

REVIEW

By Prof. Sonya Kostadinova Trifonova, PhD

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of the materials submitted for participation in the competition

for the academic position "**Professor**"

in Sofia University “St. Kliment Ohridski”

field of higher education 4. Natural sciences, mathematics, and informatics

professional field 4.3. Biological Sciences (Microbiology - Antibacterial effect of nanomaterials)

In the competition for "Professor", announced in the State Gazette, No. 103 from 12.12.2023 and on the website of Sofia University "Kliment Ohridski" for the needs of the Department of "General and Industrial Microbiology" at the Faculty of Biology, the only candidate participating is Associate Professor Dr. Iliana Atanasova Ivanova from the Sofia University "St. Kliment Ohridski".

General presentation of the materials

By order No. RD-38-93 of 14.02.2024 of the Rector of Sofia University "St. Kliment Ohridski" (SU) I have been appointed as a member of the scientific jury of a competition for occupying the academic position of "**Professor**" at the Sofia University "St. Kliment Ohridski" in the field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.3. Biological Sciences (Microbiology - Antibacterial effect of nanomaterials). Only one candidate has submitted documents to participate in the announced competition: Associate Professor Dr. Iliana Atanasova Ivanova at the Faculty of Biology, Sofia University "St. Kliment Ohridski".

The set of electronic materials presented by Assoc. Prof. Dr. Iliana Ivanova is in accordance with the Law on the Development of the Academic Staff of the Republic of Bulgaria, the Regulations for its implementation and the Regulations on the terms and conditions for acquiring academic degrees and occupying academic positions at Sofia University "St. Kliment Ohridski".

The total scientific production of Assoc. Prof. Dr. Iliana Ivanova includes 68 publications (including scientific publications indexed in Web of Science & Scopus – 36 issues), 1 dissertation abstract, 1 monograph, 1 textbook, 30 participations with reports and posters in national and international scientific forums.

The total impact factor of the presented scientific production is over 31.6 and the citations are 165. The Hirsch factor of Assoc. Prof. Dr. Iliana Ivanova is 8, according to Scopus data.

Brief biographical data of the applicant

Assoc. Prof. Dr. Iliana Ivanova was born on July 29, 1959 in the city of St. Zagora. She graduated in 1986 as a Master's degree in Biochemistry and Microbiology at Sofia University "St. Kliment Ohridski". In 1994 she successfully defended his doctoral thesis "Microflora of the Tyulenovo oil field and opportunities for its practical application" and received PhD. From 1986 to 1989, she worked as a specialist-biologist at the Institute of Genetics and Algology of the BAS and the "Plant Physiology" department of the SU, and in the period 1994 - 1997, as an ecologist-biotechnologist. Since 1997, she has successively held the academic positions of assistant (1997-1999), senior assistant (1999-2002), chief assistant (2002-2014). Since 2014, she has been an Associate Professor at the Sofia University "St. Kliment Ohridski".

Evaluation of the candidate's scientific and scientific-applied activity

In the competition for the academic position "Professor", the candidate Assoc. Prof. Dr. Ivanova has submitted a total of 22 publications (15 of which have been published in journals, refereed and indexed in world databases), 1 monograph, 1 book chapter, 1 textbook, which have not been reviewed in previous competitions for awarding degrees or academic positions. The distribution of scientific publications referenced and indexed in world databases is as follows:

- Q2 – 6 pcs.;
- Q3 – 8 pcs.;
- 1 article is in a journal without quartile;
- 1 chapter of a book.

All publications are in English. In four of the publications, Assoc. Prof. Dr. Ivanova is the first author, in six – second, and in three publications – last author, which shows her leading role as a researcher in more than half of the publications. Additionally, 6 publications are presented, which are referenced but not indexed.

Assoc. Prof. Dr. Ivanova is a supervisor of the PhD course "Nanomaterials and Interaction with Cells" at a project funded under the Operational Program "Science and Education for Smart Growth 2017-2019" (BG05M2OP001-2.09-0013 "Doctoral Center "St. Kliment Ohridski"). She is a participant in 7 projects funded by the National Science Fund at the Ministry of Education and is also a leader of three and a participant in two institutional projects of Sofia University. These activities show that Assoc. Prof. Dr. Ivanova has the necessary qualities for organization and management of research.

