

Review

of the materials submitted for participation in the competition for occupation of academic position "**Associate Professor**" in the professional field 4.2. "Chemical Sciences", scientific specialty "Theoretical Chemistry", for the needs of the Institute of Organic Chemistry with Centre of Phytochemistry (IOCCP), Laboratory "Structural Organic Analysis", published in the State Gazette, issue 79 of 08.10.2019.

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The only candidate in the announced competition is **Chief Assistant Dr. Nadezhda Vasileva Markova** (IOCCP- BAS).

1. General characteristics of the received materials. The set of materials submitted for participation in the competition is in full accordance with the requirements of the Law for the development of the academic staff in Republic of Bulgaria and the Regulations for its implementation, the Rules for the conditions and the order for acquiring scientific degrees and for occupying academic positions in BAS and meets the specific criteria of IOCCP for taking the academic position of "Associate Professor". All competition materials have been prepared very carefully, clearly and in detail, with relevant supporting materials and leave no doubt as to their authenticity. The report on the implementation of the minimum national requirements under Art. 2b of the Law for the development of the academic staff in Republic of Bulgaria for Professional field 4.2. "Chemical Sciences", when taking the academic position of "Associate Professor", shows that the candidate fulfills and by some of the indicators significantly exceeds the required minimum points. Detailed analysis is given below, Section 3.

2. Brief CV, education and professional experience. Chief Assistant Dr. Nadezhda Markova has completed her higher education in 2000 at the "Konstantin Preslavski" University of Shumen, where she graduated with a Master's Degree in Organic Chemistry. In the period 2002-2005, the candidate is a full-time PhD student in the PhD program "Theoretical Chemistry" at IOCCP-BAS. In 2006, after successful defending of her PhD thesis on the topic: "Theoretical studies of tautomeric equilibria in organic molecules, taking into account the specific influence of the solvent", Nadezhda Markova obtained the educational and scientific degree "doctor" in the scientific specialty "Theoretical Chemistry". In the same year she was elected as "Chief Assistant" at IOCCP, where she continues to work. In 2006, Dr. Markova specializes at the University of Namur, Belgium.

3. Evaluation of scientific activity

Scientific publications, Habilitation work, Projects, Scientific forums. The full list of publications of Dr. Markova contains 34 scientific works, of which: 27 (79%) have been published in reputable scientific journals with impact factor, falling in quartiles Q1-Q4, 2 are in journals with SJR, but without impact factor, 2 are in non-impact factor journals and three are in conference

proceedings. The total impact factor of the journals is 51.73. In 11 of the scientific papers, Dr. Markova is the first author, and in 4 - she is the corresponding author. The distribution of the 27 articles by quartiles of the journals, in which they are published, is as follows: in journals of the category Q1 - 7, in Q2 journals - 8, in Q3 journals – 6 and in Q4 journals - 6. The number of the citations noted on the publications is 314 according to Google scholar (h-index = 9) and 261 according to Scopus (h-index = 7).

To participate in the "Associate Professor" competition, Dr. Markova has selected 18 original scientific papers, all on the topic of this competition. Five of them present in-depth theoretical studies, done at high scientific level and combined by one theme: "Water as a medium and catalyst for tautomeric reactions". These publications form the basis of the candidate's Habilitation work (Group indicators "B"). In four of the habilitation papers, Dr. Markova is the first author and principal performer of the research. The articles included in the habilitation work have been published in reputable international journals, four of them in Q1 journals and one in Q2 journals. With these publications the candidate completely satisfies and even exceeds the requirements of Art. 2b of the Law for the development of the academic staff in Republic of Bulgaria and the Regulations of BAS and IOCCP for its implementation – she has reached 120 points with the required minimum of 100 points per group of indicators "B". The other 13 publications that meet the requirements of group "G(Γ)" indicators present theoretical studies, in some cases supported by experimental measurements, aimed at clarifying the structure and properties of various compounds and structural fragments of particular importance for the medicinal chemistry and biology. The distribution of the articles included in the "G(Γ)" group indicators by quartile of the journals, in which they are published, is as follows: one publication is in Q1 journal, 5 publications are in Q2 journals, 4 are in Q3 journals and 3 publications are in Q4 journals. With these publications the candidate has met the requirements for a minimum of 220 points. For the group "D(Δ)" indicators she presented 100 citations on the publications for participation in the present competition, which has achieved 200 points, and thus, the requirements for a minimum of 70 points for this group of indicators have been repeatedly exceeded. *For review, I accept all the 18 scientific publications, presented by Dr. Markova for participation in the present competition.*

