REVIEW

of the materials submitted for participation in a competition for the academic position "**professor**" in the field of Higher education 4. "Natural Sciences, Mathematics and Informatics", Professional field 4.2. "Chemical Sciences" (**Analytical Chemistry**), announced in State Gazette, issue 57 of 26.06.2020, for the needs of the Faculty of Chemistry and Pharmacy at Sofia University "St. Kliment Ohridski"

Reviewer: Natasha Trendafilova, Prof. Dr., IGIC-BAS

The only candidate in the announced competition for the academic position (AP) "Professor" is **Assoc. Prof. Dr. Ivayla Nedyalkova Pantcheva-Kadreva** (SCOPUS Author ID 6506664875).

Assoc. Prof. Dr. Ivayla Pantcheva was born on May 12, 1969. She graduated from the Faculty of Chemistry (FC) of Sofia University (SU) "St. Kl. Ohridski" in 1993 with a qualification "Master of Chemistry" and specialization in inorganic and analytical chemistry. In 2001, Ivayla Pantcheva was awarded with the educational and scientific degree "Doctor" at the Faculty of Chemistry at Sofia University. In the period 1997-2006, as an assistant in the FC, she conducted research, supervised graduates and was a scientific consultant for PhD students. In the period 2006-2011, the scientific activity of the candidate continues as a senior assistant, and after 2011 as an associate professor at the FC at SU and a lecturer in analytical, coordination and bioinorganic chemistry, as well as a supervisor of graduates and PhD students. In her research and teaching she is a very active and promising researcher and lecturer. In 2011-2019 period, Assoc. Prof. Dr. Pantcheva became a participant in the management of the Faculty of Chemistry and Pharmacy at SU as Deputy Dean, responsible for PhD students, international activities, research project financing and accreditation. She was the Erasmus+ faculty coordinator and has extensive experience in the preparation, organization and management of projects funded by the National Scientific Found and the EU's Seventh Framework Program for Research and Technological Development.

General characteristics of the received materials. The set of materials presented by Assoc. Prof. Dr. Pantcheva for participation in this competition is in full compliance with the requirements of the Low for Development of the Academic Staff in Republic of Bulgaria and the Regulations for its application, the Regulations for the Terms and Conditions for Acquiring Scientific Degrees and Occupying Academic Positions at Sofia University "St. Kl. Ohridski" and the Recommended Criteria for Acquiring Scientific Degrees and Occupying Academic Positions at Sofia University "St. Kl. Ohridski" for Professional field 4.2 "Chemical Sciences". The reference of Assoc. Prof. Dr. Pantcheva for the implementation of the minimum national requirements under Art. 2b of the Law in the field of Higher education 4. "Natural sciences, mathematics and informatics" in Professional field 4.2. "Chemical Sciences" *for AP "professor"* shows that the candidate meets and exceeds the required minimum for all groups of indicators: under group of indicators "В", 115 points were achieved with a required minimum of 100 points; under "Г" - 251 points were achieved at a minimum of 220 points; 138 points were achieved under "Д", at a minimum of 120 points; 209 points were achieved under "Е", at a minimum of 150 points; and 180 points were achieved under "Ж", at a minimum of 120 points.

Scientific publications. The main scientific interests and research of Assoc. Prof. Dr. Ivayla Pantcheva are in the field of biocoordination and bioanalytical chemistry and consist in the