The presented scientific production and the scientific data of Assoc. Prof. Dr. Ivanova fully comply with the minimum requirements for awarding the academic position "Professor" in professional field 4.3. Biological sciences, defined in the Law on the Development of the Academic Staff of the Republic of Bulgaria and in the Regulations on the Terms and Conditions for Acquiring Scientific Degrees and Occupying Academic Positions at Sofia University:

Indicator	Minimum requirements	Assoc. Prof. Dr. Iliyana Ivanova
A. Indicator 1. Dissertation for awarding the NSA "Doctor"	50 pts.	50 pts.
B. Indicator 4. Habilitation work	100 pts.	100 pts.
G. Indicator 7. Scientific publication in publications that are referenced and indexed in world-renowned databases with scientific information (Web of Science and Scopus) Indicator 8. Chapter of a book	200 pts.	Total 255 pts. Q2: 6 x 20 pts. Q3: 8 x 15 pts. 1 x 15 pts.
Д. Indicator 10. Citations in scientific publications, referenced and indexed in world-renowned databases of scientific information 1 quote = 2 pts.	100 pts.	314 pts. 157 citations x 2 pts.
E. Indicators from 12 to 20.	150 pts.	155 pts.
Total points	600 pts.	874 pts.

Evaluation of the candidate's scientific activity and contributions

The scientific interests of Assoc. Prof. Dr. Ivanova and the published scientific results are entirely in the field of the announced competition and their main focus is on establishing the antimicrobial activity of nanomaterials and nanocomposites, including newly synthesized ones, and elucidating the molecular mechanisms of their action.

Candidate Assoc. Prof. Iliana Ivanova presented a monograph "Biological Effects of Nanomaterials" (Publisher: Print Sofia, Bulgaria, ISBN: 978-954-9897-56-2, 137 pages), in which she is an independent author.

Nanotechnology is a rapidly developing field of modern science, with increasing application in various spheres of human activity - medicine, cosmetics, electronics, food industry, shipping, ecology, etc. In the field of medicine, nanomaterials can be applied as antimicrobial agents, as carriers for the delivery of medicinal substances in the body, for gene therapy, diagnosis and treatment of diseases, as well as for research and clinical application. The increasing number of pathogens with resistance to antibiotics used in practice is a major modern problem, with a great effect on public health, economy and ecology, which requires the search for new antimicrobial agents. This defines the topic in the scientific production of Assoc. Prof. Dr. Iliana Ivanova as relevant and with perspectives for application.

The monographic work is well structured. After a brief historical overview of the development of nanotechnology, physical, chemical and biological methods for the synthesis

of nanoparticles are reviewed. Logically, the main attention is paid to "green" synthesis, with the use of biological objects - bacteria, actinomycetes, yeasts, mold fungi and extracts of plants.

The main emphasis of the monographic work is on the antimicrobial action of nanoparticles, one of the aspects of which is the possibility of inhibition of biofilm formation through surface modification. The advantages of nanomaterials, such as antimicrobial agents, compared to antibiotics are outlined and the possibility of combining classic antibiotics with nanomaterials.

In the monographic work are systematized the molecular mechanisms of antimicrobial action of different types of nanoparticles and nanocomposite materials, which are related both to their physico-chemical properties and to the structural and physiological features of microorganisms. The complex action of metal nanoparticles (silver, copper, titanium, zinc, iron-oxide, aluminum, gold, etc.), metal oxide and graphene materials on microbial cells is clarified. Special attention is paid to the mechanisms of action of silver nanoparticles against various Gram-positive and Gram-negative bacteria, but also comment on the disadvantages of their use, which requires their combination with other materials. The author also includes results from his own studies on the resistance of two strains of *Escherichia coli* to thin silver films, and shows significant bacterial resistance due to the activation of efflux pumps. The results showed a higher sensitivity of *Bacillus cereus* compared to *E. coli* to mono- and bimetallic nanoparticles of starch-stabilized gold and silver, or deposited on submicron silica spheres, indicating the antimicrobial action is not only related to an effect on the bacterial cell walls.

The monograph also discusses the properties of non-metallic nanocomposites, paying attention to silicates and carbon nanotubes as promising carriers of metal and metal-oxide nanoparticles and their application. A detailed characterization of the physical properties of carbon nanotubes is made and emphasis is placed on the applications of metal-oxide composite materials with carbon nanofilaments in optoelectronics (lasers, photodetectors, optical modulators, waveguides and polarizers), for the storage of electrochemical energy (supercapacitors and batteries) and in the so-called the smart sensor systems that are used in biosensors, fuel cells, photovoltaic cells, hydrogen storage, catalysis and drug delivery. The properties of graphene materials, hypotheses about their antimicrobial action and their potential for biomedical applications are also analyzed. Own results of the antimicrobial action of developed RGO (reduced graphene oxide) composite versus Gram-positive (*Bacillus cereus*), Gram-negative (*E. coli*, *Salmonella*, *Pseudomonas*) bacteria and *Candida* are presented.

