R E V I E W

Concerning: competition for the occupation of the academic position "PROFESSOR" in professional field 4.3. Biological sciences (Microbiology and antibacterial effect of nanomaterials), for the needs of the Department “General and Industrial Microbiology”, presented to a scientific jury, formed by order No. RD-38-93/14.02.2024 of the Rector of Sofia University "St. Kliment Ohridski"

Reviewer: Prof. Maria Angelova-Dyankova, DSc, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Scientific area 4. Natural Sciences, Mathematics and Informatics, 4.3. Biological sciences, Scientific specialty Microbiology

For participation in the announced competition (State Gazette, no. 103 of 12.12.2023) a single candidate Associate Professor Iliana Atanasova Ivanova, PhD, from the Department of General and Industrial Microbiology of the Biological Faculty of the Sofia University "St. Kliment Ohridski" has submitted documents. The set of materials submitted by the candidate is under the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB) and the Regulations of SU.

I. General presentation of the candidate

Associate Professor Iliana Ivanova graduated from the Faculty of Biology at the University of St. Kliment Ohridski", majoring in "Molecular and Functional Biology" in 1986, after which she worked successively as a Biologist at the Institute of Genetics at the BAS and the Department of Plant Physiology at the Biological Faculty at SU. In the period 1989 - 1994, the candidate was a doctoral student at the Faculty of Biology, SU. In 1994, she defended her doctoral thesis on the topic "Microflora of the Tyulenovo oil field and possibilities for its practical application" and received the scientific and educational degree "Doctor". Her scientific career began in 1997, first as an Assistant, and then as a Senior and Senior Assistant Professor in the Department of General and Industrial Microbiology of BF, SU. Since 2014, Dr. Ivanova is an Associate Professor in the same department.

II. Fulfillment of the requirements for occupying the academic position

II.1. General characteristics of the works presented

For the competition, the candidate Assoc. Prof. Dr. I. Ivanova submitted 21 scientific papers, 1 book chapter, 1 stand-alone monograph, and 1 textbook for students. All works are outside the dissertation for ONS "Doctor" and the scientific title "Associate Professor". Of the 15 scientific articles, 13 are in editions referenced and indexed in Web of Science and Scopus and 2 are outside this classification (G7- №№. 13 and 14). In my opinion, these two articles cannot be included in the list of minimum national requirements. The competition documents include 6 more articles that are referenced but not indexed. They also include the results of Assoc. Prof. Ivanova's activity,

and can be reviewed, but without being included in the criteria regarding the requirements of the LDASRB.

Most of the articles are in renowned and specialized scientific journals, with corresponding quartiles such: Materials (Basel) Q2, IF 3.4; Chemical Papers Q2, IF 2.146; Coatings Q2, IF 4.158; Archives of Microbiology Q2, IF 2.552; Acta Chimica Slovenica Q3, IF 1.524, etc. All articles from criterion D7, as well as the book chapter (D7-16) are in international editions and are printed in English. The monograph and the textbook have been published in Bulgarian. Results of the Assoc. Prof. Ivanova have resonated among the international scientific community, they have been cited 165 times and have an h-index of 8 (Scopus). The total Impact Factor of all articles is 25.597, and of the articles submitted for the competition is 21.53.

II.2. Fulfillment of the requirements according to the individual criteria

The comparative analysis of the materials submitted for participation in the competition in accordance with the requirements of LDASRB and its regulations and the additional requirements in the regulations of SU "St. Kliment Ohridski" for the implementation of the LDASRB is reflected in the table.

Group of Indicators	**Contents**	**Requirements for a professorship**	**Applicant data**
Indicator A	Successfully defended thesis for ONS and awarded the degree of "Doctor"	**50**	**50**
Indicator C4	Stand-alone monograph: "Biological Effects of Nanomaterials"	**100**	**100**
Indicator D7	13 scientific papers (Q2 - 6; Q3 – 7) and 1 Book chapter	**200**	**240**
Indicator E	165 citations (Scopus)	**120**	**330**
Indicator F total		**150**	**175**
Indicator F14	Supervisor of 1 successfully defended PhD student		25
	Participation in national scientific or educational projects 3 x 10		30
	Participation in international scientific projects with national co-financing 4 x 20		80
Indicator F19	Published university textbook		40
Total points		**600**	**895**

As can be seen from the reference made, Assoc. Prof. Dr. Iliana Ivanova meets and exceeds the requirements of the LDASRB, as instead of the required 600 points, she presents evidence for 895 points. In addition, the candidate also fulfills the additional criteria of SU "St. Kliment Ohridski".