Participation in scientific forums. Dr. Markova included in the materials enough data and sufficient evidences for participations in scientific forums, at which she has presented 18 oral reports and 40 posters. Some of the participations were awarded: Certificate in the Competition for Excellence of the Union of Scientists in Bulgaria for 2011; Crystal prize "The best paper" for best report and article presented at Scientific Conference with International Participation in 2015, University of Rouse and Crystal prize "The best paper" for best report and article presented at Scientific conference with International participation in 2019, University of Rouse. Dr. Markova participated in the organization of two scientific forums: IEEE EUROCON 2017, 17th IEEE International Conference on Smart Technologies, Special Session SS-7: "E-Infrastructure for Scientific Excellence", 6-8 July 2017 Ohrid, Macedonia and International Conference on Smart Technologies IEEE 2019, 1-4 July, 2019, Novi Sad, Serbia.

Participation in projects and teaching. An integral part of Dr. Markova's scientific activity is her participation in the development and implementation of scientific projects and the training of graduate and doctoral students. Dr. Markova is the head of three projects at the Scientific Research Fund (FNI) and the Ministry of Education and Science and a participant in three projects at the FNI, in one project between BAS and MANU and in three EBR (EBP) projects with Belgium. She was an individual participant in a project of the Agricultural University in Plovdiv. She is also involved in Project BG051PO001-3.3.07-0002 "Student Practices" with 6 students (2 from Sofia University, 3 from the University of Chemical Technology and Metallurgy and one from Sofia Medical University. Within Project BG05M20P001-2.002-0001 " Student Practices - Phase 1", the candidate participated in the training of 3 students (2 from Sofia University and one from Medical University). In 2016, Dr. Markova was the supervisor of the diploma work for Bachelor's Degree of a student from Sofia University - Nina Stoyanova-Nankova (specialty "Comp. Chemistry") and later, in 2018, of her diploma work for the Master's Degree (specialty "Med. Chemistry"). Dr. Markova has participated in the education and improvement of the qualification of two PhD students: one full-time PhD student at Southwestern University for the period 2015-2017 (Boyka Miladinova Stoykova) and one PhD student of individual program at the Agricultural University, Plovdiv, for the period 2015-2017 (Rumyana Prodanova-Kamalieva).

Original Scientific Contributions in the Habilitation Work (Publications in the "B" Group of Indicators). Dr. Markova's Habilitation Work combines systematic theoretical studies motivated by the idea of investigating the specific role of the water as a medium and catalyst in tautomeric reactions. *Studied are the thermodynamic stability, structural and kinetic characteristics of compounds and structural fragments, as well as the possible tautomeric forms and transformations of three main groups of compounds: nucleic bases derivatives, nucleosides and salicylideneanilines. Original and reliable computational procedures have been proposed to study in detail, in one or several steps, the mechanisms of reactions with water-assisted proton transfer. Through comprehensive molecular modeling at high levels of theory, new data have been obtained to prove stable molecular structures and their tautomeric forms. The role of water as a catalyst in structural fragments of DNA and RNA, their derivatives and Schiff bases with intramolecular hydrogen bonds has been studied. The obtained theoretical data on the structure and mechanisms of proton transfer reactions explain and predict the existence of new structural forms and are of particular importance as they are inaccessible for experimental determination.* The specific scientific contributions, some of the more important results and conclusions are summarized below.