After the initial introduction to the nature of biofilms and the stages of their formation, the monographic work again comments on cellular and environmental factors on which biofilm formation depends. Special attention is paid to the effect of surface topography for the bacterial attachment. Presented own data with *E. coli*, *Listeria innocua* and *P. fluorescens* strains and test surfaces - nano-sized silica and alumina, which are generally recognized as safe (GRAS), and can be used in a wide range of biomedical and nutritional applications. The relationship between the topography of the substrate, the orientation of bacterial cells to the surface, and the number of attached cells is proven.

Another fundamental contribution of the monograph is the elucidation of the interaction of nanoparticles with bacterial cells. Metal nanoparticles (e.g. silver) induce changes in the

permeability of the cytoplasmic membrane, and the subsequent "leakage" of the cytosol, but also inhibit the activity of the enzymes of the electron transport chains, thereby blocking respiration and ATP synthesis. Nanoparticles can also generate free radicals that are highly reactive, can interact with cellular components such as DNA and proteins, and can initiate redox processes, ultimately resulting in cell destruction. The monographic work comments on the effect of nanoparticles on gene expression and presents its own results on the impact of silver and copper nanocompositions on the industrial strain *E. coli* ATCC 10536. Activation of copper oxidase expression and *cusA* and *cusF* genes related to Cu/Ag exposure and providing the protection of bacteria by "ejection" of copper ions from the periplasmic space and outer membrane was found. By simultaneous loading with copper and silver ions, their synergistic action and the use of the same efflux system for the export of both ions are demonstrated.

So far, no such in-depth analysis of the mechanisms of action of nanoparticles and nanomaterials, which is also linked to the possibility of their application, has been done in the literature.

The monographic work concludes with a commentary on the safety of nanomaterials. Risks from the mass application of nanotechnology and the need for adequate physico-chemical characterization and evaluation of the effect of nanoparticles in living organisms and the environment are outlined/ indicated.

The monographic work includes own, unpublished data and has fundamental theoretical contributions, with the potential for practical application, which is why I highly appreciate it.

The publications presented in Group Γ (15 publications and 1 chapter of a collective monograph) summarize results that can be attributed to three main directions, which are related to each other and to the theme of the monographic work.

The first thematic area includes the topic of antimicrobial action of metallic, metaloxide and non-metallic newly synthesized nanomaterials. Contributions of theoretical and applied importance in this scientific field are:

- Synthesis of new nanomaterials - ZnO-nanocluster blocks, nanocomposites of reduced graphene oxide and combinations with Ag and Cu; collagen nanocomposites based on graphene, graphene oxide, ZnO and metal nanoparticles, collagen/reduced graphene oxide (RGO):ZnO:TiO₂, etc. (publications Γ 7.1., Γ 7.5., Γ 7.6., Γ 7.10.);
- Analysis of the antimicrobial effect of nanoparticles (Se, Au, Fe₃O₄, TiO₂, ZnO, SiO₂, graphene oxide) and nanomaterials against Gram-positive (*S. aureus*, *S. epidermidis*, *B. cereus*), Gram-negative bacteria (*E. coli*, *S. enterica*, *P. putida*) and yeast (*Candida*, *Saccharomyces*) (publications Γ 7.1., Γ 7.3., Γ 7.5., Γ 7.6., Γ 7.10., Γ 7.11., Γ 7.13.);
- Demonstration of the prooxidative and antioxidant activity and cytotoxicity of nanomaterials (publications Γ 7.5; Γ 7.6., Γ 7.11.);
- Elucidation of the antimicrobial action mechanisms of nanoparticles and nanocomposites that make them a possible alternative to antibiotics (publication Γ 7.8.);
- A description of the applications of nanomaterials (publications Γ 7.8., Γ 7.16.).

The second scientific thematic direction is related to the inhibition of adhesion and biofilm formation by nanocomposites (publications Γ 7.2., Γ 7.3., Γ 7.4., Γ 7.12). The following main contributions can be mentioned in this thematic area:

- Novel composites incorporating fucoidan and antioxidants into siloxane polymers have been developed that have anti-adhesive properties and inhibit biofilm formation;
- The ability of fucoidan to inhibit the growth of Gram-positive and Gram-negative bacteria on medical siloxane coatings of catheters and implants has been established for the first time;
- For the first time, the effect of 6 compounds with antioxidant activity in the composition of siloxane coatings on biofilm formation by the bacterium *Marinobacter hydrocarbonoclasticus* was investigated. Different antioxidants have been shown to alter surface characteristics, resulting in different effects on bioadhesion and bacterial growth. Ethylcinnamate at a concentration of 2 wt.% has an inhibitory effect, while L-ascorbic acid and dodecyl gallate stimulate biofilm formation on low-adhesive siloxane coatings.

