

REVIEW

by Prof. Dr. Ivelina Mircheva Georgieva

on the Habilitation Thesis of **Dr. Ivayla Nedialkova Pantcheva-Kadreva**

Professor at Sofia University "St. Kliment Ohridski"

Faculty of Chemistry and Pharmacy, Department of Analytical Chemistry

title "**Metal complexes of carboxylic polyethers monensin and salinomycin:
structure, properties and biological activity**"

for acquiring DSc Degree

General description of the submission and the applicant. By Order No. RD-38-608/13.11.2023 of the Rector of Sofia University (SU) "St. Kliment Ohridski", Prof. Anastas Gerdzhikov, I have been appointed as a member of the scientific jury in connection with the defense of the habilitation thesis of Prof. Dr. Ivayla Pantcheva-Kadreva for acquiring Scientific Degree (SD) "Doctor of Sciences" (DSc) in the Professional Field (PF) 4.2. Chemical Sciences, Doctoral Programme (DP) "Analytical Chemistry". The submitted materials are in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its implementation at the SU and the recommended criteria of the Faculty of Chemistry and Pharmacy (FCPh) - SU on PF 4.2. Chemical Sciences. The report showed that Prof. Dr. I. Pantcheva-Kadreva fulfilled the necessary requirements. The candidate for the DSc degree presents a habilitation thesis on original scientific results published in **27 scientific papers** (full text) in the period 2008-2023, which do not repeat the content of the Dissertation for the PhD degree, entitled "Complexes of copper(II) with antihypertensive drugs", defended in 2001. The two Dissertations add 100 points to the group of indicators (GI) "**B**" and 50 p. to the GI "**A**", respectively. Nine (9*) of the publications in the habilitation thesis were not submitted in the candidate's competitions for the Academic Position (AP) "Associate Professor" (2011) and "Professor" (2020). From the list of papers for the current procedure, 20 were published in refereed and indexed scientific journals, 4 - in chapters of collective monographs and 3 - in non-indexed journals. The scientific publications are distributed by quartiles of the journals (for the year of publication) and points as follows: 6 in Q1 (150 p.) (2*), 6 in Q2 (120 p.) (1*), 6 in Q3 (90 p.) (1*), 2 in Q4 (24 p.) (1*), 4 in chapters of monographs (60 p.) (3*) and 3 in non-indexed

journals (0 pts.) (1*). With her publishing activity, prof. Pantcheva-Kadreva fulfils: 1) the minimum criteria for the GI "D" for the scientific works that are not used for obtaining the PhD degree, as well as for holding the APs "Associate Professor" and "Professor" (required 100 p./ fulfilled 142 p.); 2) the additional requirements of the FCPh-SU for the GI "D" for scientific works not used for acquiring PhD degree (required 300 p. / fulfilled 444 p.); 3) the minimum criteria under the GI "E" for 63 selected SCOPUS/WoS citations (out of 122 available) on the included publications (required 100 p. / fulfilled 126 p.). According to the StrikePlagiarism system report for the habilitation thesis, no evidence of plagiarism was found and no such reports were received on the submitted documents (research papers) for the defense of the dissertation (according to Art. 26, paragraph 4).

The habilitation thesis of prof. Pantcheva-Kadreva is a comprehensive, thorough and original study of the coordination ability of the polyether ionophore antibiotics monensin (Mon) and salinomycin (Sal) to a series of metal ions in order to obtain new effective bioactive substances. The accumulated data summarized and analyzed in the literature review provide a good basis and motivation for the directed research in this dissertation. The influence of metal ions in different oxidation state on the structure, properties and biological activity of monensin and salinomycin has been systematically evaluated in two directions. The first direction involves the synthesis, isolation and characterization of the structure and properties of newly obtained metal complexes of Mon and Sal in the solid state and testing their biological activity. The second strand is aimed at elucidating the behavior of antibiotics in solution in the presence of metal ions as a surrounding medium. The uniqueness of the fundamental research carried out lies in the development and application of a robust multidisciplinary approach interweaving knowledge of coordination chemistry, theoretical chemistry, biochemistry and spectroscopy. To characterize the newly synthesized compounds, processes and properties, a wide range of appropriate state-of-the-art experimental methods and apparatus for elemental analysis, X-ray structure analysis, transmission electron microscopy (TEM), thermogravimetry (TG-DTA/MS), spectroscopic methods (infrared (IR), electron (UV-Vis), electron paramagnetic resonance (EPR), nuclear magnetic resonance (NMR), circular dichroism (CD), fast atom bombardment mass spectrometry (FAB-MS)), bioassays and theoretical methods and approaches (empirical and density functional theory methods) were used. The thesis contains a complete research cycle of

