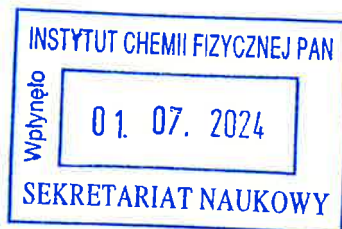




**Politechnika Łódzka**  
Wydział Chemiczny



**Beata Brozek-Płuska, PhD, DSc, Eng.**  
Associate Professor  
Institute of Applied Radiation Chemistry  
Faculty of Chemistry  
Lodz University of Technology

**Review of the doctoral dissertation**  
**„Spectroscopy, photolysis, tautomerism, and photodegradation of amino and nitro derivatives of porphycene” of Ph.D. Candidate Idaresit Mbakara**

Ph.D thesis was prepared within International Doctoral Studies of the Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland

Supervisor: Prof. Jacek Waluk

The doctoral dissertation of Mrs. Idaresit Mbakara entitled "*Spectroscopy, photolysis, tautomerism, and photodegradation of amino and nitro derivatives of porphycene*" was performed in the Photophysics and spectroscopy of photoactive systems Research Group at the Institute of Physical Chemistry, Polish Academy of Sciences, under the supervision of prof. dr hab. Jacek Waluk.

The Ph.D. thesis focuses on developing of new photosensitizers for PDT applications. In detail, Mrs. I. Mbakara studied a series of porphycene derivatives, substituted either with nitro, amino, or mixed nitro and amino groups. Within the thesis experiments and calculations regarding spectral, photophysical, and photostability characterization of porphycene derivatives were performed using different techniques steady-state electronic absorption, steady-state electronic emission, magnetic circular dichroism spectroscopy, and quantum chemical calculations. Moreover, time-resolved studies were employed to characterize the excited state dynamics of porphycene derivatives. The specific influence of electron-donating and withdrawing groups and bulky tert-butyl groups, as well as the position of the substituent, were also investigated.

The research outlined in the Ph.D. thesis aligns very well with the current trend of searching for new photosensitizers that can be used in the fight against cancer in PDT.

The origins of light as a therapy in medicine and surgery are traced from antiquity to the modern days. Phototherapy began in ancient Greece, Egypt, and India but disappeared for many centuries, only being rediscovered by Western civilization at the beginning of the twentieth century through Niels Finsen, Oscar Raab, and Herman von Tappeiner.

The revised doctoral dissertation comprises eleven chapters: 1. Introduction; 2. Goals; 3. Concepts of photochemistry and photophysics; 4. Materials and methods; 5. Spectroscopy, photophysics, and tautomerism in amino and nitroporphycenes; 6. Spectroscopy, photophysics, and tautomerism of 2-nitro-7,12,17-tri-tert-butylporphycene; 7. Instability of 9-aminoporphycenes; 8. Photostability in 9-substituted porphycenes: Possible agents for photodynamic therapy?; 9. Spectroscopic and solvent studies of the pull, push, and push-pull derivative of 9,19-disubstituted porphycenes; 10.  $\beta$  and  $\beta'$  nitro porphycenes: position effects in meso-tetraphenyl derivatives of porphycene; 11. Putting it all together including Future outlook (a critical chapter, rarely found in PhD theses), References (248 items).

Additionally, the doctoral dissertation covers the Abstract (in English and in Polish) and Table of contents. In the Abstract, the aim of the research and relatively detailed description of the performed experiments are given.

The entire doctoral dissertation (excluding Abstract and Contents) consists of 154 pages. The work was written in English and enriched with charts and numerous diagrams/drawings. On the editorial side, I would rate the thesis well. The thesis is written in the correct language and the number of typos, incorrect wordings, and linguistic errors is small. At the same time, I must emphasize that the layout of the main part of the doctoral thesis is not typical, throughout the dissertation, fragments constituting literature content are mixed with fragments reflecting experimental content e.g. the sequence of chapters 2 and 3 should be changed.

The first part of the doctoral dissertation is the Introduction including the description of porphycene structure, properties and reactions, studies of isolated porphycene molecules, dichroism and tautomerization of these molecules, their applications, and photostability. It covers 29 pages.

Generally, the introductory part should introduce the thesis topic and show the newest achievement in the field to which the submitted work relates. In the reviewed work in the first chapter of the Introduction, the Ph.D. Candidate describes porphyrins and their properties useful in PDT. This part is also dedicated to a short photodynamic therapy characterization. In this chapter porphycene derivatives are also described with special attention paid to the properties important to effective applications in PDT.