The third thematic area in the scientific production of Assoc. Prof. Dr. Ivanova is preparing thin films with antimicrobial action. The films obtained by magnetron sputtering have different compositions and ratios between nanoparticles (publications Γ 7.7., Γ 7.14., Γ 7.15.). Their antimicrobial activity against Gram-positive and Gram-negative bacteria and cytotoxic activity were analyzed. A complete inhibition of bacterial growth on thin films of TiO₂:Ag:Cu was reported. The developed thin films have potential for application as an antibacterial coating for various medical devices and surfaces.

Significance of the contributions and reflection of results in the works of other authors

The presented citations of the candidate's works are a total of 165, of which 157 are in publications referenced and indexed in world-famous databases of scientific information (Scopus and Web of Science). The cited citations are in the field of scientific activity of Assoc. Prof. Dr. Ivanova, the citing authors are mainly foreign. The recognition of the candidate among the scientific circles, based on the above citations, is indisputable.

Assessment of educational and pedagogical activity

Assoc. Prof. Dr. Iliana Ivanova is also engaged in active teaching. Her average annual classroom time over the 5-year period (academic years 2017/2018 - 2021/2022) is 419 hours. She led lectures on the disciplines "General Microbiology with Virology", "Microbiology", "Bioethics", "Ecotoxicological Tests for Environmental Control" to students from Bachelor programs and "Sanitary Microbiology", "Physico-chemical and Biochemical Control" (compulsory) to students from Master programs.

Assoc. Prof. Dr. Ivanova has developed 3 new lecture courses - "Physico-chemical control of food and food products (45 hours; for the Master's programme "Food Quality and Safety"); "Sanitary Microbiology" (30 hours; for the Master's program "Microbiology and Microbiological Control"); "Nanomaterials and interaction with cells" (30 hours; for PhD students from all specialties of Sofia University), as well as has developed and conducted new exercises in the disciplines "Microbiology and Virology" (in Bulgarian and English), to students

from the speciality "Pharmacy", and "Physical and Chemical Control of Food and Food Products", and "Summer Scientific Seminar" – to students from the Master's degree programs.

Assoc. Prof. Dr. Ivanova is the author of the textbook "Ecotoxicology and Nanotechnologies" (224 pages; University Press "St. Kliment Ohridski"), which can be used by students of all specialties of the Faculty of Biology of the University of Sofia, but also by other students studying biological sciences

In the period of the 2017/2018 – 2021/2022 academic years, Assoc. Prof. Dr. Ivanova is the supervisor of 12 graduates in the Bachelor's and Master's Degree and co-leader of 1 successfully defended a PhD student in 2019. Currently, she is the supervisor of an active doctoral student.

Assoc. Prof. Dr. Iliana Ivanova is a lecturer with intensive educational and teaching work in the field of Microbiology, including the development and implementation of new lecture courses and practical classes, publication of a textbook for the preparation of students and scientific management of graduates and PhD students.

Critical remarks and recommendations

I have no critical remarks or recommendations to the candidate's scientific works and activities.

CONCLUSION

The documents and materials presented by Assoc. Prof. Dr. Iliana Atanasova Ivanova meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and its Regulations for the Implementation and the relevant Regulations of SU "St. Kliment Ohridski".

The candidate in the competition **has submitted a sufficient number** of scientific works published after the materials used in the defense of the PhD and occupying the academic position of Associate Professor. The candidate's works contain original scientific and applied contributions that have received international recognition, as a representative part of them are published in journals issued by international academic publishers. The theoretical developments have practical significance and the possibility of application. The scientific and teaching qualification of Assoc. Prof. Dr. Iliana Ivanova is **undoubted**.

After reviewing the materials and scientific works presented in the competition, analyzing their significance, scientific, methodological, and applied contributions, I find it reasonable to give my **positive** assessment and **to recommend** the Scientific Jury to prepare a report-proposal to the Faculty Council of the Faculty of Biology for the election of Assoc. Prof. Dr. Iliana Atanasova Ivanova to the academic position "Professor" at Sofia University "St. Kliment Ohridski" in: field of higher education 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological Sciences (Microbiology – Antibacterial effect of nanomaterials).

08.04.2024

Reviewer:
(Prof. Sonya Kostadinova, PhD)