synthesis, characterization, biological activity of a series metal complexes of Mon and Sal, inferred correlations and directions for future prospective research.

The results in the thesis are divided into three sections. The first section presents all the synthesized, isolated and characterized complexes of Mon and Sal with metal cations in the second (M^{2+}), third (M^{3+}) and fourth (M^{4+}) oxidation states. The unambiguous characterization of the solid-state structure of monensinate complexes with metal cations in the second oxidation state ($M^{2+} = \text{Mg, Ca, Co, Mn, Ni, Zn, Cd}$) was achieved by X-ray diffraction analysis of their single crystals. At the same time, detailed spectroscopic characterization of these complexes in the solid state has been used to establish a correlation between structure (in particular the coordination mode of the ionophores), $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, EPR features (where possible) and vibrational properties (especially the behavior of the carboxylate and hydroxyl group). The derived relationships, together with the results of elemental and thermal analysis, and model calculations were used to predict the molecular structure of metal complexes for which no suitable single crystal was obtained for XRD structural determination. The comparison of NMR, EPR and IR data in solid state and in solution was applied to evaluate the degree of structure change of metal complexes (coordination mode, protonation) in solution, which is also the biologically active medium. The characteristic fragmentation patterns and possible association/dissociation processes occurring at the metal complexes in the gas phase are described by MS measurements. The analyses performed show that all newly synthesized metal complexes of the studied ionophores are mononuclear. For a larger group of metal complexes, the two deprotonated ionophores were found to coordinate bidentate to the metal cation via carboxylate and hydroxyl oxygen, head-tail type, and form a deformed macrocyclic structure. These complexes (32 in number) are called classical and metal ions with the same degree of oxidation form isostructural complexes: *cis*- and *bis*- $[\text{ML}_2(\text{H}_2\text{O})_2]$ complexes of the alkaline earth and transition metal cations ($L = \text{Mon}$, $M^{2+} = \text{Mg, Ca, Co, Mn, Ni, Cu, Zn, Cd}$), ($L = \text{Sal}$, $M^{2+} = \text{Mg, Ca, Sr, Ba, Co, Ni, Cu, Zn, Cd}$); *tris*- $[\text{ML}_3(\text{H}_2\text{O})_3]$ complexes with lanthanide cations ($L = \text{Mon}$, $M^{3+} = \text{Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Er}$) and ($L = \text{Sal}$, $M^{3+} = \text{La, Pr, Nd}$), and *cis*- and *bis*- $[\text{ML}_2(\text{OH})_2]$ complexes with Ce^{4+} . Under specific conditions, two other types of metal complexes, called non-classical, were obtained and characterized: three cyclic and one non-cyclic *mono*-complexes in an ionophore:metal cation ratio of 1:1 and three mixed-metal complexes of monensin sodium. These complexes differ in composition and structure from classical