III. Evaluation of the teaching activity

Teaching work is one of the main activities of the candidate and is entirely within the field of the competition. It can be analyzed in several aspects – as a teacher, as a compiler of study

programs and teaching aids, and as a mentor of young staff. Assoc. Prof. Iliana Ivanova has been a teacher for 27 years. She teaches students in the Bachelor and Master Programs in the BF and FCP of SU and teaches a summer seminar to doctoral students. In BP Ivanova conducts lectures on General Microbiology with Virology, Microbiology, Biotechnology, Bioethics and Ecotoxicological tests for Environmental Control. She participates as a teacher in the Master's Program with the courses "Sanitary Microbiology" and "Physico-Chemical and Biochemical Food Control". In numerical terms, the total annual workload of the candidate for the last 5 years varies between 648 and 844 hours (752 hours on average), with the classroom occupancy being 446 hours on average.

Dr. Ivanova's activity in this section also includes the development and launch of new lecture courses and a new cycle of exercises, namely: Physico-Chemical Control of Food and Food Products for the MP "Food Quality and Safety"; Sanitary Microbiology for MP "Microbiology and Microbiological Control"; Nanomaterials and interaction with cells for MP "Microbiology and Microbiological Control" and PhD students. Here are also added the exercises in Microbiology and Virology for Masters in "Pharmacy" in English (regular) at FCP, as well as a Summer Scientific Seminar for Masters in Microbiology and Microbiological Control. I also want to note her activity in the preparation of tests for state exams.

At the same time, Assoc. Prof. Ivanova is very intensively involved in the training of young staff. She is the supervisor of 12 graduates who have successfully defended their degrees in the bachelor's and master's program in the Faculty of Arts of the University of Warsaw, as well as 1 successfully defended doctoral student.

This section also includes the textbook "Ecotoxicology and Nanotechnologies" presented by the candidate and the monograph "Biological Effects of Nanomaterials", which can be used by doctoral students and students in universities or teachers in secondary schools. The textbook offers students historical data on the formation of the science of ecology, its development, and its prospects. A number of basic concepts from ecology and ecotoxicology approach to their determination, examples of the transformation of used substances in living organisms and in the environment are explained here. A very useful idea is to include relevant international standards. It is also interesting to acquaint the audience with indicator organisms and their use. Protocols of various experiments illustrated with photographs are added to the textbook.

Based on the above, I highly appreciate the educational and teaching activity of the candidate, I consider it to be significant in volume, cover important directions in the field of the announced competition, and fulfill the mission of a university teacher.

IV. Characterization of the presented scientific works and evaluation of scientific and applied contributions

All scientific works presented for the competition correspond to its theme. They are focused on a very current and promising problem. Today's era is considered to be the era of nanotechnology. Nanomaterials have gained great importance in the fields of technology, engineering, and medical development due to their tunable physical, chemical, and biological properties. From an economic point of view, they are also of interest, as the size of the global market for nanomaterials is constantly expanding. For 2027, it is predicted to reach over $14

billion. Due to the growing interest in nanomaterials and their versatile applications, it is very important to study their biological properties, such as antimicrobial activity, pro-, and antioxidant ability, their impact on microbial biofilms, their involvement in the fight against antibiotic resistance, etc. Assoc. Prof. Ivanova's scientific and research activity is aimed precisely at these current problems. Her scientific works submitted for the competition can be grouped and analyzed in the following 4 sections:

IV.1. Reviews on the biological effects of nanomaterials

This section includes the monograph from criterion C4 and articles D7-8 and D7-15. The presented monograph analyzes the data published so far in several aspects: (i) the methods for creation of nanoparticles, including biological ones using bacteria, fungi, plants, and their extracts; (ii) the antimicrobial properties of nanomaterials with the presence of metal ions and non-metallic nanocomposites; (iii) the mechanisms of interaction between nanoparticles and bacteria; (iv) the effect of nanoparticles on the expression of a number of genes from the bacterial genome; (v) the importance of biodegradable nanomaterials for the pharmaceutical industry, as well as (vi) the ethical issues regarding their safe application. The monograph is compiled based on 426 literary sources, more than 25% of which are from the last 5 years, which emphasizes the relevance of the material. The historical data, as well as the Bulgarian experience in this direction, are also very useful. However, I would like to point out that the section "Mechanisms of interaction of nanoparticles with bacteria" is not developed to the same extent as the other sections. Here, the sub-sections "Membrane disruption", "Metal ion toxicity" and especially "Nanomaterial-induced free radical toxicity (oxidative stress)" are only hinted at. I recommend the author to develop them further in the next release.