The next part of this chapter, which is devoted to the description of the structure and chemical properties of porphyrins and I consider particularly valuable. The Ph.D. Candidate described the stability of porphycenes molecules and many applications of Pcs not only in medicine, but also in photovoltaics, photocatalysis, and information storage. Subchapter 1.2.2. is dedicated to the absorption properties of porphycene, which are compared to porphyrins; the orbital energy level diagram for the two HOMOs and LUMOs in porphyrin and porphycene is also presented and discussed in detail. Moreover, fluorescence characteristics, triplet state, and singlet oxygen generation were described with important discussion related to the relation between the singlet oxygen efficiency generation and Pcs PDT effectiveness related to the high absorption in the red region of the spectrum. Most importantly Mrs. Mbakara described the influence of substitution on porphycene photophysical and photobiological properties. This part is especially important because the Ph.D. Candidate investigated Pcs after inner core substitution.

The contribution of the prof Waluk's group to the knowledge in the discussed area also has been highlighted.

One of the experimental techniques used by the Candidate was MCD therefore the chapter 1.4. is dedicated to the magnetic circular dichroism and the Perimeter Model data interpretation.

In subparagraph 1.5. the tautomerization in porphycenes has been discussed including the role of prof Waluk's group in a multidimensional tautomerization pathway interpretation, which is vibrational mode dependent.

Especially important, in the context of the final Ph.D. thesis conclusions is chapter 1.6.1. where PDT mechanisms are more intensively discussed with special attention paid to the properties of an effective photosensitizer. Subchapter 1.7 is dedicated to the photostability, mechanisms of photodegradation, and the comparison of photodegradation of porphyrins and porphycenes. The last part of Chapter 1 is dedicated to an explanation why the substitution with nitro and amino groups can be interesting for PDT applications.

Chapter 2 reveals the main research objectives, which include answers to the following questions regarding the Pcs • What are the tautomeric properties and timescale?, • Are these compounds suitable for application in PDT? • What is the degree of photostability of these systems if employed as photosensitizers in phototherapy? • Are they rather useful as dyes in applications requiring low stability? • What role do electron donating or electron withdrawing groups play in the photophysics, photochemistry, and phototautomerization of these systems? • What role does alkyl substitution (such as bulky tert-butyl, and n-propyl groups) play in the photophysics, photochemistry, and phototautomerization of these systems? • How does the nature (viscosity, polarity, and ability to form hydrogen bonds) of the environment/solvent influence the photophysics and photochemistry of the studied systems? • What pathway does systems take during degradation upon illumination with UV/visible light? • What is the mechanism of phototautomerization of the studied systems? • What is the position dependence of a substituent on the photochemical properties of these systems?

Chapter 3 describes light and matter interactions, light absorption, electronic state, some fundamental laws, the basics of the fluorescence and phosphorescence phenomena, non-radiative deactivation, and solvation dynamics.

Chapter 4 contains information about materials and methods used to study porphycene derivatives e.g. Table 4.1. shows the list of solvents used during research work, part 4.1.2. describes the samples, subchapters 4.2.1. – 4.2.7. describe techniques used in experiments, the last part of Chapter 4 is dedicated to short information related to the quantum chemical calculations.

Chapter 5 in Section II is dedicated to experimental results obtained by the Ph.D. Candidate. All performed experiments can be summarized as follows: substitution of porphycene with nitro and amino group at the meso position leads to changes in the absorption spectra; new red-shifted bands are observed for the aminoporphycenes; the specific influence of alkylation is not observable in the spectra of tprAPc, ttAPc, tprNPc, and ttNPc, as such influence is overshadowed by that of nitro and amino groups; only the trans-1 form is responsible for the emission in the amino series since the trans-2 form quickly transforms to the trans-1 in the lowest excited state; previous

misconception of dual emission in amino derivatives is demonstrated; fluorescence is confirmed to be single emission, with two other bands being a consequence of the degradation of aminoporphycenes; single emission is also observed for nitro derivatives; both nitro and amino porphycenes presented in the thesis are moderate or weak emitters with substantial rates of non-radiative processes; both derivatives are attractive models for more detailed studies on ground and excited state tautomerization mechanisms, 9-nitroporphycene is the highest emitter among the studied compounds, with a 10% yield of emission; the tert-butyl derivatives are the weakest emitters in both the nitro and amino categories; this weak emission is associated with the steric interactions between the 9-substituent and the bulky tert-butyl groups; this interaction varies substantially in  $S_0$  and  $S_1$  states, as suggested by calculations; nitroporphycenes are moderate emitters with a 50% yield of singlet oxygen generation and, therefore, qualify as photosensitizers in PDT, with the exception of the tert-butyl nitro derivative.