complexes. Two *mono*-complexes of Mon with mercury(II) ions, [HgMon(H₂O)] and of Sal with lead(II) ions, [PbSal(NO₃)] have been isolated and characterized. The structure of the former complex, determined by X-ray diffraction, demonstrates the ability of the monensinate dianion to bind tetradentate to Hg²⁺, with an unusual pseudo-bidentate coordination of the carboxylate group. *Mono*-complexes of Mon and Sal anions were also obtained with a lanthanide ion with a fourth oxidation state (Ce⁴⁺), [CeL(NO₃)₂(OH)]. Different coordination behavior of the ligands, pentadentate Mon with cyclization and tetradentate Sal without cyclization, was predicted using molecular dynamics and quantum chemical calculations. Of particular interest was the study of monensin sodium solution in the presence of metal ions, Co²⁺, Mn²⁺ and Cu²⁺, to assess the possibility of transition metal ions displacing Na⁺ from the Mon cavity. Mixed-metal complexes [M(MonNa)₂Cl₂] were obtained in which neutral monensin sodium coordinates bidentate to M²⁺ but Na⁺ cannot be displaced.

The second section of the thesis covers a systematic study of the complexation process in solution of Mon and Sal with metal ions under different reaction conditions (ligand:metal molar ratio and solvent type). The contribution of the thesis, both methodological and scientific, is the development of experimental approaches based on circular dichroism (CD, SRCD - with synchrotron radiation, Vis-CD, NIR-CD) to study the behavior of Mon in solution in the presence of single-, double- and triple-charged metal ions. It has been found that due to the chiral activity of metal complexes of Mon, CD spectroscopy appears to be a suitable method to evaluate the interaction of mono-charged cations with monensin, which is also sensitive to the nature of the mono-charged ions (Li⁺, Na⁺, K⁺, Rb⁺, Ag⁺) in the complexes and can distinguish between them. A better understanding of the spectral behavior of the complexes and interpretation of the nature of the one-electron transition is aided by calculations of the absorption and SRCD spectra of the complexes in solution with the time-dependent density functional theory. A complex evaluation of the factors governing the selectivity of polyether ionophores towards IA and IB metal ions is performed using DFT/PCM calculations and theoretical analysis. The thermodynamics of the competitive processes between Na⁺ and the other metal cations (Li⁺, K⁺, Rb⁺, Cs⁺, Cu⁺, Ag⁺ and Au⁺) in complexation with Mon and Sal were evaluated on the basis of the calculated free energies for the model reactions in condensed media. The metal ions of IA and IB group with the highest affinity to Mon and Sal have been identified and factors such as M-L bond type, dielectric properties of the medium, flexibility of the polyether chain, and balance between electronic effect

and solvation effect on the stability of the complexes have been determined. In the study of the interaction between M^{2+} (Co, Mn, Cd, Ni, Mg, Zn, Cu) and Mon, it was found that the SRCD spectral characteristics are sensitive to both the metal ion type and the complex particle type. This allows the determination of the *mono*- and *bis*-metal complexes formed and the equilibrium between them with changing the ligand : metal ratio, in the case of one or two metal ions. In the case of the colored monensinate complexes of Ni^{2+} and Co^{2+} , a complementary approach has been developed based on circular dichroism in the visible (Vis-CD) and near-infrared (NIR-CD) regions and more accurately estimate the complexes formed was achieved. The formation of two types of complexes, $[MMon_2(H_2O)_2]$ and $[MMon(H_2O)]^+$, in solution of Mon and M^{2+} , depending on the M:Mon ratio, 1:10 ÷ 1:2 and 1:1 ÷ 6:1, respectively, has been predicted, i.e., the formation of a new charged metal complex has been found in case of metal cation excess. The spectral study of two metal ions (M^1 and M^2 in different ratios) in Mon^- solution highlights clear trends for the possible interaction of the M^1L_2 complex with the metal ions (M^{2+}) that are in excess in its close vicinity and for the formation of the M^2L^+ complex. The equilibrium between the neutral and charged complexes is controlled by the type of metal ion in excess. When both metal ions are in excess the competition in M^2L^+ formation is determined by the specific affinity of the metal ion, which decreases in the order $Ni^{2+} > Co^{2+} \sim Ca^{2+} > Mn^{2+} \sim Mg^{2+} \sim Zn^{2+} \sim Cd^{2+}$. No mixed-metal coordination compounds were observed. The complexation of Mon with three-charged lanthanide ions (Gd, La, Nd, Pr, Sm, Eu) was investigated by UV-CD spectroscopy and the ability of the method to distinguish different complex particles but not complexes with different lanthanide ions was established. At certain Mon:Ln³⁺ ratios, three possible complex particles with the composition $[MMon_3(H_2O)_3]$, $[MMon_2(H_2O)_2]^+$ and $[MMon(H_2O)]^{2+}$ were observed.