Tautomeric equilibria at nucleic bases derivatives: 5-fluorouracil, 5-azauracil and 6-azauracil. Dr. Markova's contributions in these studies consist in the accumulation of new theoretical data on tautomeric equilibria in 5-fluorouracil, 5-azauracil and 6-azauracil in aqueous solution, which shed light on the mechanisms of proton transfer reactions and clarify the role of water molecules as a medium and catalyst. For the first time, using *ab initio* quantum-chemical calculations and NMR spectroscopy, Dr. Markova examined the neutral and charged structures of 5-fluorouracil formed at different pH values in aqueous solution (*Publication 2*). The influence of water

molecules on the tautomeric reactions between the various forms has been established and a mechanism is proposed, in which several protons are transferred. The conversion between the two tautomeric forms (oxo and hydroxy) and the two dioxo form anions has been studied by Raman spectroscopy. By applying accurate *ab initio* quantum-chemical calculations (GAMESS) for vibration frequencies calculations in the gas phase and for the four-hydrated cluster, Dr. Markova proved various forms of *5-fluorouracil*. The effect of the solvent on the tautomeric conversion of *5-fluorouracil* and its anions was studied using a “supermolecule” model and by mechanism that involves the transfer of several protons. At a high level of theory, CCSD(T)/6-31+G(d,p)//MP2/6-31+G(d,p), the free energies (ΔG_{298}) are calculated and the order of stability of the investigated forms is predicted.

By a combined computational procedure based on molecular dynamics and quantum chemistry methods, the tautomeric equilibrium of *5- and 6-azauracils* in the presence of water molecules as well as the mechanism of the proton transfer reactions in solution were correctly and thoroughly studied (*Publication 3*). A model of four water molecules attached to *azauracil* tautomers (specific solvation model) was created for reliable investigation of the effect of hydration. The role of water as a medium (global solvation) has been evaluated by the method of self-consistent reaction field and the PCM model. The calculations included Monte Carlo simulation, analysis of solvent-solute interaction models, and quantum-chemical modeling of the cluster formation process in the presence of water. With the reliably calculated Gibbs free energies of different *azauracil* tautomers, their order of stability in the gas phase and in aqueous solution is predicted. The possibility of intermolecular proton transfer in *5- and 6-azauracil* with the participation of water molecules was investigated. The energy barriers of tautomeric reactions were calculated at C-PCM/MP2/6-31+G(d,p) level of the theory. It has been predicted that in the reactions studied, the transfer of proton assisted by water molecules is carried out by a concerted mechanism.

Tautomeric equilibrium in nucleosides. Guanosine, acyclovir and inosine. Dr. Markova's scientific contributions to this topic consist in a detailed and correct theoretical study of the structure and stability of various tautomeric forms of guanosine, acyclovir and inosine. A mechanism for proton transfer reactions involving water molecules is proposed. The relative energies of the *guanosine* and *acyclovir* tautomers in the gas phase and in solution were calculated and their stability order was evaluated (*Publication 5*). By examining various conformations of the ribose ring in the tautomeric structure of *guanosine Gs-A*, it has been found that the conformation of the sugar residue in question is close to that observed in native RNA. Accurate calculations at MP2/6-31+G(d,p) level predicted a *guanosine* structure close to the experimentally established one. Three types of *acyclovir* structures have been exhaustively considered in relation to the side chain that is attached to N14. Specific solvation is simulated by explicitly incorporating water molecules that model the first hydration shell around the molecule. The position of these water molecules is specified by classical Monte Carlo simulation of dilute *guanosine* (Gs) and *acyclovir* (ACV) aqueous solutions and solvent-solute intermolecular interaction analysis. The mechanism of the water-assisted proton transfer was investigated at two levels: MP2/6-31+G(d,p) taking into account only the short-range interactions between the solute and the water molecules and

optimization of the cluster with four water molecules using the conductor-like polarizable continuum model (C-PCM/MP2/6-31+G(d,p)) level, taking into account both short- and long-range interactions. The results predicted that in the gas phase and in aqueous solution, the 1H-2-amino-6-oxo form is the most stable tautomeric structure for *guanosine* and *acyclovir*, followed by the 2-amino-6-(sZ)-hydroxy form. The energy barriers of the reaction of water-assisted proton transfer in *guanosine* and *acyclovir* are very close (11.74, 11.16 kcal mol⁻¹), and the corresponding rate constants are large enough to form 2-amino-6-(sZ)-hydroxy tautomer. The reaction profiles of the reactions were investigated and it was predicted that when only the short-range interactions with the solvent (MP2/6-31+G(d,p)) were taken into account, the proton transfer proceeds in one step without intermediates, whereas when using in addition the model of long-range interactions (global solvation), the reaction profile turns out to be different. The reaction profiles predicted that proton transfer processes proceed through an asynchronous concerted mechanism.

