

R E V I E W

of PhD Dissertation

for obtaining the educational and scientific degree "Doctor"

Professional Field: 4.1 Physical Sciences

Scientific specialty: Biophysics

Author of the dissertation: Desislava Henri Lazarova

Topic: "Contrast-enhanced magnetic resonance techniques for visualizing pathologies associated with cellular redox status disorders"

Reviewer: Academician Boris Tenchov

Desislava Lazarova is a Ph.D. student at the Department of Condensed Matter Physics at the Faculty of Physics of Sofia University St. Kliment Ohridski. Its supervisors are Prof. Romyana Bakalova and Assöc. Prof. Genoveva Zlateva. The experimental research in her dissertation was conducted at the National Institute of Radiological Research (NIRS) in Chiba, Japan.

The aim of Desislava Lazarova's dissertation is to develop methods for contrast enhancement in the magnetic resonance methods used for visualization of various pathological processes associated with the oxidative-reductional balance of the cells. An approach based on the use of stable nitroxyl radicals as contrast-enhancing agents in magnetic resonance imaging has been used to achieve this goal.

Lazarova's dissertation is written in 95 pages and contains 39 figures and 1 table. It is structured in a standard way with an introduction, an overview of the literature, purpose and tasks, materials and methods, results and discussion, conclusions and contributions. The reference list contains 144 titles.

The literature overview, written on about 30 pages overall, presents the theoretical background and physical principles of the magnetic resonance methods ESR and NMR as imaging techniques, as well as their applications for visualizing the redox status of the biological systems, including the use of nitroxyl radicals for enhancing the image contrast. A well-structured overview of the key information on the cellular redox status as well as on the main sources and metabolism of endogenous free radicals, such as, for example, reactive oxygen species (ROS), has also been made.

Overall, the literature review is well written and illustrated. It demonstrates a profound knowledge of the literature and the ability of the author of the dissertation to present concisely, yet in a clear, comprehensible and sufficiently detailed way all information from published work needed for the purposes of the dissertation.

In the work on the dissertation, an impressively large number of experimental methods described in the Materials and Methods section (20 pages) has been used. These methods include work with laboratory animals, with normal and cancer cell lines, also with cell-free aqueous solutions for ROS generation. Experimental models have been developed to induce various diseases in mice (hypercholesterolemia and Parkinson's disease). A number of analytical methods have been used, for example, to determine cell vitality, ROS and antioxidant capacity, and many other methods. For recording free radicals in cell suspensions and in cell-free model systems, EPR spectroscopy was used, while magnetic resonance imaging (MRI) was used in the studies of the experimentally induced diseases in laboratory animals. This rich set of experimental techniques is adequate to the assigned tasks and shows that the dissertation work was carried out at a very high methodological level.

The results obtained in the dissertation are presented in the Results and Discussion section (about 40 pages). These results refer to the categories of developing new methods and obtaining new facts about known from previous work problems. In accordance with the expectations and literature data, it is confirmed that both nitroxyl and endogenous radicals, for example, superoxide radicals reduce the spin-lattice relaxation time T_1 and noticeably enhance contrast in the MRI images. In fact, the dissertation is largely based on this effect. The MRI results for the tested tissues in experimental disease models in experimental animals are ultimately effects of enhancing the contrast by the exogenous and endogenous paramagnetic substances (free radicals) by way of their influence on the T_1 time. These effects have been extensively studied in model systems and there is no doubt that the results obtained are fully reliable. It has also been found that EPR spectroscopy allows to identify the differences in proliferative activity of normal and cancer cells that are indicative of the level of cell reduction capacity. From the professional description of the results and from their in-depth discussion, I have the impression that Desislava Lazarova has made a significant personal contribution to obtaining the results presented in her dissertation. As reflected in the contributions, these results represent significant interest from the viewpoint of developing new diagnostic approaches. I have no remarks about the conclusions and contributions in the work and I accept them as they were written.

From what has been said so far, it can be concluded that Lazarova's dissertation work is at a high scientific level. They are up to date and fully in line with the modern trends in imaging diagnostics. A convincing testimony to this is the high number of citations of her papers, which is rare and unusually high for PhD dissertations.

The dissertation of Desislava Lazarova is very well written and shaped. The language is clear and comprehensible, the figures are well made and clearly illustrate the results. There are almost no technical and typographical errors (I will just note that it should be ACS, and not ASC in paper no. 4 of the list). The synopsis presents correctly and in a sufficiently comprehensive manner the content of the dissertation. The list of Lazarova's publications on results from the dissertation is impressive. It contains 6 articles, 4 of which are in journals with IF (2 of them are accepted for publication). These articles have a total IF around 9. The two articles published in 2013 (No. 3 and No 4 in the list) have been so far cited 73 times. In the two papers accepted for publication, Lazarova is the first author, that emphasizes her personal contribution. The requirements of the Faculty council of the Physical Faculty of Sofia University for PhD dissertains are fulfilled. Also, a list of 4 participations in national and international conferences is provided. The full list of Lazarova's publications is also impressive and deserves to be noted. This list contains 26 articles, 16 of which are with IF and one is with SJR. To of the date of submission of the dissertation, these publications have been cited 104 times. Also included are lists of 21 participations in scientific forums and 14 participations in scientific projects.

In conclusion, new methods of contrast enhancement have been developed and interesting new results have been obtained in the field of magnetic resonance imaging in the reviewed here PhD work. These results have been published in authoritative journals and have already found a great impact in the world scientific literature. I believe that this dissertation undoubtedly fulfills all the necessary requirements and I offer with full conviction to the honorable jury to award to Desislava Henri Lazarova the requested educational and scientific degree "doctor".

31 March 2019

Reviewer:

(academician Boris Tenchov)