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REVIEW OF THE DOCTORAL DISSERTATION
ENTITLED "DESIGN AND SYNTHESIS OF MICELLAR NANOCARRIERS FOR INTERNAL RADIOTHERAPY"
BY MATHILDE PONCHELLE

The PhD project entitled "Design and Synthesis of Micellar Nanocarriers for Internal Radiotherapy" was conducted by Mathilde Ponchelle under the supervision of Dr. Eric Doris from the Alternative Energies and Atomic Energy Commission (CEA Paris-Saclay) and Dr. Marek Pruszyński, DSc, affiliated with both the Institute of Nuclear Chemistry and Technology (IChTJ) and the National Centre for Nuclear Research (NCBJ).

This review was prepared at the request of the Discipline Council of the Institute of Nuclear Chemistry and Technology in Warsaw, within the field of natural and exact sciences.

1 Scientific value of the thesis

The main objective of this work was to develop and evaluate multifunctional nanostructures that combine advanced chemical synthesis with radiolabelling techniques for potential applications in cancer therapy, and this goal has been successfully achieved. The research demonstrates the candidate's ability to integrate complex synthetic methodologies with radiochemical approaches and to assess the physicochemical and biological properties of the resulting nanostructures. The thesis is composed of two main parts, preceded by an introduction that provides fundamental information on cancer, nuclear medicine, radiotherapy, and nanoparticles.

The first part focuses on the synthesis of a fluorinated amphiphile, which was used to formulate perfluorinated micelles designed for tumor radiosensitization. In addition to their oxygen-carrying capacity, these micelles were engineered to incorporate gold nanoparticles for radioenhancement purposes. Gold nanoparticles coated with fluorinated ligands were synthesized and successfully encapsulated within the perfluorinated micelles. Furthermore, the toxicity of the obtained nanostructures was evaluated in two cancer cell lines — murine melanoma (B16F10) and human breast cancer (SKBR3). The radioenhancement efficiency of the nanostructures was also investigated by irradiating these cells with 9 MV electron beams under two experimental conditions: acute exposure, where cells were incubated with nanoparticles for 24 hours before the medium was replaced with fresh culture medium prior to irradiation, and chronic exposure, where nanoparticles remained in the culture medium throughout the entire experiment. Three concentrations of the micelles, with and without gold nanoparticles, were tested to assess the concentration-dependent effects on cytotoxicity and radiosensitization performance.

The second part describes the integration of lutetium-177 into the micellar systems for internal radiotherapy applications. The [^{177}Lu]-labelled micelles were tested *in vitro* for their radiotoxicity against B16F10 and SKBR3 cancer cell lines, using non-radiolabelled analogs as negative controls. A preliminary biodistribution study was conducted on healthy C57Bl/6 mice to determine the pharmacokinetic parameters of [^{177}Lu]AuNP@Micelles following intravenous injection. In the final stage of this work, direct intratumoral administration (nanobrachytherapy) was implemented as an alternative route to systemic delivery.

The results contribute to the growing body of knowledge in the field of theranostic nanomaterials and may provide a foundation for future translational studies. This work is further supported by a review article authored by the PhD candidate, entitled "^{177}Lu–Gold Nanohybrids in Radiotherapeutic Approaches Against Cancer", published in Small Science, which is closely related to the topic of the dissertation and reflects her comprehensive understanding of the current state of research in this area.

2 Editorial correctness of the dissertation

The dissertation begins with a list of abbreviations, followed by a general introduction providing essential background information on cancer, nuclear medicine, radiotherapy, and nanoparticles. The main body of the thesis is divided into two chapters. The first chapter focuses on the synthesis and characterization of fluorinated micelles and their encapsulation of gold nanoparticles for tumor radiosensitization, including studies on their oxygen-carrying capacity, radioenhancement properties, and *in vitro* toxicity. The second chapter addresses the incorporation of lutetium-177 into the micellar systems for internal radiotherapy, presenting radiolabelling procedures, stability assessments, in vitro radiotoxicity studies on B16F10 (murine melanoma) and SKBR3 (human breast cancer) cell lines, as well as preliminary *in vivo* biodistribution and tumor-retention experiments in C57Bl/6 mice, including initial nanobrachytherapy approaches. The dissertation concludes with a general summary, bibliography, and annexes containing experimental details, list of figures, and supplementary data.