This section outlines the following contributions:

1. A large volume of data on medically relevant nanomaterials has been summarized and analyzed, offering the scientific community and the student audience information on one of the most pressing issues of today.

2. Valuable information is presented regarding the methods for obtaining biologically active nanomaterials.

3. Material is presented that contributes to the clarification of:
   - the impact of nanomaterials on cells with different structural organization;
   - the mechanisms of action of hydrophobic nanomaterials on living cells;
   - the mechanisms of action of nanoparticles at the gene level.

4. Author's contributions are:
   - the established photodynamic effect of silver nanoparticles on titanium dioxide coatings, which enhances the antibacterial effect on Gram(-) bacteria.
   - the created composition of coatings for medical devices, antibacterial surfaces, and hospital clothing to reduce the spread of nosocomial infections.

IV.2. Investigation of the antimicrobial effect of newly synthesized nanomaterials and nanocomposites (monometallic, metal oxide and non-metallic)

Nowadays, drug resistance is emerging as a global threat to the treatment of microbial infections. This phenomenon is not only a serious complication in medical practice but is also one of the greatest challenges for public health worldwide. It also sets new goals for scientists – the development of new antibacterial strategies and new therapeutics. Among them are nanotechnologies, which have proven to be a promising tool for creating new therapies and for the advanced treatment of various diseases. Dr. Ivanova's works are a contribution in this direction. Articles D7-1, D7-3, D7-5, D7-6, D7-7, D7-9, D7-10, D7-11, D7-16, D7-17, D7-18, D7- 19, D7-21 (numbering is in accordance with Appendix 16), as well as reports and posters from scientific forums (№№ 1, 3, 4, 6, 7, 9, 14, 15, 17). They reflect the largest part of the results submitted for the competition. They are aimed at finding new drugs based on nanoparticles to combat drug resistance. The candidate presents data on the antimicrobial properties of a large number of nanoparticles with different compositions. Here have to be mentioned nanoparticles with metal ions or metal oxides such as e.g. Ag, Se, Au, TiO_2, Fe_2O_3, SiO_2, GO, etc., effective against Gram (+) and Gram (-) bacteria and yeast (*Candida lusitaniae*). The created graphene nanocomposites with added zinc oxide, silver and copper nanoparticles are effective against the bacteria *Staphylococcus epidermidis* and *Escherichia coli* and against cancer cells. Broad-spectrum antibacterial activity was found using the porous collagen/($ZnTiO_3$/SiO_2)/fucoidan composite, ZnO nanocluster blocks and silica-coated ZnO nanoparticles (ZnO-SiOA, ZnO-SiOB). Also of interest are the benzimidazole complex with silver, collagen/$ZnTiO_3$, collagen/RGO, collagen/(Ag/RGO), collagen/(Ag/RGO/SiO_2), and collagen/($ZnTiO_3$/SiO_2) composites, as well as Fe_3O_4 and TiO_2 nanoparticles, and talicarpine. I want to emphasize that the newly created materials have been studied in many ways. Data are presented on their morphology (with electron microscopy), on their physical, chemical, cytotoxic, and chemiluminescent properties, on their impact on cancer cells, and on their pro- and antioxidant action. I appreciate the activity of Prof. Ivanova in this section as large-scale and multidisciplinary. Based on a large number of newly created nanomaterials of medical importance, original contributions have been made in theoretical and applied aspects.

The most important of them are the following:

1. A very detailed characterization of the newly created nanoparticles and composites has been made in terms of structure, morphology, and impact on bacteria, yeast, and cancer cells.

2. The antimicrobial effect of newly created nanomaterials with different compositions and physico-chemical properties has been proven.

3. Increased antibacterial activity of polymer-stabilized nanoparticles has been found.

4. The bactericidal effect of the particles depends on the ratio of the components in them and on the administered dose.

5. Comparative evaluation of commercial nanoparticles (selenium, gold, iron oxide, silicon oxide, and graphene oxide) in the form of dispersions proves their dose-dependent antibacterial effect.

6. New collagen/(ZnTiO3/SiO2)/fucoidan composites with broad antimicrobial effect against bacteria and yeast have been created.

