

REVIEW

in the competition for the academic position "Associate Professor"

in professional field 4.2. Chemical sciences (Analytical chemistry)

for the needs of the department of Analytical Chemistry at Faculty of Chemistry and Pharmacy

at Sofia University St. Kliment Ohridski,

announced in SG no. 104 / 15.12.2023

By Assoc. Prof. Dr. Violeta Milenkova Stefanova

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The only candidate who has submitted documents for participation in the announced competition is chef. assist. prof. Dr. Veronika Valentinova Mihaylova.

1. General presentation of the received materials

The candidate has submitted a complete set of materials on electronic media that meet all the requirements of the *Law on the Development of Academic Staff in the Republic of Bulgaria* (LDASRB) and the *Regulations for the Terms and Conditions for Acquiring Scientific Degrees and for Holding Academic Positions at Sofia University and the Specific Requirements of the Faculty of Chemistry and Pharmacy (FCPh)*.

The documents are organized in 18 files, containing reference on the fulfillment of the minimum national requirements under Art. 2b of the ZRASRB and the regulations of the FHPH; evidence materials for academic development; scientific guidance of graduates; list of published articles and corresponding citations; an archive containing full texts of the selected publications for participation in the contest; participation in scientific forums; participation in scientific projects; habilitation thesis and author reference for the contributions of the scientific works. The presented materials are well organized and provide comprehensive information about the candidate's scientific and teaching activities.

In the Register of academic staff of the Republic of Bulgaria, maintained by the National Centre for Information and Documentation (NACID), there are available data on the scientific degree "Doctor" of Veronika Valentinova Mihaylova as well as a list of 23 registered publications for the period 2009-2019., which testify that there is no repetition of the materials used in the current competition with previous procedures for awarding of PhD (<https://ras.nacid.bg/dissertation-preview/40153>).

2. Education and scientific development

V. Mihaylova graduated with honors from her high school education, majoring in biology, at the Natural and Mathematical High School "Hristo Smirnenski", Pernik, in 2003. graduated as the top of the class for the Master of Science in Chemistry at the Faculty of Chemistry of the University of St. Kliment Ohridski" in the master's program "Ecochemistry". In 2013, he successfully defended his doctoral dissertation on the topic: "**Investigation of changes in the ionome of *Taraxacum officinale* under the influence of anthropogenic factors with ICP-MS**" with supervisor: Prof. DSc R. Djingova.

Immediately after graduation, V. Mihailova started working as a chemist at FHF, and from 2013 until now she is the main assistant in the Department of Analytical Chemistry. From 2019 ch. assistant professor Dr. V. Mihaylova was involved as a R2 researcher under project BG05M2OP001-1.002-0019 on the topic "Clean technologies for a sustainable environment - water, waste, energy for a circular economy", financed within the framework of the OP "Science and Education for smart growth".

3. Scientometric data

✓ **General characteristics of the applicant's activity**

In her documents ch. As. Prof. V. Mihailova presented a total list of 27 scientific publications, of which 26 are indexed in the scientific databases *Web of Science* (WoS): 17 articles; 94 independent citations; h-index -5) and *Scopus Science* (24 articles; 92 independent citations; h-index -6) with the

following quartile distribution: 9 publ. – Q1; 6 publ. – Q2; 7 publ. – Q3; 4 publ. – Q4). The dissertation work for obtaining the educational and scientific degree "doctor" includes 3 publications, which are not repeated with those selected for participation in the current competition.

Over 50% of the publications are in highly reputable scientific journals with (Q1) such as: *Applied Spectroscopy Reviews* (TAYLOR & FRANCIS INC); *Journal of soil and sediments* (Springer Heidelberg); *European Journal of Pharmaceutical Sciences* and *Journal of Trace elements in Medicine and Biology* (ELSEVIER); *Journal of Food Composition and Analysis* (Academic Press INC Elsevier Science); *Molecules, Water and Pharmaceuticals* (MDPI).

According to the reference database *Web of Science*, V. Mihaylova is the first author in 29%, second author in 47% and corresponding author in 18% of all reflected articles, which testifies to her active role in the process of conducting and presenting scientific research. A general assessment of the quality of the published works compared to other publications in similar scientific fields is given by the percentile of citations, which according to WoS data for V. Mihaylova is the 35th.