The stability and structural characteristics of the possible tautomeric forms of *inosine* in the gas phase and in aqueous solution were investigated using analogous calculation procedures (*Publication 4*). The Gibbs relative free energies calculated at MP2/6-31+G(d,p) and SCS-MP2/6-31+G(d,p) levels of theory predicted the relative order of stability. The proton transfer with the participation of water molecules was investigated. Clusters, containing an *inosine* molecule and five water molecules were modeled at C-PCM/MP2/6-31+G(d,p) level. The calculated energy barrier of the proton transfer reaction for *inosine* (12.9 kcal mol⁻¹) and the calculated rate constant ($k = 6.68 \times 10^1 \text{ s}^{-1}$) predicted the formation of the 6-enol tautomer. The reaction profile showed that the proton transfer proceeds through an asynchronous concerted mechanism. According to theoretical predictions regarding the kinetics of tautomeric conversion of *inosine* in water, and according to vibrational frequency calculations, it is predicted that the hydroxy-tautomer may be present in solution, although the experimental IR and Raman spectra of *inosine* in water have not shown this. *The exhaustive calculations presented in this section for different nucleosides show that if the calculated energy barriers of water-assisted proton transfer reactions are in the range of 12–17 kcal mol⁻¹, then tautomeric transformations are kinetically possible processes.*

Keto-enol tautomerism in salicylideneanilines. Using sequent computational procedures at different levels of theory (HF, B3LYP, MP2, and MP4//MP2), Dr. Markova examined in details the keto-enol tautomeric equilibrium, and in particular the keto-amine/enol-imine equilibrium in a series of substituted salicylideneanilines (*Publication 1*). The most stable structures in the gas phase and in solution are predicted depending on the calculation methods used. The dependence of the calculated energy barriers of the tautomeric transformations on the used structural model and calculation method has been established. It is important to conclude that in order to correctly predict the stability of structures and energy barriers of proton transfer reactions, combined models including both specific solvation and global solvation need to be applied in the calculations.

Original scientific contributions to publications in Group Indicators “G” (,,Γ“). *The scientific contributions in the 13 scientific articles outside the Habilitation work are in the field of theoretical modeling (DFT and ab initio) and the study of the structure, physicochemical and kinetic*

properties of neutral and charged structures and biomolecular fragments, various tautomeric forms and equilibria with consideration of the solvent role and the possibility of hydrogen bonding and proton transfer with the participation of water molecules. Two scientific publications are devoted to the study of mechanisms of organic reactions. A new tautomeric system is proposed with a small difference in the energies of the tautomeric forms and a relatively low activation barrier. In the presence of an external electric field, depending on its direction and amplitude, different tautomeric forms stabilize, which is why this system could be used in the design of new molecular electronic devices (*Publication 3*). The absorption and fluorescence properties of a newly synthesized compound, 3,6-bis(4,5-dihydroxyoxazo-2-yl) benzene-1,2-diol in ethanol, were studied, showing an unusually strong Stokes shift. DFT/TDDFT calculations were applied to investigate all possible tautomeric forms in the ground and excited states. A mechanism for intramolecular proton transfer in the first singlet excited state is proposed, in which two successive tautomerization reactions are carried out (*Publication 4*). Another group of publications is devoted to the study of the structure of organic and coordination compounds. Through ab initio quantum chemical calculations (MP2, MP4) and IR spectroscopy, structural studies have been performed to show that 2-amino-thiazolidin-4-on and its isomer 4-amino-thiazolidin-2-on exist in solution in the form of an *amino* tautomer. This result refutes the claim of other authors that 4-amino-thiazolidin-2-on exists as an imino tautomer. It is theoretically predicted and proven by IR spectra that two tautomeric forms of pseudothiohydantoin are present in D₂O: 2-amino-4-oxo-thiazolidine, which dominates, and 2-imino-4-oxo-1,3-thiazolidine, which is found in small quantities (close to the measurement limits of the method) (*Publication 11*). Semi-empirical (PM3) and *ab initio* (HF/3-21G, HF/6-31G**//HF/3-21G) quantum-chemical calculations have been used to study the tautomeric forms of the neutral molecule of *daunorubicin*, their complexes with one DMSO molecule and three dimeric structures of *daunorubicin*, evaluating the effect of the medium on the relative stability of the tautomers. GIAO calculations were performed to explain the experimental NMR spectra. Quantum-chemical modeling, together with NMR spectroscopic measurements and calculations, have been used for structural characterization and determination of the preferred distribution of the stereochemical sequence of the monomer units in the homopolymer chains of poly(butyl- α -cyanoacrylate) nanoparticles (carriers of the active drug substance *5-fluorouracil* and *daunorubicin*). The presence of *5-fluorouracil* in the reaction medium (saline, pH 10-11) has been shown to influence the polymerization and nanoparticle formation, affecting the initiation of the polymerization reaction. *Fluorouracil* acts as an initiator of the anionic polymerization of the n-butylcyanoacrylate monomer via its nucleophilic nitrogen atoms (*Publication 1,2*). Using quantum-chemical calculations, infrared spectra and NMR parameters, the structure of a number newly synthesized derivatives of brominated amide of cinnamoyl amino acids, spirohydantoin, 1,8-naphthalimides and hydrazine with various applications in medicine as anticancer, antimicrobial and anticonvulsant drugs. (*Publications 7, 8, 9, 10 and 13*). The photophysical properties of the two tautomeric forms of 2-carbamido-1,3-indanedione (CAID), which could be a proton donor in hydrogen bonds and a potential biomarker and sunscreen reagent, were investigated through theoretical and experimental studies. The UV and fluorescence spectra were

