Warsaw, August 1, 2024

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Report on the thesis

entitled 'A study of selected endocrine disrupting chemicals and their binding to host molecules with molecular modelling'

by Anna Helena Mazurek

The PhD project entitled 'A study of selected endocrine disrupting chemicals and their binding to host molecules with molecular modelling' was carried out by Anna Helena Mazurek under the supervision of Prof. Łukasz Szeleszczuk from the Medical University of Warsaw and Prof. Thomas Simonson from the École Polytechnique de Paris. The thesis was written based on five publications (three original research papers and two review articles) preceded by a comprehensive discussion of the results of the PhD project with an introduction and a description of the materials and methods.

1 Scientific value of the thesis

The doctoral student's research project, which concerns the development of a complex of estradiol and β-cyclodextrin, represents a novel undertaking in the field of pharmaceutical science. This work necessitated an interdisciplinary approach, drawing upon elements of chemical, biological, and physical knowledge. Cyclodextrins (CDs) are frequently employed as drug delivery agents, as they enhance the solubility of pharmaceutical ingredients (such as estradiol), thereby increasing their bioavailability. Encapsulation in CDs may also be utilised as a means of removing toxins, as illustrated in the subject matter of the thesis.

The structural and functional characterisation (study of physico-chemical interactions) of the 17-β-Estradiol-β-Cyclodextrin complex was investigated using experimental and theoretical methods. The various theoretical approaches tested by Anna Helena Mazurek made it possible to obtain a crystalline and amorphous system and analyze the complex in the aqueous solution. It was investigated by Anna Helena Mazurek using two types of quantum mechanical (QM) approaches: Density Functional Theory (DFT) and semi-empirical computational methods. The QM-based parameters were chosen based on the studies of the author of this work (Mazurek, A. H., and Szeleszczuk, Ł. (2022). Current status of quantum chemical studies of cyclodextrin host–guest complexes. Molecules, 27(12), 3874). The characteristics of

estradiol-cyclodextrin complex in the aqueous solution (structural and physicochemical) was described theoretically, taking into account the molar ratio and stability of the complex, using molecular dynamics simulations and Molecular Mechanics (MM) Generalized Born Surface Area calculations (Mazurek et al. (2024). 17-β-Estradiol-β-Cyclodextrin Complex as an aqueous solution: Structural and Physicochemical Characterization supported by MM and QM calculations. Journal of Molecular Structure, 138710). Based on theoretical studies solid form of 17-β-Estradiol-β-Cyclodextrin complex (DFT and nuclear magnetic resonance (NMR) parameters calculations) was synthesised and verified experimentally. In order to achieve this Anna Helena Mazurek employed a range of measurement techniques, including X-ray diffraction method, NMR imaging, infrared spectroscopy, thermogravimetric analysis, differential scanning calorimetry, Cryo-SEM (Mazurek et al. (2023) 17-β-Estradiol-β-Cyclodextrin Complex as Solid: Synthesis, Structural and Physicochemical Characterization, Structural and Physicochemical Characterization. Molecules, 28(9), 3747). The QM approaches were compared with AMOEBA force field parametrization for selected EDCs and cyclodextrin, demonstrating a high level of agreement (Mazurek et al. Polarizable models for selected Endocrine Disrupting Chemicals and their hosts, Journal of Computational Chemistry, under review). Modelling molecular dynamics was also discussed by the author of this work as a method for analysing complexes containing EDCs and CDs or estrogen receptors (ER) (Mazurek at al. (2020). Application of Various Molecular Modelling Methods in the Study of Estrogens and Xenoestrogens. International Journal of Molecular Sciences, 21(17), 6411). While Steered Molecular Dynamics and Free Energy Perturbation calculations, described therein were not used in the thesis, they could be applied to planned studies in the near future.

The tool developed by the doctoral student as part of her doctoral project, together with her expertise in experimental methods, provide a foundation for the analysis of other complexes of stereoidal hormones and cyclodextrins that may have potential applications in pharmacy and toxicology.