The candidate's active participation in other national and international scientific forums is proven by the list of 21 poster and 5 oral reports presented at conferences and seminars.

V. Mihailova participated in 16 scientific projects, 3 of which were financed by EU programs.

✓ ***Assessment of the implementation of the minimum national requirements (Art. 2b of the LDASRB) and the specific requirements of the FCPH for occupying the academic position (AP) Associate Professor***

21 articles, divided into two groups of indicators, have been selected for participation in the current competition for AP "Associate Professor".

Group of indicators C: Includes a total of 6 articles in international scientific journals referenced in *the Web of Science* and *Scopus* databases with distribution by quartiles as follows: Q1(2 points); Q2 (1); Q3(1); Q4(2). The specified publications carry 109 points, which exceeds the 100 points required for this indicator.

The habilitation work is thematically focused on "Investigation of the impact of anthropogenic factors on changes in the elemental composition of various natural matrices by means of inductively coupled plasma mass spectrometry" and covers 4 types of real natural matrices: water, plants, soils and sediments.

The work presented by the candidate is laid out in 35 pages, 5 tables and 19 figures. It includes research published in 6 articles [B1-B6]. V. Mihailova is the first author in 3 of the articles, and in the remaining 3 she is the second author.

Group D, indicator 7: Includes a total of 15 articles [B8-B21] in international scientific journals referenced in the *Web of Science* and *Scopus* databases with distribution by quartiles as follows Q1(6); Q2 (2); Q3(5); Q4(2). V. Mihaylova is first author on 2 of the above articles, second author on 4 and last author on 1 publication.

The above publications carry 289 points, which significantly exceeds the 220 points required for this indicator. It should be noted that more than 50% of them were published in highly regarded scientific journals (Q1 and Q2).

Group of indicators E:

The applicant has submitted a total list of 92 citations of 16 articles, while according to a reference in WoS, the number of noticed independent citations reaches 94. The declared sum of points in this group of indicators is 184, which far exceeds the required 70 points from citations (LDASRB).

Group of indicators F:

According to the additional specific requirements of the FCPH for professional field 4.2 chemical sciences, this group unites: h-index (indicator 21); number of defended diplomas (ind. 23), participation in scientific projects (pos. 25), etc.

Currently, the applicant's h-index is 5 - WoS and, accordingly, 6 - Scopus (60 points).

During the period 2016-2023 ch. assist. prof. dr. V. Mihailova has been a supervisor of 5 bachelor/master diploma theses (50 points). Documents for the candidate's participation in the scientific teams of 11 projects (55 points) have been presented as well.

The total number of points in this group (165) far exceeds the required 70 points.

The above-mentioned comparison shows that the candidacy of the head assistant, Dr. V. Mihailova, fully complies with the requirements laid down in the ZARSRB and the rules of the FHF for holding the academic position Associate professor. It should be noted that for some indicators the achieved results significantly exceed the national minimum requirements.

4. Scientific contributions

The main scientific contributions of the candidate can be divided into the following areas:

