OPINION

about the dissertation entitled "***Metal complexes of carboxylic polyethers monensin and salinomycin: structure, properties and biological activity***"

by the candidate Prof. Dr. Ivayla Nedialkova Pantcheva-Kadreva,

within the procedure for acquiring the "Doctor of Science" degree

in the professional field of 4.2. Chemical sciences (Analytical chemistry)

By the reviewer: Assoc. Prof. Dr. Neli Nikolova Mintcheva-Peneva,

from the Department of Chemistry, University of Mining and Geology, Sofia,

a member of the academic committee, appointed with the order RD-38-608/13.11.2023 by the Rector of the Sofia University "St. Kliment Ohridski"

The documents submitted for the procedure include a DSc thesis, an abstract (in Bulgarian and English), a certificate of compliance with the national minimum requirements and the criteria of the Faculty of Chemistry and Pharmacy, Sofia University, a certificate for the originality of the works, all scientific papers included in the dissertation, a curriculum vitae, and diplomas. The abstract fully reproduces the content of the dissertation, and the English version corresponds to the Bulgarian one. The originality of the works has been confirmed and there is no evidence of plagiarism.

The data and results presented in the DSc thesis were already published in 27 scientific papers, including 21 publications in peer-reviewed and indexed journals and 4 chapters of collective monographs. Among the prestigious international scientific journals are such as *BioMetals, Inorganica Chimica Acta, Journal of Inorganic Biochemistry, Molecules*, etc. A total of 204 citations were found for these publications, and 63 citations are presented in the current procedure "Doctor of Science". The materials used in the DSc dissertation do not repeat results and papers applied for the acquisition of the "Doctor" degree. The total number of points from the presented publications and citations exceed the minimum national requirements and the recommended criteria of the Faculty of Chemistry and Pharmacy, Sofia University in the professional field 4.2. Chemical sciences.

In my opinion, this dissertation represents an extensive and in-depth study of the structure and properties of numerous metal complexes with the antibiotics monensin and salinomycin. The

reported results are original and demonstrate novel structures of metal complexes proven by crystallographic data and theoretical calculations, as well as their antibacterial and anticancer activity, so that these findings have a significant contribution to the development of modern coordination and medicinal chemistry.

In the DSc Thesis, the results are summarized in three groups:

- Preparation, structure, and spectral properties of monensin and salinomycin complexes with metal ions in different oxidation states.

- Complexation of the monocarboxylic polyether ionophores monensin and salinomycin in solution.

- Biological activity of polyether ionophores and their metal complexes.

The study is based on the 39 complexes of the ligands monensin and salinomycin with many metal cations (alkaline earth, transition metals and lanthanoids) in their (+2), (+3) and (+4) oxidation states, that were synthesized, isolated in solid state, and structurally characterized. The structure of a part of these complexes was determined by X-ray crystallography, while for other complexes spectroscopic study and theoretical calculations were combined to optimize the molecular structure. Many spectroscopic data (IR, UV-Vis, NMR, EPR, MS) have been collected to characterize the as-prepared complexes and relationships between the structure and spectral parameters have been deduced. The information has been published in several papers and could be a benefit to other researchers working with polyether ionophores. In this aspect, the scientific contribution of the author consists in the methodology development for synthesis of various metal complexes with bidentate and polydentate coordinated ionophores, that implement the complexation capability of the polyether ionophores. Additionally, the formation of metal complexes can be used to explain the effect of metal ions on the antibacterial activity of the studied antibiotics.

The author has also studied the complexation equilibria in solution of mono-, di- and trivalent metal ions with the ionophore monensin by means of circular dichroism (CD), which turns to be a very useful method for studying the coordination ability in solution of polyether ligands (especially monensin) towards metal ions with different charge and in different concentration. Thus, by using the circular dichroism and the theoretical modeling, it has been proven that M^{2+} ions form positively charged complexes with monensin, $[MMon(H_2O)]^+$ in solution together with the neutral complexes, $[MMon_2(H_2O)_2]$, which were isolated in the solid

