STATEMENT

By Assoc. Prof. Dr. Violeta Milenkova Stefanova

Department of Analytical Chemistry and Computer Chemistry,

Faculty of Chemistry, Plovdiv University "Paisii Hilendarski"

on a PhD thesis for acquisition of educational and scientific degree "Doctor"

in the area of higher education: **4. Natural sciences, mathematics and informatics**

professional field: **4.2. Chemical sciences**

doctoral program: **Analytical Chemistry**

Author: Lidia Ivanova Kaynarova

Title: Investigation of the analytical capabilities of inductively coupled plasma mass spectrometry for identification, characterization and determination of nanoparticles

PhD Supervisors: Assoc. Prof. Dr. Violeta Stefanova and Assist. Prof. Dr. Deyana Georgieva - Plovdiv University "Paisii Hilendarski", Faculty of Chemistry, Department of Analytical and Computer Chemistry

1. General description of the procedure and the PhD student

According to order № РД 21-1304 from 13.07.2022. of the Rector of University of Plovdiv "Paisii Hilendarski" (PU) I have been appointed as a member of the scientific jury under the procedure for defence of PhD thesis entitled "**Investigation of the analytical capabilities of inductively coupled plasma mass spectrometry for identification, characterization and determination of nanoparticles"** for awarding the educational and scientific degree "Doctor" in the higher education field **4. Natural sciences, mathematics and informatics**; professional field - **4.2. Chemical sciences**; doctoral program - **Analytical Chemistry**. The author of the thesis is **Lidia Ivanova Kaynarova** - PhD student in full-time education at the Department of Analytical Chemistry and Computer Chemistry, Faculty of Chemistry, PU "Paisii Hilendarski".

The set of materials presented by Lidia Kaynarova on paper and electronic media is in accordance with all the requirements specified in Article 36 (1) of the Regulations for the Development of the Academic Staff of the PU. All presented documents are perfect and correspond to the requirements of PU "Paisii Hilendarski".

Lidia Kaynarova graduated with a bachelor's degree in medicinal chemistry (2016) at the Faculty of Chemistry of the "Paisii Hilendarski" PU with a thesis on the topic: "New multicapillary nebulizer for introduction of liquid samples into ICP-MS", result from a joint research with the University of Alicante, Spain, where she studied, within the framework of the ERASMUS international student exchange program. In 2017, Lidia Kaynarova also acquired a Master's degree in Spectrochemical Analysis at the Faculty of Chemistry of PU "Paisii Hilendarski. The part-time form of study in the master's program allows her to gain professional experience as an analytical chemist in the laboratory of Biovet AD, Peshtera. By order of the Rector of PU No. P33 – 863 of 23.02.2018, Lidia Kainarova has been enrolled as a full-time doctoral student in the doctoral program of Analytical Chemistry. Since February 2019 till now, she has been appointed as an assistant professor on a fixed-term contract in the Department of Analytical Chemistry and Computer Chemistry

2. Relevance of the topic

Over the past two decades, the multidisciplinary fields of nanoscience and nanotechnology have been at the forefront of modern research. Due to their high surface/volume ratio, which leads to a change in their properties, nanomaterials (NM) have proven to have the potential to revolutionize many technological and industrial sectors: information technology, medicine, pharmacy, cosmetics, energy, food industry, etc., but in addition, their growing applications pose risks of potentially toxic effects on human health and the environment. This requires the development of both adequate methods and relevant regulations and legislation for their control. In 2020, two regulations of the European Commission, (EU) 2018/1881 and (EU) 2020/878 came into force, introducing special requirements regarding the import and use of nanoform substances and applying explicit legal requirements, according to REACH. Therefore, it is recognized that the development of innovative analytical methods is necessary to monitor the presence of NM in real objects, their potential risk assessment and ensure a control according to legal regulations. In this context, analytical chemistry is facing new challenges by regarding nanomaterials as analytes. The problem is further exacerbated by the lack of standard methods for sample preparation, calibration, processing, interpretation, and validation of measured parameters in real objects.

Inductively coupled plasma mass spectrometry (ICP-MS) is capable to provide information related to the analysis of individual structural units (nanoparticles, cells or micro/nanoplastics). This new approach, called spICP-MS, has been intensively developed in the last decade, due to the possibility of modern analytical instruments to provide high frequency of detection, satisfactory sensitivity, and the development of the relevant theoretical bases that ensure the coupling of the fast transient signals, generated by single events, with the elemental composition of target objects.