1. Development and application of ICP-MS to study the elemental composition of objects from the environment [B1-8, B10, B12, B14-15, B17-19, and 10 scientific conferences]
- ✓ An approach to extend the linear dynamic range in the determination of macro elements in natural matrices by ICP-MS is proposed. The capabilities of a dynamic reaction cell (DRC) were used for the reduction of high signals by optimizing the RPa (Dynamic Bandpass Tuning parameter) coefficients. The approach has been applied to the determination of: Na, K, Ca, Mg and Si in waters; and Na, Mg, Al, P, K, Ca, Ti, Mn and Fe in plants, sediments and soils. The selection of the optimal value for the RPa coefficients (for each studied isotope) is consistent with the expected concentration intervals of the particular matrix element. It has been shown that the introduction of RPa coefficients can shift the linear dynamic range by 2-3 orders of magnitude (from ppb to ppm). It is noted that signal suppression also reflects on increasing detection limits. The adequacy of the proposed corrections has been verified by analysis of CCM.
- ✓ An ICP-MS method has been developed for the simultaneous determination of macro-, micro- and trace element composition of Bulgarian mineral and spring waters:
 - A first-of-its-kind study was conducted to characterize the chemical composition of Bulgarian brands of bottled water. The contents of 69 elements have been determined, most of which are not yet considered by environmental legislation (Bulgarian and European) and for now there is no data on them.
 - The results are presented in concentration cadasters, which present an elemental "fingerprint" for the particular samples and allow easy comparison of a large group of elements whose concentrations vary over wide intervals of (3-8 orders of magnitude).
 - A group of elements (potentially toxic - PTE) has been identified for which there are no regulated maximum permissible contents for drinking water in Europe, but which are found in real samples and can be harmful to human health.
 - Statistical analyzes of Bulgarian bottled waters were performed (in terms of elemental composition): the cluster analysis shows that the samples form three significant clusters, well corresponding to their type ($\text{HCO}_3\text{-Ca-Mg-SO}_4$; $\text{HCO}_3\text{-Na}$; $\text{HCO}_3\text{-Na-SO}_4$); and the conducted discriminant analysis highlights Ca, Mg, K, Ti and Fe as discriminating elements for their grouping.
 - Statistical analyzes of the results for 17 mineral and 8 spring waters were performed, which divided the samples into 2 main clusters, well corresponding to their origin, and the discriminant analysis showed that the macroelements Na, Ca, K, Si and Mg were responsible for the clustering of the samples. Trace element cluster analysis separated the samples into four clusters with mixed similarity groups (mineral/spring waters).

- ✓ The optimized methods for multi-element analysis have been adapted to other natural matrices (sediments; soils and plants used as bioindicators). The characterization of the elemental composition has been successfully complemented with ecotoxicity tests, and statistical approaches for the analysis of experimental data allow to determine the degree of risk of anthropogenic impact:
 - The dynamics in the macro- and microelement composition of sediments from the Pchelina dam, Bulgaria, were evaluated. Depth distribution profiles of 20 elements are shown (Ti, Mn, Fe, Zn, Cr, Ni, Cu, Mo, Sn, Sb, Pb, Co, Cd, Ce, Tl, Bi, Gd, La, Th and U). Anthropogenic influence on PTE accumulation in sediments was assessed using enrichment factors, geoaccumulation indices, bioassays and Phytotoxkit F and statistical approaches
 - The risk of contamination of a region near the closed Tsar Asen mine was assessed by determining the elemental composition of soils and plant samples from a suitable biomonitor - *Taraxacum officinale*. The multivariate statistical processing of the obtained data allows to reveal some relationships between the factors of transfer and/or accumulation of PTE in the environment. The response of different plant species to environmental pollution was investigated and the bioavailability of PTE was determined
 - The transfer factors (TFs) of 52 elements from soil to plants (*Taraxacum officinale*) were determined for 27 background and anthropogenically affected areas in Bulgaria. By means of cluster and factor analysis, the dependence between TFs and soil pH, cation-exchange capacity (CEC) and element concentration has been proven. It has been shown that in polluted areas, major pollutants are more bioavailable
 - ✓ Application of ICP-MS methods for assessment of bioavailability and bioaccumulation in fruits, vegetables and medicinal plants
 - The ability of annual (maize, sunflower) and perennial (apple, pear) plants to accumulate PTE- Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn was compared. The obtained values for AF indicate a higher accumulation of Pb and Cd in the annual plants. Accumulation of PTE in the fruit and grain than in the corresponding leaves has been shown, indicating the existence of mechanisms that the plant uses to protect itself from anthropogenic pollutants
 - Generalized procedures for the extraction and fractional analysis of 8 elements (Cd, Co, Cr, Cu, Mn, Ni, Pb Zn) in two varieties of apples are proposed, which allow to evaluate the bioavailability of PTE by the human organism. A consistent analytical approach was applied, determining the total catalytic concentrations and extractability of the metals in different media (water, ethanol, acetone, n-octanol and mimic media - HCl+pesin). The results between a physiologically based extraction test (PBET for gastric and intestinal digestion) and extraction with HCl+pepsin and n-octanol were compared. The studied elements are divided into 2 groups - with high bioavailability in the gastrointestinal tract (Cu, Mn, Zn and Cd), while for Ni and Pb only <30% of the total concentration is bioavailable
 - In ICP-MS analysis of the medicinal plant *Clinopodium vulgare L.* from 15 regions of Bulgaria and the corresponding infusions. The concentration of the elements extracted in the water decreases in the order Ca > K > Mg > Al > Fe > Na > Zn > Mn > B > Sr > Cu > Cr > Ni > Pb > Ce > La > Cd. It has been shown that it is necessary to control the concentration of Cd in the herb due to its almost complete extractability in water.
2. Assessment of the impact of treatment plants on adjacent water bodies and determination of organic pollutants in water [B12, B14, B15, B19 B16, B18, and 2 scientific forums]

