SMT-Ag) to the maghemite particles based on host-guest interaction of this highly hydrophobic drug and β -CD. Relevant NMR data were included in the supplementary materials of the publication no. 3, based on which, e.g., important ratios of selected organic moieties were quantified to confirm the structure of the desired compounds. Nevertheless, some questions still arise, and some data are not that convincing.
- a. According to the mentioned scheme, β -CD-VS was synthesized in the fourth step by reacting divinyl sulfone with ethanolamine-functionalized dextran (β -CD-EA) via the aza-Michael reaction. One could speculate that some fraction of resulting β -CD-VS molecules underwent another reaction with the starting compound β -CD-EA, whereby a bis(β -CD) derivative with a sulfone bridge was formed. Is it possible that a mixture of the desired β -CD-VS and bis-derivative was formed? Were any relevant characterizations applied to resolve this issue and possibly to analyse the molecular weight of the product?
- b. The thesis (and the experimental section of the publication no. 3) describes that the maghemite nanoparticles coated with the DPA-Dex- β -CD conjugate were further mixed with SMT-Ag to achieve the host-guest interactions and loading of the cargo onto the magnetic carrier. Considering the given experimental procedure and the very low solubility of SMT-Ag, one could speculate that the final product may be, at least partially, formed by a mixture of the drug-free Fe_2O_3 @DPA-Dex- β -CD particles and free (uncomplexed) SMT-Ag. In contrast, attempts to increase the solubility of SMT-Ag by ammonia

(which forms the well-known $[\text{Ag}(\text{NH}_3)_2]^+$ complex cation) may lead to another pitfall (entrapment of Ag-free SMT as a cargo, whilst Ag^+ ions are washed away as the soluble ammine complex). Could the candidate comment on these ideas, please, including the issue of possible protonation of the SMT anion formed upon dissociation of SMT-Ag?

Some comments will be provided regarding selected formal issues.

- 1) Going back to the point of distinguishing the candidate's effort and the work of others, I wonder if the candidate can briefly summarize, please the different methods employed in these studies and role of individual researchers.
- 2) The quantity D_w defined by the expression (2) on p. 23 should not be primarily denoted as the "weight-average diameter" but as the volume-weighted mean diameter. The former designation is based on a rather rough approximation.
- 3) Unlike the stereochemical descriptors of the Cahn–Ingold–Prelog system and unlike the rather rarely used *d/l* descriptors (dextrorotatory/levorotatory), the D- and L-descriptors, relating the configuration to the prototypic glyceraldehyde isomers, should not be italicized.
- 4) The binominal names of organisms, including bacteria and fungi, with the generic name being abbreviated, such as *A. niger* and *C. albicans* should not be formally included in the list of abbreviations. The use of the abbreviated forms of binomens is regulated by respective nomenclature codes (for example, the International Code of Zoological Nomenclature covers this issue in the Recommendation 25A). Similarly, IGEPAL CO-520 is not an abbreviation but a brand name (IGEPAL is a registered trademark) and thus it should not be included in the list of abbreviations.
- 5) Several abbreviations used in the text are missing in the list of abbreviations, e.g., SEC (size exclusion chromatography), IS (icosane), VDPA (vinylidene 1,1-diphosphonic acid).
- 6) Hydrates should not be written with the symbol "x" (cross product) - see, e.g., " $\text{FeCl}_3 \times 6\text{H}_2\text{O}$ " on p. 20.

Irrespective of the objections above, the thesis by MSc. Anastasiia-Bohdana Shatan summarized an ample amount of work and meets all requirements imposed on the thesis. It is ready to be defended.

Prague, 19 April 2024



Ing. Mgr. Ondřej Kaman, Ph.D.

Institute of Physics of the Czech Academy of Sciences