synthesis and modification of biologically active substances by coordination to metal ions in order to increase their therapeutic properties. She is a co-author of 53 scientific papers, of which 33 (over 62%) have been published in refereed international journals, 19 are in non-refereed journals, proceedings with an editor/publisher and one auto-reference. So far, 336 citations (251 according to Scopus) have been noticed on the publications of Assoc. Prof. Pantcheva. The Hirsch index is 9. The results of the research with the participation of the candidate have been presented many times at scientific forums in Bulgaria and abroad. A list of 102 conference entries is presented, of which 45 are oral presentations and 57 are poster presentations. For participation in the current competition, Assoc. Prof. Dr. Pantcheva has selected 22 original scientific papers that have not been used in her dissertation for "Doctor" degree and in the competition for occupation of the AP "Associate Professor". These works describe current and extremely interesting thematically scientific problems, research and results. 19 scientific papers (over 86%) have been published in peer-reviewed authoritative journals in the field of her research, with impact factor and quartile, and 3 are scientific papers in full text in collections with editor/publisher. The distribution of the scientific articles by quartiles of the journals in which they are published is as follows: 2 articles are in Q1 journals, 5 are in Q2 journals, 12 are in Q3 journals and 3 articles are in Q4 journals. In 12 of the scientific articles the candidate is a corresponding author, and in 6 - a first author. The review of the scientific publications of Assoc. Prof. Dr. Pantcheva shows that they correspond in number, topic and quality to the conditions and the topic of the competition. On these publications, as of the date of submission of the documents, 40 citations were noted (28 in Scopus). For the purposes of the competition, a total of 69 citations after taking the academic position "Associate Professor" are included. The results of the research activity of Assoc. Prof. Dr. Pantcheva after her habilitation were presented at 16 national and 30 international scientific forums with 20 oral reports and 26 posters.

Teaching. The report of Assoc. Prof. Dr. Pantcheva shows that the scientific disciplines in which she is a lecturer, Analytical Chemistry I and II and Analytical Chemistry (compulsory), are in full accordance with the theme of the announced competition for AP "Professor" - *Analytical chemistry*. Her teaching activities at the Faculty of Chemistry and Pharmacy and at the Biological Faculty include training of students in the specialties of Pharmacy, Biology and Chemistry and Biology for a bachelor's degree and a master's degree after secondary education. In the period 2012-2020, under her leadership, 6 diploma theses and 2 dissertations were successfully accomplished. Currently, the candidate is the supervisor of the PhD student Radoslava Stamboliyska (part-time study).

Projects and other activities. The materials of the competition contain data on the participation of Assoc. Prof. Dr. Pantcheva in 10 research projects in the period 2012-2020. Two of the projects are international, and of three of them the candidate is a supervisor. As of the date of submission of the documents, she has three ongoing research projects funded by the National Science Found, one of which is headed by her. It should be underlined her active participation in the project under the Operating Program “Science and Education for Intelligent Development” (2017), related to education and lifelong learning of students, PhD students and young scientists, which is extremely important for achieving an optimal environment for training, research, innovation and sustainable development of human potential in the field of chemical sciences, for adaptation of education

today, for tomorrow. To the scientific activity of Assoc. Prof. Dr. Pantcheva should be added her participation as a member of the editorial board of The Scientific World Journal (Inorganic Chemistry). Together with colleagues (M. Nedyalkova, H. Alexandrov, P. Petkov) she participated in the compilation of a Book of Abstracts of the International Scientific Conference "Chemistry Today for Tomorrow". Since 2011, Assoc. Prof. Dr. Pantcheva has carried out a number of visits and specializations at the University of Szeged, Hungary (2014, 2016, 2018, 2019). Her participation in the international cooperation and projects with Aarhus University, Denmark, allows her to conduct cutting-edge and specific experiments. The conducted specializations and collaborations with leading universities in Europe have contributed to the increase of her qualification and scientific growth and have permanently shaped the image of a specialist - researcher in the field of modern biocoordination and bioanalytical chemistry.

The habilitation work of Assoc. Prof. Dr. Pantcheva is on the topic: "The diversity in the properties of the natural antibiotic *monensin*". It describes interesting in thematic terms and significant scientific results of the candidate. Based on six publications it is dedicated to the diverse coordination chemistry and promising biological properties of the complex compounds of the polyether ionophorous antibiotic *monensin*. The properties of the isolated *monensin* as well as its ability to interact with metal ions, in varying degrees of oxidation, are described in detail. Quantum chemical calculations have been performed, the results of which have contributed to understanding the nature of complex formation processes and properties. The characteristic changes that occur in the conformation of the ligand *monensin* and its electronic structure upon its binding to the metal ion, as well as the changes in the electronic structure of the latter are traced using CD spectra and DFT calculations. As a final phase of the research, the isolated ligand and its coordination compounds were subjected to biological tests to assess their ability to affect the viability of bacterial strains and cell lines.