Research on chemical characterization of regions around water treatment plants combined with statistical analysis of the experimental data, allows to create flexible approaches for planning sampling process, ways to assess impacts on the condition of receiving water bodies, as well as prioritization of quality indicators of the waters

- ✓ Methods for instrumental determination of chemical and physicochemical parameters necessary for the characterization of surface water and the efficiency of operation of wastewater treatment plants (WWTP) located near 21 large cities in Bulgaria are applied.
 - ✓ Chemometric tests were applied, combining data for chemical indicators with those from various ecotoxicological impact tests. The Partial Least Squares with Discriminant Analysis (PLS-DA) method was used for the overall assessment of the impact of WWTP on surface water quality.
 - ✓ A chromatographic method with (HPLC) was developed for the determination of pesticides (imidacloprid, cypermethrin and chlorpyrifos ethyl) in water, which was applied to the analysis of river (Struma) and drinking (Pernik) waters
3. Archaeometric studies by spectral and X-ray methods [B9, B11, B20 and 2 scientific forums]
- The creation of an extensive database on the chemical composition of archaeological finds from different geographical regions of Bulgaria creates prerequisites for subsequent statistical processing of results, which can reveal some important chronological indicators useful in the study of historical processes and periods. The capabilities of appropriately selected spectral methods for archaeometric studies (non-destructive - XRF and multi-element methods - ICP-AES and ICP-MS) were used to characterize the elemental composition of archaeological finds from the territory of Bulgaria.
- ✓ By means of XRF, the contents of Au, Ag and Cu were determined in 96 gold objects from the 4th century BC. The selected analysis method does not cause visible damage to the studied object therefore it is suitable for chemical characterization of finds with high historical and museum value.
 - ✓ For the first time, a large-scale archeometric study of finds from the III-VII centuries AD was conducted to determine the macro- and microelement composition of 219 artifacts (belt accessories - buckles, buckles, etc.) made of silver, bronze, brass, etc. copper alloys.
4. Development and application of inductively coupled plasma mass spectrometry for the analysis of anticancer platinum complexes. [B13, B21 and 3 scientific forums]
- The experimental study of drug uptake by cancer cells allows to clarify some of the reasons for the change in pharmacotherapeutic potential, as well as to define future structural modifications aimed at optimizing anticancer activity.
- ✓ An ICP-MS method was established and validated for the determination of trace concentrations of Pt in different types of cell cultures and cell fractions. Sample preparation, instrumental measurement and method validation are tailored to the specifics of the objects to be analyzed. For the correction of the non-spectral matrix influence, internal standard calibration was used (IS - Ir), and the quality of the analytical results was proved by standard addition method.
 - ✓ The method allows experimental evaluation of the efficiency of accumulation in cells of different types of chemotherapeutic drugs based on Pt(II) or Pt(IV) complexes and comparison of new formulations with the traditionally used carbo- and cis-platin. Newly synthesized supramolecular Pt(II) nanocapsules and Pt(IV) pyrenebutyric complexes were found to show much higher accumulation in two types of cancer cells, compared to cisplatin

5. Educational and teaching activities

During the period 2018-2024, Ch. assist. prof. Dr. Veronika Mihailova is a lecturer of two compulsory courses for bachelor students from the FCPH and the Faculty of Biology (BF) of the SU - "Analytical Chemistry" and "Applied Statistics".