The above proves the relevance of the present PhD research, dedicated to evaluating and expanding the analytical capabilities of the single particle inductively coupled plasma mass spectrometry (spICP-MS) method, as a new promising approach allowing the determination and characterization of nanoparticles (NPs) with different composition in colloidal suspensions.

3. Knowledge of the problem

The detailed literature review - 366 sources, of which more than 75% were published after 2010, shows that the PhD student is deeply familiar with the current state of scientific achievements in the thematic area of the dissertation work. The critical review of the advantages and limitations of the ICP-MS method, in terms of nanoparticle analysis and characterization, combined with a systematically planned study of the contributions of each step of the analytical process to the analytical characteristics of spICP-MS show that the PhD student gained deep knowledge in this new and unconventional application area of elemental mass spectrometry. A convincing proof of this is that Lidia Kaynarova is the first author in all the posters presented at scientific forums, two of the oral reports and one of the published articles.

4. Research methodology

The main part of the study was carried out with one of the most widespread analytical instruments – a single quadrupole ICP-mass spectrometer operating with integration times in the millisecond range. The possibilities for characterizing silver and titanium dioxide NPs, which are among the most widely used nanomaterials in various areas of industry – cosmetics, pharmaceutical and medical products, food technologies, etc., have been studied in detail. All stages of the analytical process in the characterization of NPs with the spICP-MS method were systematically studied: sample preparation, homogenization and stabilization; optimization of the instrumental conditions for measurement; statistical approaches to signal processing; calibration by certified reference materials or standard solutions of the monitored element and validation of methods by comparison with certified reference materials and/or alternative spectral and microscopic analyses.

The developed method was applied to the analysis of real samples containing Ag NPs.

5. Characteristics and evaluation of the dissertation

The PhD thesis is structured in five main sections: literature review (48 pages); goals and objectives of the research (1 page); an experimental part that covers the instruments used, reagents, standard solutions, certified reference materials, silver NPs synthesis procedure, sample preparation for analysis, and signal processing approaches (9 pages); results and discussions on the conducted research (69 pages), conclusions are summarized in 1 page and a list of cited references (366 sources,

26 pages). A list of used abbreviations and designations is attached (2 pages). The entire study is visualized in 34 tables and 38 figures.

The goal and corresponding research tasks of the PhD thesis are clearly stated. The systematically planned research strategy and thoroughly discussed results convincingly demonstrate the role and influence of each investigated step, or parameter, on the analytical performance of the spICP-MS method. Some of the contributions of the dissertation work go beyond the specifically studied nanomaterials and are of a fundamental nature concerning the analytical capabilities of inductively coupled plasma mass spectrometry for the characterization of NPs. The most important of them can be summarized as follows:

- For the first time, a computational model has been proposed that allows: selection of an appropriate dilution factor for a sample containing NPs; a module based on Poisson statistics for assessing the probability of coincidence of NP signals, as well as the possibility of particle concentration correction, given specific data on the number of registered peaks in the sample; calculation of the concentration of ionic standards of the monitored element corresponding to spherical NPs with predetermined diameters. The model is developed as an active spreadsheet incorporates data characterizing the particle material, preliminary information on the analysed nanocolloid suspension, and instrumental conditions of the specific measurement, making it applicable to all types of nanomaterials containing an element measurable by ICP-MS.
- The analyte-directed optimization of instrumental parameters in spICP-MS analysis allows sensitivity improvement, which directly reflects on reducing the limit of determination, in terms of NPs' size. The sampler position and the nebulizer gas flow rate have the most significant impact on the signal-to-noise ratio.
    - ✓ An approach is developed for the experimental evaluation of signal noise due to the processes of ionization in plasma and transport of ions through the mass spectrometer, based on time segmentation of a continuous signal recorded for standard solutions of the monitored element. For the first time, the contribution of signal noise is included in the combined uncertainty estimate and the following general trends are established, with respect to individual nanoparticle diameters determined by spICP-MS:
    - ✓ the half-intervals are asymmetric with an extension towards the lower limit, more pronounced for small diameters;
    - ✓ as the size of the NPs decreases, the interval estimate widens;
    - ✓ the width of the interval estimates depends on the mass fraction of the analyte and the density of the nanoparticle material
- The mean value of confidence intervals is proposed as an overall estimate for the spICP-MS method size resolution, concerning NPs with a specific composition and size range.