The report on the scientific contributions of Assoc. Prof. Dr. Pantcheva is comprehensive and very correctly outlines her own contributions, which are in the field of design (directed synthesis), structural, spectral and biological characterization of the newly synthesized biocoordination compounds. The specific scientific contributions, some more important results and conclusions from them are presented briefly below, within the scientific topics defined by Assoc. Prof. Dr. Pantcheva in two directions - biocoordination and bioanalytical chemistry.

Complexes of natural biologically active substances. The coordination properties of the *salinomycin* sodium ionophore with respect to heavy metal ions were studied. A different way of *salinomycin* coordination has been established: bidentate with Cd(II) [$Cd(Sal)_2(H_2O)_2$] and polydentate with Pb(II) [$Pb(Sal)(NO_3)$]. The studies were performed in connection with the potential antidote activity of the ligand (*publ. 1*). Coordination of *salinomycin* sodium with double-charged metal ions (Mn, Co, Cu, Ni and Zn) has been demonstrated. Sterically stabilized DPPC:CHOL:DSPE-PEG-2000 liposomal formulations of the lipophilic complexes of *salinomycin* with Na(I), K(I), Mn(II), Co(II), and Ni(II) ions were prepared by film-hydration method at different drug-to-DPPC molar ratios. The cytotoxicity of the complexes and liposomal *salinomycinates* has been demonstrated in three human tumor cell lines (*publ. 4*). The coordination behavior of the antibiotic *monensin* acid with respect to La (III), Nd (III) and Gd (III) was studied. Reliable information on the coordination polyhedron and composition of the isolated neutral

complexes, $[Ln(Mon)_3(H_2O)_3]$, was obtained by applying appropriate spectral techniques (IR, ESI-MS, FAB-MS, EPR, SRCD). The formation of charged complex particles has been demonstrated by circular dichroism spectra with synchrotron radiation (*publ. 17, 22*).

Biological activity of metal complexes of natural antibiotics. The biological activity of *monensin* acid and its complexes with double-charged ions of Ca, Mg, Co, Mn, Ni, Zn, as well as of *monensin* with La(III) and Nd(III) was studied against a series of different cell lines. All compounds were found to significantly reduce the viability and proliferation of the treated cells. The main goal of the research has been achieved – the isolated metal complexes are more effective than the uncoordinated ligand (*publ. 2, 3, 17, 20*). The ability of the *monensin* complexes with double-charged ions of Ca, Mg, Co, Mn, Ni and Zn to visibly inhibit the growth of bacterial cells against the Gram-positive anaerobic bacterial strain *Clostridium perfringens spp,* has been proved. It was found that despite the similar structure, the activity of monensinate complexes against the studied bacterial culture varied, but in most cases the newly obtained compounds had better *in vitro* activity than the acidic form of the antibiotic (*publ. 6*). The acute toxicity of the polyether ionophores *monensin* and *salinomycin* with Na(I), Mg(II), Ca(II), Mn(II), Co(II), Zn(II) was evaluated in *in vivo* experiments in mice. *Salinomycin* complexes with Ca(II) and Mg(II) have been shown to have the highest toxicity among the tested compounds (*publ. 8*). In studying the coordination properties of the macrolide antibiotic *tylosin* with respect to Cu(II), two types of complexes have been isolated, depending on reaction conditions: mononuclear $[Cu(Tyl)_2]$ and binuclear $[Cu_2Tyl_2X_2]$, X = Cl^-, NO_3^-. The complexes turned out to be relatively non-toxic compounds in white male ICR mice. The *in vitro* determined antibacterial and SOD-like activity were evaluated and the complexes were found to be more promising agents than the uncoordinated antibiotic *tylosin* base (*publ. 21*).

Complexation of ionophore antibiotics in solution. For the first time, circular dichroism spectra (CD/SRCD) were used to prove the changes in the spatial conformation and electronic structure of the ligand during coordination. Studies with *monensin* and its complexes with single- and double-charged metal ions have been performed. It was found that the CD spectra of the ligand (acid) in the presence of Li(I), Na(I), K(I), Rb(I), Ag(I), Et_4N^+ in methanol are very sensitive to the coordinating cations and can be used to identify them. To predict the structure in solution, computer modeling was performed within the density functional theory and the continuum solvent model (PCM). This study showed that the structure of *monensin* in solution is very close to that determined in the solid state (*publ. 11*). Calculations within the time-dependent density functional theory (TDDFT) have indicated that the spectral changes were caused by a combination of conformational changes upon the monovalent cation binding and a direct involvement of the metal electrons in *monensin* electronic transitions. It was found that, depending on the metal-ligand molar ratio, the *monensinate* anion forms neutral and positively charged complex species with double-charged metal ions (Ca, Mg, Co, Mn, Ni, Zn and Cd), the coordination of which characteristically affects the SRCD spectrum of the ligand. The particle distribution, the individual spectra of the two complex types, as well as their stability constants are calculated. An estimate was given of the relative affinity of *monensin* to coordinate double-charged metal ions (*publ. 18*). The affinity of *monensin* to coordinate single-charged metal cations was assessed within the density functional theory and the PCM model. The factors that can lead to the formation of

complexes between the *monensinate* A anion and metal ions of groups IA and IB have been identified. Gibbs free energy was used to evaluate the preferred coordination of the ligand with Na(I), Li(I), K(I), Rb(I), Cs(I), Cu(I), Ag(I) and Au(I). A relationship between the size and selectivity of metal ions was found and the role of the solvent was studied for the first time. It has been found that the polyether ionophore binds selectively to Na(I) in polar solvents, while in less polar solvents it can become selective with respect to Li(I) or Cu(I) (*publ.19*).

Metal complexes of acetylcholinesterase reactivators. Within this topic, the coordination ability of a series of oxime-containing acetylcholinesterase (ACE) reactivators with respect to Pd(II) and Pt(II) was studied (BT-07 (K005), BT-08 (K033), BT-07-4M (K074) and *obidoxim*). Experiments have shown that the formation of Pd(II) complex species proceeds faster than that of Pt(II). Biological tests for *in vitro* reactivation of *paraoxone*-inhibited rat brain acetylcholinesterase (*obidoxime*) showed that the new complex particles had significantly lower activity than the noncoordinated *obidoxim* ligand, which is explained by the stability of the complexes in solution and with the deprotonation of the ligand oxime groups required for the recovery of the enzyme activity (*publ. 9, 13*).

Properties of phospholipase A_2 isolated from vipoxin. The ability of secreted phospholipase A_2 ($sPLA_2$) to catalyze the hydrolysis of natural and artificial substrates was assessed in the presence of divalent Ca, Mg, Sr, Ba and Cd ions. It has been found that the hydrolysis of natural glycerophospholipids proceeds at a higher rate when the enzyme is activated by Ca ions, while the hydrolysis of the artificial substrate is facilitated by metal ions with ionic radii larger than that of Ca(II), such as Sr(II) and Ba(II). Conformation changes have been shown to occur during metal ion coordination prior to subsequent hydrolysis (*publ. 5*). In another study, the hemolytic activity of secreted phospholipase ($sPLA_2$) was examined in the presence of saturated (palmitic) and unsaturated (oleic) fatty acids, and oleic acid was found to increase the hemolytic activity of $sPLA_2$ depending on the concentration. Studies have shown that the addition of *heparin* to red blood cells (RBC) suspension (containing $sPLA_2$ or a mixture of $sPLA_2$ and the corresponding fatty acid) led to an inhibition of hemolytic activity. The effect of $sPLA_2$ on RBC morphology resulted in formation of *echinocytes* (spherocyte subtype) suggesting that RBC could be the possible targets, attacked by the $sPLA_2$. *Vipoxin* $sPLA_2$ has also been shown to inhibit the platelet aggregation in a dose-dependent manner when *arachidonic* acid and *collagen* are used as inducers, while in the case of adenosine diphosphate (ADP) its inhibitory effect was inappreciable (*publ. 7*).