theoretically calculated and compared to the experiment. It was shown that both enol forms, 2-(hydroxylaminomethylidene)-indane-1,3-dione and 2-carboamido-1-hydroxy-3-oxo-indane, exist in solution (*Publication 5*). The structures of N-(4-benzalaniline)-15-crown-5 and its complexes with Li^+ , Na^+ , K^+ , Be^{2+} , Mg^{2+} , Ca^{2+} , Sr^{2+} and Ba^{2+} were optimized and compared using ab initio and DFT methods. From theoretical and experimental NMR spectra, it has been found that in all cases the distances $\text{M}^{n+}\text{-N}$ ($n = 1,2$) are longer than the average $\text{M}^{n+}\text{-O}$ distances in the respective complexes and the distances between metal and nitrogen in the alkali metal complexes are longer than alkaline earth ones. Li^+ , Na^+ , Mg^{2+} and Ca^{2+} are inserted into the cavity of the crown, while K^+ , Sr^{2+} and Ba^{2+} , whose size is larger than the diameter of the macrocycle cavity, are positioned outside it. The conformation of the crown ether does not change except for the Be^{2+} complex where the macrocycle conformation is highly deformed (*Publication 12*). Two newly synthesized compounds have been proven to exist in the form of various tautomers in solid phase and in solution: 2-(methylthio)-1,3-diazaspiro[4.4]non-2-en-4-on and 2-(methylthio)-1,3-diazaspiro[4.4]non-2-en-4-thion. It has been suggested that this phenomenon could be called desmocatropia (from the Greek words: δεσμός (bond), κατάσταση (state) and τρόπος (change)) (*Publication 6*).

4. Final Opinion. In the competition for the academic position “Associate Professor”, Chief Assistant Dr. Nadezhda Markova has presented a sufficient number of scientific papers published in reputable international journals with an impact factor, which fully comply with the Law for the development of the academic staff in the Republic of Bulgaria and the specific requirements of BAS and IOCCP for its application. The scientific output presented, undoubtedly proves the applicant's competence to perform and to conduct valuable theoretical studies in the future. The scientific achievement of Dr. Markova, her teaching activities, as well as her participation in a number of scientific projects and scientific forums, outline the profile of a young and in-depth scientist, with well-defined research interests and achievements in the field of theoretical and computational chemistry. *After analyzing the materials presented and based on my excellent personal impressions, I find it justifiable to give my positive assessment by voting "yes" for the selection of Chief Assistant Dr. Nadezhda Markova in the academic position "Associate Professor" in Professional Field 4.2. "Chemical Sciences", specialty "Theoretical Chemistry".*

23.01.2020

Reviewer:

(Natasha Trendafilova, Prof. Dr. IGIC-BAS)