Regarding the contributions of the thesis related to the specific NPs (Ag and TiO_2), the following should be mentioned:

- Targeted optimization of the instrumental conditions towards to the observed element in spICP-MS analysis leads to an increase in operating sensitivity by factors of 2 for Ag and 3 for Ti.
- For the first time, the possibility of characterizing TiO_2 NPs with a cubic shape is shown. The selection of an adequate mathematical model for size calculation is related to preliminary verification of the shape of NPs by microscopic techniques.
- For the first time a systematic study was conducted about the influence of carrier solvents, washing reagents and homogenization approaches on the reproducible introduction of Ag NPs from dilute suspensions.
- Calibration by silver ion standard solutions is used to calculate the analytical characteristics of other Ag-based nanomaterials (AgCl, AgI, Ag_2O Ag_2S). It is shown that the size detection limits and resolution of the spICP-MS method strongly depend on the mass fraction of Ag and the density of NPs material.
- For materials possessing surface plasmon resonance, the UV-Vis method enables obtaining preliminary information about the average diameter of the NPs in the sample, which allows refinement of spICP-MS analysis parameters.
- The developed spICP-MS method has been validated and successfully applied for the characterization of Ag NPs in real samples.

6. Evaluation of the scientific publications and the contribution of the PhD student

Two articles have been published on the subject of the dissertation in refereed scientific journals with an impact factor, in which Lidia Kainarova is a co-author:

1) V. Stefanova, L. Kaynarova, D. Georgieva, „*General characteristics of silver nanoparticles analysis by Single Particle Inductively Coupled Plasma Mass Spectrometry"*, Bulgarian Chemical Communications 51(D):77-84 (Q4; IF 0.398)

2) L. Kaynarova, D. Georgieva, V. Stefanova, „*An approach to estimate the contribution of signal noise to the diameter uncertainty of individual silver nanoparticles and resolution of spICP-MS analysis",* Journal of Analytical Atomic Spectrometry, 2022, 37, 1484–1500, DOI: 10.1039/d2ja00039c (Q1; IF 4.351).

On the recommendation of the editor and reviewers, the publication in the prestigious journal of the Royal Society of Chemistry – JAAS, is featured on the cover of vol. 37, issue 7, 2022 and included in the thematic collection „JAAS HOT Articles 2022".

Research results have been presented in 7 posters and 5 oral reports at scientific conferences and seminars in Bulgaria and abroad.

Lidia Kainarova is a participant in the research teams of two scientific projects that are related to the topic of the dissertation:

1) DN19/9 2017-2020 "Investigation of nanoscale materials through innovative spectrochemical analyzes (INISA)", financed by the National Science Fund.

2) National program "Young scientists and postdoctoral fellows - 2020", financed by the Ministry of Education and Science

7. Abstract

The abstract systematically, correctly and clearly represents the main achievements and contributions of PhD thesis of **Lidia Kaynarova** and meets the requirements of the Regulations of the Plovdiv University "Paisii Hilendarski"

8. Critical remarks and recommendations

I have no critical remarks concerning the PhD-Thesis, the abstract, and the materials presented by PhD student. Given the topicality of nanomaterials research, I recommend Lidia Kaynarova to continue research in the field of mass spectrometry with inductively coupled plasma, as a method for characterizing new types of nanomaterials and single cells.

CONCLUSION

The PhD-Thesis contains ***scientific and applied results which are original contributions to science*** and **meet all the requirements** of the Law for Development of Academic Staff in the Republic of Bulgaria, the Regulations for implementation of the Law for the development of the academic staff in the Republic of Bulgaria and the respective Regulations of the Plovdiv University “Paisii Hilendarski”. The presented materials and the results obtained completely correspond to the specific requirements of the Faculty of Chemistry accepted according to the Regulations of Plovdiv University “Paisii Hilendarski”.

The PhD thesis **demonstrates** that **Lidia Ivanova Kaynarova** has acquired in-depth theoretical knowledge and professional skills on the field of Analytical chemistry which shows that she has developed qualities and skills for planning, conducting, and reporting independent scientific research.

Due to the above, I am convinced to give my **positive assessment** to the research presented by the above reviewed PhD-Thesis, abstract, results, and contributions, and ***I propose the esteemed scientific jury to award the educational and scientific degree "Doctor"*** to **Lidia Ivanova Kaynarova** in the field of higher education: **4. Natural sciences, mathematics and informatics;** professional field **4.2. Chemical sciences**; doctoral program **Analytical Chemistry**.

07. 09. 2022 г. Reviewer: .....

/Assoc. Prof. Dr. Violeta Stefanova/