The information presented in each section is comprehensive, and the conclusions are clearly formulated, reflecting sound reasoning and scientific rigor. The overall organization, depth of analysis, and integration of relevant literature demonstrate a high level of research competence. Moreover, the dissertation is written in a clear and accessible manner, evidencing the candidate's deep understanding of the subject. The structure of the work is coherent and logical, with only minor formal issues and critical remarks discussed in the following section.

3 Critical remarks

The dissertation has been carefully edited; however, a few minor editorial issues and research-related questions have been identified and are listed below for the sake of clarity.

1. The manuscript shows some inconsistency in the use of abbreviations. Several abbreviations are reintroduced multiple times, despite the guideline that they should be defined only upon their first occurrence (e.g., ROS appears repeatedly on pages 38, 40, 55, and 90; PET on pages 26, 29, and 61). Additionally, certain abbreviations are used without prior definition—for example, red blood cells on page 57, while the abbreviation RBC appears later in Figure 8 without introduction. Although RT is commonly used to denote room temperature, it should still be defined before its first appearance, which occurs in Figure 32. Furthermore, the acronyms of the research institutions—CEA (Commissariat à l'énergie atomique et aux énergies alternatives) and NCBJ (National Centre for Nuclear Research)—are used without prior explanation. Consistent and proper introduction of abbreviations would enhance the clarity and readability of the manuscript.

2. In several places, sentences are missing a period at the end, e.g., the fourth line on page 35, the third line on page 63, and the third line on page 66.

3. The bibliography is organized in a non-standard way. References appear in footnotes throughout the text. In some cases, multiple sources are grouped under a single reference number, while in other cases, one source corresponds to one number, resulting in inconsistency. Additionally, a list of references is repeated at the end of the dissertation without a title and is formatted in a different font style, which affects the visual coherence of the document.

4. On page 109, in section IV.B.3 "Stability study of the radiolabeled micelles," the text is centered instead of justified, disrupting visual consistency. This likely occurred during the transfer of the figure, whose caption is correctly centered. In Figure 32, the caption appears both above and below the image, whereas it should only appear below.

5. The definitions of radiosensitizing parameters are provided only on page 85, although the author refers to them earlier (page 64), citing values from other research groups. For better readability and logical flow, these parameters should be defined upon their first mention.

6. The term "damages" is incorrectly used in the context of DNA. The correct term is "damage," which is uncountable in this context. The plural "damages" is appropriate in legal or financial contexts (e.g., "financial damages").

7. There are minor typographical issues regarding spacing: on page 100, there is no space before the "%" symbol, and on page 122, there is no space between the numeral and "h" in "72h."

8. Figure editing and labeling issues:

    - Figure 10: The x-axis for zeta potential is missing the unit (mV). It is also unclear in which units the intensity is expressed, as the values appear as fractions in Figure 10 and in tens in Figure 14.
    - Figure 11: The units for C and Surface tension on the x- and y-axes contain unnecessary dots.

- Figure 16: There is inconsistency in the use of decimal points and commas. The figure caption should indicate that the test was performed after 24 h incubation, as is done for Figure 21.
- Figure 25: The title is misleading; the figure does not represent "acute irradiation" alone but a comparison between acute and chronic irradiation.

9. In Section II.B, "Main types of radiation," beta decay is described without mentioning the neutrino. Beta decay produces three particles—an electron (or positron), a daughter nucleus, and a neutrino or antineutrino. Even if the neutrino's effect is negligible, it should be mentioned to satisfy lepton number conservation.

10. In the discussion of the "Rs" of radiobiology, the dissertation correctly cites Withers' 1975 formulation of the first four Rs (Repair, Reassortment, Repopulation, and Reoxygenation). However, it should be noted that since then, the concept has been expanded to include a total of seven Rs, with intrinsic Radiosensitivity introduced as the fifth R (Steel et al., 1989), immune Reactivation as the sixth R (Boustani et al., 2019), and Tumor Microenvironment Reinforcement as the seventh R (Taghizadeh-Hesary, 2024).