7. Data were obtained on the relationship between the antibacterial activity and the pro-oxidant nature of the nanoparticles.

8. The bactericidal and cytotoxic effect of nanomaterials depends on the method and starting materials for synthesis, production, and storage conditions, their structure and size.

IV.3. Prevention of microbial adhesion and biofilm formation

A serious part of Assoc. Prof. Ivanova's works is related to the study of the effect of nanomaterials on the adhesive ability of bacteria and their ability to form biofilms (articles D7-2, D7-4, D7-7 and reports 11, 18, and 20). I highly appreciate the candidate's activity and large volume of experimental work in this direction. Moreover, infections of medical implants and devices are one of the most common and dangerous complications in medical practice. Their treatment is a great challenge, and relapses are common. Microorganisms that have formed biofilms exhibit a greatly reduced sensitivity to antimicrobial agents compared to single bacterial cells. For example, biofilms older than 7 days are 500 to 5000 times less susceptible to bactericidal action. The reasons are many, including biofilm structure, reduced growth rate, the presence of drug-degrading enzymes in the matrix, the expression of genes for antioxidant defense, the presence of genes for the horizontal transfer of antimicrobial resistance, etc. The aim of the research in this section is to characterize the antibacterial properties of non-toxic natural compounds concerning biofilm formation. Results were obtained on the effectiveness of fucoidan (a polymer from brown algae) in inhibiting the growth of bacteria on medical coatings. Its action on bioadhesion was found to be more significant in Gram-positive than in Gram-negative bacteria. The candidate and her partners have found an unexplored niche in the problem of adhesion and biofilms. They have created medical coatings with antioxidant substances included in siloxane - natural and synthetic, active against monospecies biofilms (*Marinobacter hydrocarbonoclasticus*). The results show that effective antioxidants reduce adhesion by changing the structure of siloxane composites. Ag and Cu doped TiO2/Ag/Cu magnetron coatings were developed. These coatings demonstrate a broad spectrum of antimicrobial activity against Gram-negative (*E. coli*, *P. aeruginosa*) and Gram-positive (*P. putida, B. cereus, S. epidermidis*) bacteria and provide protection for at least 12 hours.

The contributions made can be formulated as follows:

1. New coatings for medical purposes based on natural and non-toxic substances have been developed. They inhibit the adhesion of bacteria and yeast and the formation of biofilms for a long period.

2. New fucoidan-containing anti-adhesive composites have been developed for the first time.

3. For the first time, anti-adhesive composites have been created with the addition of antioxidants to siloxane polymers.

4. The mechanism of action of the new composites has been studied. The relationship between their effectiveness and the change in the vulcanization network has been established.

5. An effective method for obtaining composites for medical purposes by magnetron sputtering has been established. The resulting coatings exhibit high antibacterial activity through direct contact and elution of Ag and Cu ions.

IV.4. Creation of biologically active thin films with antimicrobial action

Antibacterial thin coatings deposited on biomedical devices and implants can eliminate or reduce bacterial colonization on surfaces, thereby preventing nosocomial infections. They have the potential to play a key role in infection management in healthcare settings. This is an argument for increased interest in creating methods to limit antibacterial infections by minimizing bacterial growth in hospital settings. The efforts of scientists in the last decade have been focused on the development of new antibacterial materials for medical devices or medical equipment. In this direction, the activity of Assoc. Prof. Ivanova is focused in the 4th section. The included papers (Articles D7-13, D7-14, D7-20, and Reports 12, 13, and 21) are a continuation of her work on the problem of resistant microorganisms. Of interest are studies on the antibacterial effect of TiO2:SiO2:Ag thin layers. A very good idea is the comparative analysis of their impact on a resistant strain of *Escherichia coli* (highly resistant industrial) and a sensitive strain of *Pseudomonas putida*. The advantage of thin films is that they are produced without heating by magnetron sputtering of TiO2 on small wafers of quartz and Ag on the surface in the zone of maximum erosion. The data show complete inhibition of bacterial growth in the first 1-2 hours of treatment. The antibacterial and cytotoxic effects of TiO2:Ag:Cu thin films were studied. An antibacterial effect on *E. coli*, *Salmonella entherica*, *Staphylococcus epidermidis*, and *Bacillus cereus* was proven by diffusion analysis. The useful methods of microbiological staining and observation with a phase-contrast microscope prove the changes in the morphology and the degree of cytotoxicity of the new agents. Thin films composed of TiO2:Ag:Cu have the most pronounced bactericidal effect against *S. epidermidis* and *B. cereus*, which is maintained at 100% even after 24 hours of treatment. The obtained results are the basis of application in medical practice and everyday life.