She participates in conducting practical classes in the courses: "Analytical Chemistry" for students majoring in Biology and Molecular Biology (BF); "Analytical Environmental Chemistry" for bachelor students in Eco chemistry and Chemistry majors and "Instrumental Methods of Analysis I" for Chemistry majors. V. Mihailova conducts practical classes in "Analytical Chemistry I and II" with master students majoring in Pharmacy.

The average auditorium workload of the candidate for the last 5 years is more than 460 hours, and the corresponding total workload exceeds 600 h.

Despite the significant workload with classes, Ch. assist. prof. Dr. Mihailova actively works with students and on research tasks, as evidenced by the 5 diploma theses of which she is the scientific supervisor.

6. QUESTIONS AND RECOMMENDATIONS

- 1) How is the linearity of the calibration dynamic range evaluated in ICP-MS methods? The coefficients of determination of the regression equations shown in a number of studies are not a reliable indicator of linearity because they strongly depend on the number and concentration levels of the standards used.
- 2) What model is used to calculate the parameters of the regression equations in cases where the concentration range of the calibration standards is very wide (4-5 orders of magnitude)? The conventionally offered (in the instrument software) unweighted regression model is adequate only when the experimental data are homoscedastic, while over a wide calibration range the signals are highly heteroscedastic, which is a prerequisite for applying a weighted regression model – i.e. setting different statistical weights to the standards.
- 3) In most of the described ICP-MS methods, external calibration is used, even for samples with relatively "heavy matrices", which raises some questions regarding the calibration strategy:
 - ✓ At high concentrations of macroelements in the measured solutions from natural matrices, a non-spectral matrix effect is expected. Was such an effect observed and how was it corrected?
 - ✓ Sensitivity drift is fundamentally inherent to the ICP-MS method, especially when measuring long series of samples. What measures have been taken to correct this drift?

My **recommendations** to Dr. Mihailova are related to the development possibilities of two of the prospective research directions in which she works and considering the available instrumental base in the Department of Analytical chemistry.

- ✓ I believe that the LA-ICP-MS technique is much more suitable than acid mineralization for the development of archaeometric studies because it is the least destructive and allows determining the elemental and isotopic composition without visible damage to the studied artifacts
- ✓ Regarding research in the field of accumulation of drugs by different types of cells, I believe that mastering the possibilities of single cell-ICP-MS has the potential to enrich this direction.

7. CONCLUSION

The scientific and teaching qualification of Ch. assistant professor Dr. Veronika Mihailova is undoubtedly and exceeds the minimum scientometric requirements of the LDASRB and the specific requirements of the FCPH for holding the academic position of "associate professor" in the field 4.2.

chemical sciences. The presented research contains original scientific and applied contributions. A significant part of them have been published in prestigious journals and have received a wide recognition in the referential databases Scopus and Web of Science.

Ch. Assistant Professor V. Mihailova, Ph.D., is a highly qualified scientist who has undoubtedly gained expertise in the current scientific fields of modern analytical chemistry such as: 1) methodological development of plasma spectral methods for trace element analysis, which are well complemented by a variety of statistical approaches for the evaluation of arrays of experimental data. The last allows the identification of groups of similarity as well as the disclosure of some cause-and-effect relationships or trends; 2) study of processes of accumulation, transfer and assimilation (incl. Bioavailability) of substances in natural objects; 3) assessment of ecological condition of natural objects and environmental impact of anthropogenic factors; 4) application; of specific analytical techniques for archaeometric studies; 5) evaluation of the effect of drugs on cells.

Ch. Assistant Professor V. Mihailova, actively works on scientific and applied projects and successfully imparts professional knowledge and skills to students and graduates.

The above gives me reason to confidently **give my positive assessment and to propose to the scientific jury to approve the election of Ch. Assistant Professor Veronika Valentinova Mihaylova, Ph.D., for the academic position of "Associate Professor"** in professional field 4.2. Chemical Sciences (Analytical Chemistry) in the department of Analytical chemistry at the Faculty of Chemistry and Pharmacy, Sofia University "St. Kliment Ohridski".

16.04.2024 r.

Signature:
Assoc. Prof. Dr. Violeta Stefanova