Oral fluid as an object of study in forensic toxicology. A number of parameters of the oral fluid (that could serve as markers for its identity/dilution) were studied. The data obtained showed that the protein profile and α-amylase activity can be used as markers for sample identity, while creatinine levels can serve as an indicator of oral fluid dilution/stability. The oral fluid storage conditions (temperature, period, stabilizers) were also assessed (*publ. 10*). Studies of the dry oral stain, which can be used both for storage and transport, as well as for the elimination of enzymatic and hydrolysis degradation of analytes, have been performed. The procedure for extracting drugs from the oral liquid and the corresponding dry stain has been improved and the applicability of the dry stain technique has been evaluated in order to detect prohibited substances. The procedure was validated using oral fluid from subjects with recent use of illicit drugs. The conclusion is that drug

abuse can be assessed using the dry stain technique, which is a promising alternative to the liquid sample (*publ. 12*).

Anesthetics and psychoactive substances. Within this topic, Assoc. Prof. Dr. Pantcheva has participated in conducting specific experiments that have helped the investigation of alleged suicide with a strong and fast-acting hypnotic substance - *propofol*. It was quantified in all available biological samples and based on toxicological and autopsy results, it was shown that the death was caused by an overdose of *propofol* (*publ. 14*). In another study, with a suitable combination of experimental techniques (gas chromatography-mass spectrometry and nuclear magnetic resonance), the content of the synthetic cannabinoid 5F-ADB (5F-MDMB-PINACA) was found in five "herbal mixtures" (*publ. 15*). Studies have been conducted to prove drug-induced and drug-related deaths from acute intoxication with two synthetic cannabinoids, 5F-ADB and FUB-AMB (*publ. 16*).

Summarized scientific contributions. The main scientific contributions in the research of Assoc. Prof. Dr. Ivayla Pantcheva are in the field of biocoordination and bioanalytical chemistry. They consist in the detailed study of the coordination ability of biologically active compounds with respect to one-, two- and three-charged metal ions, in order to obtain new complex species with biological properties and activity better than that of isolated ligands. It should be noted the huge amount of work and contributions of the candidate in the precise and ingenious optimization of the conditions for their production and formation of the most appropriate synthesis strategy. The numerous experiments, conducted to prove and characterize the newly obtained complexes with different spectral techniques, in some cases with the use of theoretical models and calculations, are significant contributions to the coordination chemistry. Much of the research of Assoc. Prof. Dr. Ivayla Pantcheva is devoted to biological experiments, from which valuable results have been obtained for the biological activity and application of the new complexes. Her contributions consist in the planning of biological experiments and in the selection of conditions for *in vitro* and *in vivo* experiments.

Conclusion. The in-depth interdisciplinary investigation conducted by Assoc. Prof. Dr. Ivayla Pantcheva determine her as a talented scientist and expert in the field of biocoordination and bioanalytical chemistry. In the research she has conducted numerous targeted and precise experiments, proposed strategies and original methods for the synthesis of new biocompounds, for study their structure, coordination and biological properties and activity. Assoc. Prof. Dr. Pantcheva has deep fundamental knowledge and indisputable scientific qualification in the field of chemistry and biology, which provides her with the potential for conducting and leading advanced research in the future. After the analysis of the materials presented in the competition: scientific papers, habilitation work, data on teaching, expert and organizational activities, participation in research projects and scientific forums, I give my positive assessment and vote "yes" for the election of Assoc. Prof. Dr. Ivayla Pantcheva to the academic position of "professor" at the Faculty of Chemistry and Pharmacy at Sofia University, in professional field 4.2. "Chemical Sciences", scientific specialty "*Analytical Chemistry*".

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