phase. The formation of such complex cations was found for the first time by the author using CD and it could not be observed by other spectral methods. Interestingly, the CD results for the complexation between the M^{3+} ions and monensin indicate the presence of at least three types of complexes in solution: neutral $[MMon_3(H_2O)_3]$, and positively charged complex ions $[MMon_2(H_2O)_2]^+$ and $[MMon(H_2O)]^{2+}$. The CD spectra of the latter cannot be used to distinguish the complexes of a specific rare earth metal ion, while the CD spectra of monensin with M^+ ions are highly sensitive to the nature of the metal ion and show a difference in the position of the peaks and their intensities, therefore they can be used to recognize the corresponding M(I) complexes. The studies carried out in solution indicate possible changes within the complex structure in the presence of other metal ions. These results are important when the biological activity of the metal complexes is studied and in future clinical application. Related to that, the processes of coordination competition between two metal ions for binding to the ligand were evaluated by determination of the stability constants of the mono- and bis-monensinate complexes of Co^{2+} and M^{2+} (Ni^{2+}, Mg^{2+}, Ca^{2+}, Zn^{2+}) ions. Using UV-CD and Vis/NIR-CD, the formation of complexes ML^+ and ML_2 for each of the metal ions was found, and the absence of mixed-metal complexes was proven. Therefore, the author successfully applied the CD method as a useful tool for studying the complexation properties of the ligand monensin in solution. Additionally, the quantum chemical calculations allowed to determine the factors affecting the selectivity of monensin and salinomycin to group IA and IB metal ions.

The third part of the thesis presents the investigation of the biological activity of structurally characterized metal complexes against several bacterial strains and tumor cell lines. Although the metal complexes demonstrated different effect on the antibacterial and antitumor properties in comparison with the free ligands, some complexes such as Ni^{2+} and Zn^{2+} bis-monensinate complexes exhibited higher selectivity towards cervical carcinoma than that of other tested compounds. Moreover, the Ce (IV) complexes with monensin and salinomycin were found to be highly toxic to the same cancer cells and were significantly more selective than some well-established antitumor agents. In the next stage of research, the toxicity of metal complexes with monensin and salinomycin were tested on the laboratory animals and their clinical indicators were measured. The findings revealed that polyether ionophores and their metal complexes are promising candidates in the development of new molecules as potential antibacterial and anticancer drugs.

Overall, the presented materials show that during the last 15 years, Prof. Ivayla Pantcheva has developed her own topic in research. In her dissertation and published papers, she has extensively investigated the structural and spectroscopic characteristics of the studied objects, that have been determined experimentally and additionally supported by simulated spectra and optimization of molecular geometry. She has studied the complexation of polyether ionophores in solution, complexation equilibria between metal ions and complex ions and their stability constants. After synthesizing the new materials, she has continued to test their antibacterial and antitumor properties. A series of tests showed that significant biological activity was found for some of the metal complexes obtained.

Knowing and applying the basics of analytical chemistry, Prof. Pantcheva has developed comprehensive research on the theoretical and applied aspects of metal-polyether complexes, with the aim to select compounds for potential application as therapeutics. Thus, she has contributed for the development of the coordination chemistry in Bulgaria, and she has gained her place in the world scientific community.

I have known Prof. Ivayla Pantcheva very well since I was a student, later in the doctoral and post-doctoral years we studied coordination and organometallic chemistry together. She is a responsible, persistent, inquiring person, and always perfect in her work. Nowadays, she is an experienced and outstanding scientist and knowledgeable educator who actively and effectively works to improve the prestige of the Faculty of Chemistry and Pharmacy.

In conclusion, all presented materials and scientific metrics fully meet the requirements of the Law for the Development of Academic Staff in the Republic of Bulgaria, the Regulations for its implementation and the recommended criteria of the Sofia University "St. Kliment Ohridski". Consequently, I give my positive evaluation of the DSc thesis and research contribution, and confidently recommend to the respected scientific jury to award the degree of "Doctor of Sciences" to Prof. Dr. Ivayla Nedialkova Pancheva-Kadreva.

12.02.2024г. Reviewer:

Sofia Assoc. Prof. Neli Mintcheva, PhD