The third section presents results on the biological activity of polyether ionophores and their metal complexes. The effect of the metal ions in the structure of the polyether ionophores on the biological activity against gram-positive bacterial strains and/or tumor/non-tumor cell lines of human or animal origin, as well as their toxicity in *in vivo* experiments was evaluated. In addition, certain biochemical parameters were analyzed after 72-h loading of surviving individuals. Ambiguous results were obtained, revealing the specific action of certain complexes towards particular bacterial strains and tumor lines. Initial activity assessment revealed the metal complexes of ionophores that exhibited greater antimicrobial and antitumor activity compared to the corresponding ionophores. The results confirm the reliable approach for the development of

selective and gentle bioactive compounds by complexation of natural biologically active compounds with metal ions and provide a new impetus in the research of new drug formulations. The clinical picture showed non-significant effects on clinical parameters and isolated cases of dehydration and myocardial damage. It has been suggested that the fatal outcome is related to the manifestation of neurotoxicity and respiratory failure.

Comments and remarks: The UV-Vis band in the 250-350 nm range for $[\text{CeL}_2(\text{OH})_2]$ and $[\text{CeL}(\text{NO}_3)_2(\text{OH})]$ complexes is assigned as $\text{O} \rightarrow \text{Ce}$ charge-transfer electron transition, on what data is this interpretation based? In my opinion, the compound $[\text{CeL}_2(\text{NO}_3)_2(\text{OH})]$ instead of $[\text{CeL}(\text{NO}_3)_2(\text{OH})]$ is inaccurately noted in the caption of Fig. 5.2-5. Two Tables are denoted as Table 5.1-4. Is it correct to exist two types of complexes $[\text{NiMon}_2(\text{H}_2\text{O})_2]$ (p. 59) and $[\text{NiMon}_2(\text{H}_2\text{O})]$ (p. 58, 57)?

The habilitation thesis is presented in 155 pages, includes 59 figures, 31 tables, 188 references and 5 Appendices with 16 tables and 22 figures. Its Abstract is prepared in 85/79 pages in Bulgarian and English and adequately reflects the content of the thesis. Undoubtedly prof. I. Pantcheva-Kadreva is a leading researcher on the subject of the dissertation, out of 27 papers on the procedure, in 22 she is a corresponding author and in 10 - the first author. The well-systematized material, with clearly outlined objectives and thoroughly presented results of the conducted research is extremely impressive. The antibiotics monensin and salinomycin are produced in Bulgaria, therefore the purposeful research of their properties mainly by a Bulgarian team and the results obtained in the dissertation work of the leading researcher have even greater significance and contribution to the *know-how* of the native producers. The trends highlighted in the dissertation provide a direction and perspective for future research in the development of effective and sparing preparations based on carboxylic polyether ionophores with antibacterial/antitumor action.

In conclusion, the habilitation thesis contains significant results, generalizations and solutions to scientific and applied problems in the field of coordination, biocoordination and medicinal chemistry of the natural polyether ionophores and their metal complexes. The research and the results obtained are in line with the state of the art and represent a significant and original contribution to science. On the basis of the above, I give a highly positive assessment of the habilitation thesis of Prof. I. Pantcheva-Kadreva and propose to the Honourable Scientific Jury to

award Prof. Dr. Ivayla Nedialkova Pantcheva-Kadreva the scientific degree "Doctor of Sciences" in the professional field 4.2. Chemical Sciences, Doctoral programme "Analytical Chemistry".

Sofia, 15.02.2024

Signature: