

EVALUATION REPORT

on THESIS

submitted for the award of the scientific degree 'Doctor of Science'
professional field 4.2. Chemical sciences, Analytical chemistry

Title: METAL COMPLEXES OF CARBOXYLIC POLIETHERS MONENSIN AND SALINOMYCIN: STRUCTURE, PROPERTIES AND BIOLOGICAL ACTIVITY

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Reviewer: prof. Irina Bogdanova Karadjova, PhD, Faculty of chemistry and pharmacy, University of Sofia "St. Kliment Ohridski"

The review was prepared on the basis of the presented thesis, thesis summary and publication activity. The presented materials meet the requirements of the Act on Development of the Academic Staff in the Republic of Bulgaria, the Rules for its implementation, and the Specific criteria for the acquisition of the requested scientific degree in Faculty of chemistry and pharmacy, University of Sofia "St. Kliment Ohridski".

Brief biographical data for the PhD candidate

Prof. Pantcheva's entire scientific career was realized at the Faculty of Chemistry and Pharmacy of Sofia University "St. Kliment Ohridski". Both educational degrees – bachelor's and master's – were obtained as a result of research activities carried out in the Department of Analytical Chemistry of Faculty of chemistry and pharmacy. The doctoral thesis was defended in 2001 on the topic "Complexes of copper II with antihypertensive drugs". The research carried out can be characterized by several keywords (according to the candidate): Biocoordination chemistry, medicinal drugs, synthesis and structural analysis of metal complexes with biologically active ligands. Prof. Pantcheva purposefully specializes in the same field in all visits as a research scientist conducted over the years in laboratories outside the country.

Thesis structure

The thesis presented is based on 27 publications which were not used for the PhD degree. Nine of these publications were also not used to award the degrees of associate professor and professor. There are 21 articles published in refereed and indexed journals, 4 chapters of collective monographs have been published. The distribution by quartiles is as follows: 6 articles in Q1 (150 items), 6 - in Q2 (120 items), 6 - in Q3 (90 items), 3 - in Q4 (36 items), 4 -

chapters from collective monographs (60 items). All publications comply with indicator G of Act on Development of the Academic Staff in the Republic of Bulgaria, the Rules for its implementation as well as fulfills the requirements of the rules of Faculty of chemistry.

Prof. Pantcheva has clearly formulated the goals in the presented thesis and the problems she believes should be solved through the scientific activities conducted for more than 15 years on the topic of polyether ionophores, their complexes with chemical elements, the action and application of these complexes as a biologically active substances.

The appropriate modification of substances with biological activity in order to achieve higher efficiency of action is a current topic of research. Regardless of the efforts to introduce some predictability of the achieved effect depending on the change introduced in the structure of the molecule of the biologically active substance, empirical results are still relied on in many cases. The idea to investigate the possibility of changing and rather increasing the biological effectiveness of ligands by including them in complexes with metal ions is a traditional topic of research at the Laboratory of Biocoordination and Bioanalytical Chemistry. In this aspect, in the presented dissertation, this idea was developed through systematic and targeted research with ligands of polyether ionophore antibiotics in complexes with metal ions in different degrees of oxidation, different complex-forming properties, different behavior as bioelements. The assessment regarding the biological activity was completed with various studies – antibacterial activity, cytotoxicity and *in vivo* studies on the toxicity of the synthesized complexes with biometals on laboratory animals.

The antibiotic of choice, monensin, has been in use since the middle of the last century and has been very well studied both in terms of mechanism of action, efficiency and metabolites, as well as possibilities for new applications as an antitumor agent. Salinomycin has recently been increasingly used as an antitumor agent. Both antibiotics have a similar structure and contain donor groups - carboxylate and hydroxyl, located at both ends of the molecules, which provide the possibility of forming complexes with the metal ions. Monovalent to tetravalent metal ions - alkali, alkaline earth/transition metal ions M^{2+} - $[ML_2(H_2O)_2]$, lanthanide cations M^{3+} - $[ML_3(H_2O)_3]$ and Ce^{4+} - $[ML_2(OH)_2]$ are included in the study. The tasks that are set are:

- Impact of present metal ions on the effect of antibiotics
- Behavior of antibiotics in the presence of metal ions in a higher oxidation state
- Composition and structure of isolated antibiotic complexes in aqueous solution and as a crystal structure

A. The experimental results on the structure of complexes synthesized show two variants of complex formation:

- "classical" complexes of polyether ionophores, in which both substances act as a bidentate ligand, the coordination of the ligand is carried out in "head-tail" mode - donor atoms are the oxygen atoms of carboxyl and hydroxyl groups. They are characteristic of alkaline earth, transition metal ions $M^{2+} - [ML_2(H_2O)_2]$, lanthanide cations $M^{3+} - [ML_3(H_2O)_3]$ and $Ce^{4+} - [ML_2(OH)_2]$.
- "non-classical" - a specific binding method for each isolated complex: mononuclear monocomplexes of monensin with Hg^{2+} ions and of salinomycin with Pb^{2+} ions; mononuclear monocomplexes of ionophores with Ce^{4+} ; the mixed-metal complexes of monensin sodium with Co^{2+} , Mn^{2+} and Cu^{2+} .

It is likely that future studies will show the factors that determine the behavior of ligands as bi- or polydentate in complexed with the same metal ions.

C. The behavior of the ligands in solution, the composition of the complexes formed and their stability constants were investigated by Circular dichroism (CD) spectroscopy and theoretical models. The essential advantages of circular dichroism spectroscopy for the study of complexation equilibria in solution have been proven. The results show that positively charged complex species are formed in solution in the presence of +2 and +3 charged metal cations. Theoretical modeling delineates the factors responsible for the selectivity of monensin and salinomycin toward group IA and group IB singly charged metal cations.

C. In order to evaluate the biological activity and, accordingly, the effect of the inclusion of metal ions in the structure of the complex with polyether ionophores, studies were carried out for the antibacterial activity and cytotoxicity of most of the structurally characterized complexes. The results show that, in general, the coordination compounds of monensin and salinomycin change the biological activity of the ligand, but further studies are needed to reach unequivocal conclusions in the direction of higher efficiency.

D. For metal complexes of studied compounds with biometals which demonstrate antibacterial and cytotoxic properties, the toxicity studies were conducted *in vivo* on laboratory animals.

The text of the thesis is well structured, clear and precise. The conclusions drawn follow the logic of the experimental data and demonstrate the candidate's critical analysis.

Scientific contributions and significance of the results presented in the thesis

A significant volume of experimental data was obtained within the limits of the conducted research - 32 new neutral "classical" and 7 "non-classical" complexes of the natural polyether

ionophores monensin and salinomycin were isolated and structurally characterized. New scientific results can be considered:

- The monovalent polyether ionophores monensin and salinomycin form complexes with metal ions regardless of their oxidation state – even metal ions in a higher oxidation state have been shown to form neutral, coordination compounds. So far, no predictions can be made about the way the ligands bind to metal ion– diversity is observed depending on the type of metal ions.

- New data were obtained on a series of positively charged complex species with monensin thanks to the application of circular dichroism spectroscopy, a first-line method for obtaining reliable results for complex species in solution. In addition, this method, after application in the ultraviolet region, allows distinguishing the "colorless" metal ions of alkaline elements, Mg^{2+} , Ca^{2+} , Zn^{2+} , Cd^{2+} , and its application in the visible part of the spectrum makes it possible to study colored ions Co^{2+} , Ni^{2+} and the processes of competition "color-colorless" metal ion.

- The results of the conducted studies on antibacterial activity and cytotoxicity indicate the potential for biological activity of carboxyl ionophores and their complexes with metals, but the inclusion of a metal ion in the composition of the coordination compounds of monensin and salinomycin affects the biological activity in a different way compared to that of the uncoordinated ligands. So far, the conclusions regarding the biological activity of the complexes compared to that of the free ligands are not one-way and suggest additional studies. Nevertheless, there are positive results that can be used in future more serious clinical studies.

It is necessary to emphasize that in the dissertation, in addition, a significant volume of experimental data characterizing structures of complexes was obtained and summarized. The possibilities of the modern instrumental methods, their advantages and disadvantages in the evaluation of structures of complexes are shown. Approaches to evaluate experimental data from different methods to obtain unambiguous conclusions about crystal structures of complexes are demonstrated.

Assessment of publications on the dissertation work and personal involvement of the author

The thesis is based on 27 publications, which were not used for the acquisition of the doctor's degree. Nine of these publications were not used to award the degrees of associate professor and professor. All publications are published in reputable specialized journals. A total of 204 independent citations were noticed (122 of them in the Scopus database). In the present procedure, 63 citations are presented, which were received on one publication of the

candidate's doctoral student. It is undoubtedly the personal involvement of the candidate, most of the results are obtained in the frame of her own research activities or are obtained in the frame of the research studies of her doctoral students. Prof. Pantcheva has been a supervisor of 9 diploma thesis and 3 PhD thesis (4th is in preparation) on the topic of complexes of metals ions with monensin and salinomycin. The abstract presented (in Bulgarian and English) exactly follows the thesis work and correctly reflects the results of the scientific research.

Critical notes

I have no critical comments on the materials of the competition and on the conducted studies, which have been published in refereed and renowned specialized journals for the relevant field.

CONCLUSION

The doctoral thesis prepared by Prof. Pantcheva presents scientific results that represent an original scientific contribution and meet all the requirements of the Act on Development of the Academic Staff in the Republic of Bulgaria, the Rules for its implementation, and the Specific criteria of Faculty of chemistry and pharmacy, University of Sofia "St. Kliment Ohridski" for awarding the scientific degree "Doctor of Sciences". I know Prof. Pantcheva personally and I am a direct witness of the purposeful research on the topic of the thesis work. Her desire to achieve maximum clarity and credibility of the conclusions drawn deserves respect. The presented dissertation shows that she is a highly qualified researcher with her own signature and original ideas, successfully combining multidisciplinary approaches to solving scientific problems and the effective application of the obtained results in research practice. The review of the publications on the basis of the presented thesis work shows that she successfully on the achievements of the Laboratory of Biocoordination and Bioanalytical Chemistry and has a clear perspective for its future development. Prof. Pantcheva willingly works with young colleagues and knows how to pass on her knowledge and skills. In this aspect, on the basis of the current and prospective scientific topics, the quantity and quality of scientific publications, the scientific contributions in the dissertation thesis, the guidance of doctoral students, I strongly recommend to the honorable scientific jury to award the scientific degree "Doctor of Sciences" to Prof. Dr. Ivayla Pancheva in the field of higher education 4. Natural sciences, mathematics and informatics, professional field 4.2 Chemical sciences, scientific specialty "Analytical chemistry".

Sofia, 19/02/2024

Reviewer: