REVIEW

of the materials submitted for participation in the competition for the academic position (AP) "*Professor*" in the professional field 4.2. Chemical Sciences *(Analytical chemistry*), announced in the State Gazette No. 103 of 12.12.2023 (page 118) for the needs of the Faculty of Chemistry and Pharmacy (FCPh) of Sofia University "St. Kliment Ohridski"

The only candidate in the competition is Assoc. Prof. Dr. Galina Georgieva Gencheva-Kissovsky; Galina Gencheva; ORCid: 0000-0003-1696-1589; Scopus ID: 6701758294

General description of the submitted materials and the applicant. By Order No. RD-38-12/10.01.2024 of the Rector of Sofia University "St. Kliment Ohridski" (SU), Prof. Georgi Valchev, I have been appointed as a member of the scientific jury for the present competition. 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 verification of the above requirements for the AP "Professor" showed that the candidate fulfilled the required minimum for all the indicators of the groups (A, C, D, E, F, G) and collected **1077.6** pts against the total required minimum of 760 pts. There is no evidence and no reports of plagiarism have been received on the documents (research papers) submitted for the competition (according to Art. 26, paragraph 4).

Galina Gencheva completed her higher education at the FCPh at Sofia University with a Master's degree in Inorganic and Analytical Chemistry in 1986, and in 1993 she obtained the educational and scientific degree “doctor”, specialty “Analytical Chemistry”. With a PhD degree, the candidate fulfils group indicator (GI) “**A**” (**50 pts**). In her consecutive career development in the field of Analytical Chemistry, as a part-time Assistant Professor (1992-1996), Chief Assistant Professor (1996-2004) and since 2004 Associate Professor in the Department of Analytical Chemistry at FHPh-SU, Dr. Gencheva has accumulated 28 years of work experience in the specialty. She and the team she leads are worthy followers of the school of coordination chemistry in the Department. During her scientific career, the candidate has carried out three specializations: two at the University of Saarland, Saarbrücken, Germany and one at the Institute of General and Inorganic Chemistry, University of Münster, Germany. Since 2007, the scientific activity of Assoc. Prof. Gencheva is closely related to the implementation of **11 projects**: two international, three at Bulgarian National Science Fund – Ministry of education and science and six at Science Fund-SU. She has accumulated administrative experience as the Head of the Molecular spectroscopy laboratory for structural analysis in the department of analytical chemistry and merits for the establishment of this laboratory. She is a member of the General Assembly of the Faculty and is currently Deputy Dean for Quality Management, Accreditation and Employer Relations.

Research activity. In her current research activity, Assoc. Prof. Gencheva is a co-author in **52 scientific papers**, 38 of them published in indexed journals in the database SCOPUS and another

2 in the Web of Science. At the time of drafting the document, the total number of citations of the publications is **171**, h-index **8** (SCOPUS) (without self-citations of all co-authors).

In the current competition, Assoc. Prof. Gencheva participates with **21** original scientific papers and one patent, which exclude publications from her PhD thesis and the competition for AP "Associate Professor" (2004). The papers correspond thematically to the competition in the scientific specialty "Analytical Chemistry" and have been published in prestigious refereed journals such as *Journal of Inorganic Biochemistry* (IF = 3.3), *International Journal of Molecular Sciences* (IF = 5.6), *Pharmaceutics* (IF = 5.4), *Investigational New Drugs* (IF = 3.5) etc., which is a recognition of the importance of scientific research in the field of biocoordination and analytical chemistry. From the list of papers for participation in the procedure, **18** were published in refereed and indexed scientific journals, one in a non-indexed edition, in a collection with an editor and in a special book with abstracts from a congress Scientific publications distributed by quartiles (for the year of publication) and points are as follows: **6** in **Q1** (150 pts), **3** in **Q2** (60 pts), **2** in **Q3** (30 pts), **6** in **Q4** (72 pts) and **1** with **SJR** (10 pts). With her publication activity, Assoc. Prof. Gencheva fulfills the minimum criteria: 1) under GI "**C**" for habilitation thesis on 4 papers with Q1 (required 100 pts/completed **100 pts**); under GI "**D**" for scientific papers and patent (required 220 pts/completed **247 pts**); 3) under GI "**E**" for 64 citations in SCOPUS of the publications included in the competition (without self-citations of co-authors) (required 120 pts/completed **128 pts**). In 11 publications on the competition, Dr. Gencheva is a corresponding author, and on two of them she is also the first author.

In fulfillment of the requirements for the competition for AP "Professor", Dr. Gencheva presented a habilitation thesis, entitled **"Instrumental methods for determining molecular structure - application in modeling non-classical antitumor drugs"**. Thus, the author outlines her scientific contribution in a large-scale and complex study dedicated to the development of effective antitumor drugs with high selectivity and low toxicity based on metal complexes with organic molecules. Assoc. Prof. Gencheva has a leading role in defining the concept, modeling the reaction schemes, applying experimental and theoretical approaches to the analysis of the newly synthesized complexes, interpreting the data and summarizing the results in the publications. The idea underlying the choice of the ligands (natural hematoporphyrin IX (*Hp*) and *cis*-2,4,6-triaminocyclohexane-1,3,5-triol (*taci*) - analog of the biomolecule *cis*-inositol)) and metal ions (platinum, palladium, gold, copper and iron) as well as to carry out the targeted complexation process is based on known correlations between structure, mechanism and antitumor activity. The excellent knowledge of the trends in the development of selective anticancer drugs and the necessary properties that they must possess in order to exhibit antitumor activity, combined with the high competence in coordination chemistry, allow Assoc. Prof. Gencheva to determine the equilibria in the reaction system and control the complexation processes with changing starting reagents, solvent, medium acidity and metal:ligand ratio. The complex time-course processes of deprotonation in solution, complexation and redox, as well as the geometrical and electronic structure of the complexes in the solid state and in solution have been analyzed and proven by means of selected instrumental methods: spectroscopic - UV-Vis in solution and solid-state diffuse reflectance spectroscopy; ^{13}C and ^{1}H NMR in solution and solid-

state NMR for diamagnetic complexes; electron paramagnetic resonance (EPR) for paramagnetic complexes; vibrational spectroscopy (IR and Raman); X-ray photoelectron spectroscopy (XPS) and elemental analysis; thermogravimetry; differential scanning calorimetry; X-ray diffraction; magnetic measurements.

Two series of metal complexes with the two bioligands were developed as new medicinal formulations with potential pharmacological properties. The first group includes 11 new complexes of hematoporphyrin IX, which is an O and N polydentate ligand with varying ability for coordination to the metal ions, favoring redox processes, with protolytic properties and a function as a targeting agent for selective delivery of the drug and metal ions - Pt(III), Pd(III), Au(II), Cu(II), Fe(II) and Fe(III), characterized by suitable kinetic inertness and thermodynamic stability.

1) Using various starting substances in aqueous-alkaline medium, presence of oxygen from the air, scattered light and different Pt:*Hp* molar ratios, three mononuclear paramagnetic complexes of Pt(III) with *Hp* were prepared, isolated and characterized: *cis*-$[Pt(NH_3)_2(Hp_{-3H})(H_2O)_2].H_2O$ (Pt1), with bidentate coordination of two adjacent pyrrole N-atoms and two NH_3-molecules in *cis*-position; $[Pt(Hp_{-3H})(H_2O)_2].H_2O$ (Pt2), with symmetric coordination of the ligand to Pt(III) via the four pyrrole N-atoms in the porphyrin macrocycle; $[Pt((O,O)Hp_{-2H})Cl(H_2O)_3]$ (Pt3), where *Hp* is bidentate coordinated via two O-donor atoms of the carboxyl groups of the side chains of the substituents. (*publ. 3, 4, 10*)

2) In the approach to tailor metal complexes for effective antitumor drugs, complexes of Pd(III) with *Hp* are included by analogy with Pt(III) complexes. Two paramagnetic palladium complexes are isolated and their molecular structures are predicted: a mononuclear complex $[Pd(Hp_{-2H})Cl(H_2O)]\cdot H_2O$ with symmetrical coordination of the ligand to Pd(III) via the four pyrrole N-atoms in the porphyrin macrocycle (resembling Pt2) and a new type of dinuclear $[Pd_2(Hp_{-3H})Cl_3(H_2O)_5]\cdot 2PdCl_2$ complex, where *Hp* is coordinated bidentate through two adjacent pyrrole N-atoms to one Pd(III) and bidentately through two O-donor atoms of the carboxyl groups to the second Pd(III) ion. (p*ubl. 11, 16*)

3) Gold compounds are widely used for medicines and this determines the research interest of Assoc. Prof. Gencheva in the complex formation between Au(III) and *Hp*. A paramagnetic $AuHp_{-2H}\cdot 2H_2O$ complex are obtained, where the ligand is tetradentate coordinated via the pyrrole N-atoms in the porphyrin ring to the gold ion. In the process of complex formation and with the active role of the deprotonated Hp_{-2H} ligand, an redox reaction and reduction of Au(III) $\rightarrow$Au(II) take place, in contrast to Pt(II)/Pd(II), which are oxidized to Pt(III)/Pd(III). (*publ. 1, 6*)

4) The complexation of *Hp* with the essential elements copper (Cu(II)) and iron (Fe(II) and Fe(III)) in aqueous-basic media and/or both acetic acid-water and glacial acetic acid media is studied. In solution, it is found that the complex formation proceeds with the reduction of Cu(II), but in the solid state, a paramagnetic mononuclear $[Cu(Hp_{-2H}].2H_2O$ complex, with a tetradentate N-coordinated ligand is isolated. By varying the reaction conditions, four *Hp*-Fe(II)/Fe(III) complexes are synthesized and the ligand is predicted to coordinate through the pyrrole N-atoms to form metalloporphyrin or SAT (sitting-atop) type complexes. (*publ. 4, 5, 9*)

The second group includes two new complexes of Pt(IV) with the ligand *taci*, with a designed structure of a «non-classical» cytostatic. Two new complexes of Pt(IV) with the N-tridentate ligand *taci*, *fac*-[Pt(taci)I_3]I и *bis*-*[Pt(taci)$_2$]*(CO_3)$_2$ are synthesized and isolated. The determined crystallographic structures show that the ligand is tridentate coordinated to Pt(IV) through the three NH_2 groups. The vibrational behavior of *taci* and its platinum complexes are analyzed using quantum chemical calculations, and coordination-sensitive vibrations are identified.

EPR, magnetic susceptibility, IR and photoelectron measurements are used to analyze the stability of the studied complexes in polar solvents and aqueous-basic media, and it is proved that the oxidation state of the metal ion, the coordination mode of the ligands (*Hp* and *taci*) and the coordination polyhedron established in solid phase, do not change in solution with time and increasing temperature. Hydrolysis processes over time and under various factors, simulating a physiological environment, are studied by ^{1}H and ^{13}C NMR spectroscopy. Hydrolysis was shown not to affect the *Hp* and *taci* ligands, but only the monodentate ones. Correlations between the spectral characteristics and the electronic and geometric structure of the metal complexes are derived, which are a good basis for elucidating the mechanism of cytotoxic activity.

The pharmacological properties of the metal complexes with the proposed design have been proven by biological tests, including evaluation of antiproliferative potency, cell death detection and studies on mechanisms of cytotoxic activity in terms of DNA-platination, repair of damage in DNA molecules, evaluation of total toxicity, effect on resistant cell lines, etc., as well as a comparative evaluation with the mechanisms of the reference drug cisplatin. (*publ. 3, 6, 10, 11, 12, 16, 21*) Thanks to the porphyrin ligand, hematoporphyrin complexes efficiently accumulate in malignant cells, achieving greater selectivity and much lower toxicity to healthy cells (HEK-293T) compared to cisplatin. The new complexes have pronounced cytotoxic activity in selected cell lines comparable to that of cisplatin and in malignant cells of certain cell lines, they exceed it. The higher kinetic inertness of Pt(III)-*Hp* complexes in ligand substitution reactions compared to cisplatin is found to favor lower overall toxicity and a longer duration of action is required to achieve maximal efficacy. Success in the design of new structural modifications are three complexes with prominent pharmacological properties: these are the metalloporphyrin complex of Pt(III) with *Hp*, the dinuclear complex of Pd(III) with *Hp* and the complex of *taci*:Pt(IV)=1. Both metal and ligand type and coordination polyhedron are found to be important for optimizing antitumor activity. For the metal complexes with *Hp*, the coordination in the porphyrin ring is a favorable factor and they are characterized by a different mechanism of action from cisplatin, which opens new perspectives in the treatment of cancer cells. The newly designed metal complexes are specifically effective against certain cell lines (for which cisplatin is resistant), which determines their potential for specific therapies.