2 Editorial correctness of the dissertation

The dissertation was crafted with great care and attention to detail by the author. The dissertation is structured into six main parts. The initial part presents the rationale for the study and defines the fundamental terminology used throughout the remainder of the dissertation. It places particular emphasis on pharmaceuticals and pesticides as illustrative examples of endocrine-disrupting chemicals and the interactions between chemical substances and receptors based on molecular modelling approaches. In the second part of the dissertation, the author provides a comprehensive analysis of the characteristics of EDCs, estrogen receptors and cyclodextrins. The following two parts present an overview of the force field (AMOEBA and two additional ones: CHARMM and AMBER), followed by a description of the models employed to describe molecular dynamics. The fifth part is devoted to the presentation of DFT calculations, with a particular emphasis on both solid-state and solution-phase applications. The final section presents the findings, the conclusions that can be drawn from the results obtained in the dissertation, and the prospects, which are very promising and demonstrate the doctoral student's high level of scientific activity. The dissertation is accompanied by a list of the publications and an extensive bibliography. The structure of the work is clear and logical, with minor formal shortcomings described below.

3 Critical remarks

The work has been edited in an appropriate manner; however, a few minor editorial issues have been identified, which are listed herewith for the sake of order.

1. It is regrettable that the PhD student did not provide a description of the experimental methods she employed in her research. The results of her experimental measurements are, however, presented in Anna Helena Mazurek's publications.

2. The abbreviations of terms used in the paper are not consistently introduced at the first mention of the name. For example, the abbreviation MD appears on page 9, while 10 pages further down on page 19, and the abbreviation ER (Estrogen Receptor) is not introduced at all.

3. An error has been identified in the notation of units expressing the concentration of a substance on pages 7 and 8. Rather than the correct term, 'ng/L', the notation 'ng L / 1' has been used on several occasions.

4. Some equations require minor corrections, including an explanation of the symbols used (e.g., T in formula (2), K_{b0} in formula (8)). This is important, given that energy itself is described using different symbols, including U, V, and E.

5. In consideration of the vector nature of the induced dipoles, as illustrated in Figure 6, it is evident that formula (3) would necessitate the incorporation of the indices and arguments pertaining to the dipole moment vector (it should be noted this is a dipole moment) and the electric field.

6. The sequence of figures in the document is not in the correct order. Figure 7, which is on page 15, is incorrectly placed before Figure 6, which is on page 16.

7. It should be noted that the reference on page 18 is given as doi number, and several literature items containing web addresses, lack the information on the date of access. This is the case for items 12, 139 and 169, for example.

4 Final evaluation

The development of a method for modelling the crystal structure (and aqueous solution) and interactions of estradiol with cyclodextrin, together with experimental verification, represents an original solution to the scientific problem. The application of the developed methods to synthesise and characterise the structure of the 17-β-Estradiol-β-Cyclodextrin complex represents a significant scientific achievement of Anna Helena Mazurek, which is an important requirement for doctoral dissertations. The doctoral student's research outcomes, as presented in the dissertation, demonstrate her capacity for independent scientific inquiry and her comprehensive understanding of methodologies for modelling the structure and molecular dynamics of chemical substances. Furthermore, the dissertation evidences her ability to employ experimental techniques to validate theoretical postulations.

The achievements of Anna Helena Mazurek, as outlined in the review, encompass a substantial body of novel and intriguing findings that advance the technical frontiers of host-guest complex applications in pharmacy and toxicology. It should

be emphasised that progress in these fields is a significant step forward in improving human health and quality of life.

Anna Helena Mazurek is the first author of eight papers that have been published in peer-reviewed international journals (with one additional paper currently under review) and three publications where she is the second author. According to the Web of Science database, the number of citations is 192 (excluding self-citations), which is a noteworthy achievement for a young scientist. The implementation of a doctoral project in collaboration with scientists from the French École Polytechnique and the established partnership with the Agricultural University of Athens in Greece, as well as the grants obtained, demonstrate her scientific maturity. This also evinces Anna Helena Mazurek's preparedness to undertake the planned research, which is described in detail in the dissertation and will enhance her scientific output, resulting in further publications.

I, the undersigned, confirm that the reviewed doctoral dissertation of Anna Helena Mazurek 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 Council of the Discipline of Pharmaceutical Sciences of the Medical University of Warsaw to admit Anna Helena Mazurek to further stages of the doctoral proceedings and public defense.

Sincerely,

Beata Brzozowska