I consider the more important contributions in this section to be the following:

1. A method for obtaining effectively acting thin films characterized by a number of advantages has been developed:
   - the process is carried out at room temperature and is easily scaled up;
   - the resulting films are long-lasting;
   - the produced antimicrobial film can be stuck on different contact surfaces;
   - magnetron sputtering ensures uniform application on various medical devices such as catheters, drainage tubes, etc.

2. A synergistic effect of the copper and silver nanoparticles encrusted on a titanium dioxide coating has been established.

3. An increase in the antimicrobial effect has been proven when copper nanoparticles are replaced with silicon dioxide ones.

4. The resulting thin films are suitable for use in hospitals, public facilities, in the food and pharmaceutical industry.

5. Application of these thin films has a potential to reduce surgical and inpatient infections.

V. Participation in research projects

The candidate presents information on participation in 7 research and educational projects, 3 of which are national and 4 - international with national co-financing. Assoc. Prof. Ivanova is a member of the team in 6 projects and 1 she is the leader. Here I would like to mention the European Network of Multidisciplinary Research to Improve the Urinary Stents (Action DKOST SA 16217 01/14/16.08.2018); Improved protection of medical devices against infections (DKOST Action TD 1305 CA 15216 01/10/2017); European bio-adhesion network (COST Action TD D-COST 15216, 100116/4 dated 24.03.2017) and Science and Education for Smart Growth 2017-2019 "Nanomaterials and interactions with cells" BG05M2OP001-2.09-0013. All of them are in the field of the announced competition and concern its main areas of research and teaching.

VI. Critical remarks and recommendations

In addition to what was noted above in item IV.1, I would like to add the following:

1. In article #11, Fenton's reaction is incorrectly written.

2. Article No. 16 is entitled "Stress Response of Gram-Positive and Gram-negative Bacteria Induced by Metal and Non-Metal Nanoparticles". The conducted experiments do not concern the determination of the level of oxidative stress. The authors speculate in the discussion that, according to literature data, nanoparticles containing iron or selenium are involved in the generation of free oxy-radicals. Biomarkers of stress, free radical levels, and antioxidant defenses must be determined here to set this title. I believe that in this case the title completely contradicts the content of the article.

I note these inaccuracies with the presumption of being helpful to the applicant for her future employment.

VII. Personal impressions

I have known Assoc. Prof. Dr. Iliyana Ivanova for a long time, and from my contacts with her I have the impression that she is an active teacher and scientist in the field of modern areas of microbiology and a sought-after partner for joint projects. Its subject matter is modern, up-to-date and with serious potential for application.

VIII. Conclusion

The documents and materials presented by Assoc. Prof. Dr. Iliyana Ivanova meet the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for the Implementation of the LDASRB, and the relevant Regulations of Sofia University. The candidate has a sufficient number of scientific works for the competition, published after the materials used in the defense of the ONS "Doctor" and the academic position "Associate Professor". The results achieved in the educational and scientific research activities fully correspond to the minimum national and additional requirements of the SU, adopted in connection with the application of the LDASRB.

I want to emphasize that the teaching activity of Assoc. Prof. Iliyana Ivanova, PhD is in the field of the current competition and corresponds to the current requirements of higher education. She is the author of study programs, and teaching aids, and works actively with graduates and doctoral students. Assoc. Prof. Ivanova is a sought-after partner in the development of scientific projects and an active member of the teams she works with. The presented scientific works define

her as a professionally competent specialist. They have been published in reputable publications and have become known to our and the international scientific community. Formulated scientific and applied contributions are a basis for further developments.

After getting acquainted with the materials and scientific works presented in the competition, after analyzing their significance and the scientific and scientific-applied contributions contained in them, I give my positive assessment and strongly recommend the Scientific Jury to prepare a report-proposal to the Faculty Council of the Faculty of Biology for selection to **Assoc. Prof. Dr. Iliana Atanasova Ivanova** at the academic position "**PROFESSOR**" at the Sofia University "St. Kliment Ohridski" by the field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.3. Biological Sciences, Scientific specialty Microbiology.

April 9, 2024
Sofia

Reviewer: ……
/Prof. Maria Angelova, DSc/