Part of the research work of Assoc. Prof. Gencheva (*publ. 2, 8, 15*) is dedicated to the study of the coordination ability of phosphine oxide ligands to metal ions and prediction of the structure of the obtained complexes with XRD and spectroscopic methods. In the joint research, Dr. Gencheva contributed a) to determining the structure of 4 complexes of (aminoalkyloxymethyl)dimethylphosphine oxides with Pd(II) ions by means of comparative IR analysis; b) in the characterization of a series of new mononuclear and polynuclear coordination

compounds of copper(II) with bis((dimethylphosphinyl)methyl)amine obtained under various reaction conditions; c) in the theoretical and experimental study of the coordination ability of 1,4 bis(dimethylphosphinylmethyleneoxy)benzene, where lone electron pairs analysis of the ether and phosphine oxide O-atoms predicts the difference in their reactivity.

Assoc. Prof. Gencheva develops a research approach, based on vibrational spectroscopy (Raman and IR) to characterize new graphene materials for biomedical purposes and natural products (herbs). By means of spectroscopic analysis, information was extracted about the composition, the presence of O-containing functional groups on the surface and defects during reduction and functionalization of graphene oxide nanoparticles by methoxypoly(ethylene glycol)amine or with PEGylated nanographene oxide (*publ. 18, 19*). With the help of the IR spectroscopic study of biomaterials adsorbed Cu(II), it is established that there is no complex formation between the metal ion and the surface functional groups of the biomaterial, therefore the retention of copper ions on the surface is a result of physisorption. (*publ. 17*)

In three publications (*7, 13, 14*), Assoc. Prof. Gencheva demonstrated competence in the determination and interpretation of structural characteristics from XRD analysis. Her contribution is expressed: a) in the preparation and characterization of a new polymorph of cis,fac-$RuCl_2$(DMSO-S)$_3$(DMSO-O); b) in the analysis of the crystal structure and interpretation of the spectroscopic characteristics of *3-methylpyridazinium hydrogensquarate*; c) in determining the crystallographic lattice and space group symmetry parameters of the compound *3-(2-(diphenylphosphorothioyl)phenyl)-4-oxo-1-phenyl-3,4- dihydroquinazolin-1-ium perchlorate.*

The educational and teaching activity of Dr. Gencheva is impressive. It includes the development and implementation of 6 lecture courses and 6 practical classes in a bachelor's program with a number of hours, 320 of lectures and 162 of exercises. She is a co-author of a textbook for 12 class "Chemistry and environmental protection" (module 4 « Methods for control and analysis of substances»), of the guide "Tasks in analytical chemistry, Processes and their use in chemical methods of analysis" and of a protected patent. Three PhD theses were defended under the supervision (and co-supervision) of Assoc. Prof. Gencheva, dedicated to new metal complexes with potential antitumor activity. Under her supervision, 5 bachelor's and four master's theses were successfully defended. As a result of the active teaching and project activity, the candidate reported that **270.6 pts** were completed under the GI "**F**" (150 pts were required) and **282 pts** were completed under the GI "**G**" (120 pts were required).

A major contribution in the long-term research work of Assoc. Prof. Gencheva and the team led by her is the pioneering design of "non-classical" structures of metal complexes with pharmacological properties alternative to cisplatin. The successful results are due to the refinement of the reaction conditions for targeted synthesis and the detailed characterization of the structures, kinetic and thermodynamic properties of the metal complexes with the skillful application and in-depth knowledge of the capabilities of a number of instrumental methods. In summary, the scientific contributions, the successfully implemented scientific projects, the publication and teaching activity of Dr. Galina Gencheva prove that she is a highly qualified scientist in the field of analytical and biocoordination chemistry, which fully meets the scientific specialty "Analytical Chemistry" of the competition for "Professor". The presented analysis of

the competition materials and my personal impression of the candidate as a thorough and critical scientist give me reason to vote with "yes" and recommend to the Scientific Jury to propose to the Faculty Council of the FHPh at SU, Associate Professor Dr. Galina Georgieva Gencheva-Kissovsky to be elected to the academic position of **"Professor"** in the FHPh at SU in professional field 4.2. Chemical Sciences, scientific specialty "Analytical Chemistry".

19.04.2024
Sofia

Reviewer:
Ivelina Georgieva, Prof. Dr., IGIC-BAS