In Chapter 6 Mrs. Mbakara focused on characteristic of 2-nitro-7,12,17-tri-tert-butylporphycene because it has been proved that the nature, number, and position of substituents influence the spectral properties of Pcs. Performed experiments confirmed that placing the nitro group at the *meso* (9) position results in the near degeneracy of the two trans tautomers in  $S_0$ , as in the case of NPc. However, when the nitro group is introduced at the  $\beta$  position (e.g., 2NttPc), the degeneracy is lifted, resulting in a 1.5 kcal energy gap between trans-1 and trans-2. Moreover, 2NttPc has shown a single emission with monoexponential decay kinetics, with a higher yield of fluorescence and longer excited state lifetimes compared to NPc.

Chapter 7 describes properties of 9-aminoporphycenes, especially in the context of Pcs stability. The Ph.D. candidate proved that the electron-donating property of the amino group makes aminoporphycenes unstable both in the solid state and in solution, experiments showed also that aminoporphycenes exhibit 'triple' emission, with F1 being the actual emission from aminoporphycene; F2 and F3 are emissions from the degradation products of aminoporphycene. Moreover, in the case of biological applications, since the amino substituent better interacts with biological cells Mrs. Mbakara proposed the synthesis of aminoporphycenes with protecting groups to facilitate their use in applications such as PDT.

In Chapter 8 performed experiments confirmed that substitution with the amino group renders porphycene more susceptible to degradation, both as a reaction occurring in the dark and even more efficient as a light-motivated process, and that nitroporphycenes are three orders of magnitude more stable than their amino counterparts.

The next part of the Ph.D. thesis is dedicated to 9,19-disubstituted porphycenes. Particularly low QY of fluorescence was observed for the three investigated compounds (ttdNPc, ttdAPc, and ttANPc), similar to fluorescence reported for tert-butyl porphycenes with a single *meso*-substitution of nitro and amino group (ttNPc and ttAPc). The low emission yield suggested that an efficient non-radiative deactivation pathway to the ground state and/or efficient transfer of the singlet state to the triplet state is observed.

The penultimate chapter describes *meso*-tetraphenyl porphycenes substituted at  $\beta$  and  $\beta'$  with nitro groups. Performed experiments showed that such compounds exhibit the largest red shift in absorption among the reported nitroporphycenes – highlighting

the influence of *meso* substitution on the spectral properties of porphycenes. The Ph.D. Candidate showed that NPc, 2NttPc, and tprNPc are good emitters, possess excellent yields of singlet oxygen, are photostable, and are therefore proposed as candidates for 2nd-generation sensitizing agents in PDT.

The last part (in Section III) in Chapter Putting it all together summarizes all experiments and calculations.

Summarizing all the presented works the Ph.D. Candidate carried out intense work on developing new photosensitizers. Despite some questions presented work requires knowledge of different research fields and techniques. Moreover, all experiments were conducted in a way that demonstrated the researcher's maturity.

I ask Ph.D. Candidate to address my comments and questions listed below during public defense.

- 1) Why were aqueous solutions omitted in the research? Has the solubility of potential photosensitizers in water been tested?
- 2) Many different basic studies have been performed to characterize potential photosensitizers. Why preliminary phototoxicity tests have not been performed, especially for normal cells?
- 3) The Ph.D. Candidate did not personally perform quantum calculations. Calculations are an important element of the research, but since they are not an independent contribution of the doctoral student, it may be advisable to present them as an attachment rather than the main text.

### **Final conclusion**

Taking into account the substantive value of the doctoral dissertation of Mrs. Idaresit Mbakara, I conclude that the evaluated doctoral dissertation meets the criteria for candidates applying for a doctoral degree as specified in the Act of July 20, 2018 Law on Higher Education and Science (Journal of Laws of 2023, item 742), following the provisions of the Act Art. 187. pt. 1., pt.2. and the evaluation of the submitted doctoral dissertation is positive.

The presented doctoral dissertation is a very important voice in the work on a new generation of photosensitizers used in the fight against cancer.

The Ph.D. Candidate is co-author of 3 publications (2 additional in preparation) and conference proceedings related to the topic of the dissertation published in the journal from the JRC list. Mrs. Mbakara presented the results of her study also during national and international conferences she is also co-author of 4 other papers (including 1 submitted for publication). Some experimental results were performed during the short research visit at the University of Geneva under the supervision of prof. E. Vauthey.

Due to the positive assessment of the entire doctoral dissertation presented above, I am applying to the Scientific Council of The Institute of Physical Chemistry (IChF), Polish Academy of Science to admit Mrs. Idaresit Mbakara to the next stages of the doctoral process.



Beata Brozek-Pluska, PhD, DSc  
Associate Professor