11. The functional form of the fitted curve for blood clearance as a function of time (shown in Figure 43) is not provided, and the uncertainties of the fitted parameters (K, plateau, and half-life) are not reported. Providing the explicit equation of the "one-phase exponential decay" model, together with the parameter values and their associated uncertainties, would clarify the modeling approach and enable proper interpretation of the pharmacokinetic data.

12. TEM images of nanoparticles were analyzed using ImageJ; however, could the author clarify how the mean nanoparticle size and its uncertainty were determined—specifically, how many images were analyzed and how many nanoparticles were measured? Additionally, why is the size uncertainty not reported for the TEM image of AuNP@PFTD-PEG prepared via the Brust–Schiffrin method? Furthermore, regarding the DLS analysis of AuNP@PFTD-PEG in Figure 19, could the author clarify what the larger particles in the size distribution (particles above 100 nm) represent and how they relate to the main nanoparticle population? Finally, why do the particle size values obtained from DLS differ from those measured by TEM?

13. Regarding radiosensitization studies, cell viability was plotted against irradiation dose and fitted with a polynomial regression to determine radiosensitizing parameters for PFTD-PEG micelles and AuNP@Micelles on B16F10 and SKBR3 cells. However, the functional form and fitted parameter values are not provided, and the curves in Annex 4 appear as simple point-to-point connections rather than fitted functions. Providing the equations and parameter uncertainties would clarify the analysis.

14. Cytotoxicity studies: In Figure 16, both empty micelles and micelles containing gold nanoparticles show a reduction in cell viability. Could the author clarify why empty micelles are considered non-toxic under these conditions? Furthermore, in the cytotoxicity studies of irradiated cells (SKBR3 and B16F10, Figures 23–26), no clear dose–response effect is observed for untreated cells

after electron beam irradiation. Could the author provide a mechanistic explanation for this observation?

These minor shortcomings do not affect the scientific merit or substantive value of the dissertation.

4 Final evaluation

The synthesis of fluorinated micelles, their functionalization with perfluorinated ligands, and the incorporation of gold nanoparticles, as well as the integration of lutetium-177 into micellar systems and the investigation of their physicochemical and biological properties, represent a significant scientific achievement by Mathilde Ponchelle and fulfill a key criterion for the award of the doctoral degree. The research outcomes presented in the dissertation clearly demonstrate the candidate's ability to conduct independent scientific work and her strong understanding of methodologies for designing, synthesizing, and characterizing complex nanostructures. Furthermore, the thesis showcases her proficiency in applying experimental approaches to study the radiosensitization effects and radiotoxicity of nanomaterials *in vitro*, as well as their preliminary biodistribution and tumor retention *in vivo*.

The achievements of Mathilde Ponchelle reflect a high level of experimental skill, perseverance, and determination—particularly in overcoming challenges associated with the synthesis and functionalization of fluorinated micelles, the incorporation of gold nanoparticles, and the radiolabeling with lutetium-177. Notably, she conducted the synthesis of gold nanoparticles through three different methods: the Brust–Schiffrin method, the Turkevich method, and the Stellacci method, with only the final approach yielding the desired results. Her persistence in continuing experiments despite initial setbacks demonstrates a valuable scientific trait: the ability to remain motivated and not be discouraged by failure. The candidate's publication of a review article related to the dissertation topic, together with her active contribution to experimental research, highlights both her scientific commitment and her strong ability to work effectively within a research team.

I confirm that the reviewed doctoral dissertation of Ms. Mathilde Ponchelle meets the requirements specified in the Act of March 14, 2003 on Scientific Degrees and Academic Title and Degrees and Title in Art (consolidated text Dz. U. of 2017, item 1789 as amended) with consideration of Art. 179 paragraphs 1 and 2 of the Act of July 3, 2018 pre-writing the Law on Higher Education and Science (Journal of Laws 2018, item 1669, as amended), and I request the Discipline Council of the Institute of Nuclear Chemistry and Technology in Warsaw, within the field of natural and exact sciences, to admit Ms. Mathilde Ponchelle to the subsequent stages of the doctoral proceedings and to the public defense of her dissertation.

Sincerely,

Beata Brzozowska

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