REVIEW

by

Dobromir Dimitrov Enchev, doctor, professor, Shumen University "Bishop Konstantin Preslavski"

Member of the Academic Jury set to render a decision

on the competition for filling the academic position of an Associate Professor /Professor in the Professional Field 4.2. Chemical Sciences according to the Classifier of the Areas of Higher Education and the Professional Fields (Scientific Specialty “Polymers and Polymer Materials”)

This Peer Review is prepared in response to Order № РД-09-28 of 21.02.2023.
issued by the Director of the Institute of Polymers, Bulgarian Academy of Sciences, following the decision made by the Academic Jury that was held on 02.03.2023.
The Review is in compliance with *Development of Academic Staff in the Republic of Bulgaria Act (DASRB), the Rules for the Application of the Development of Academic Staff in the Republic of Bulgaria Act, the Rules of BAS and with the Rules set at the Institute of Polymers, Bulgarian Academy of Sciences, for applying the Act aforementioned.*

Biographical information about the candidate/s

According to the attached information, IVELINA TSANKOVA TSACHEVA obtained the educational and scientific degree "doctor" in 2008.
Dissertation topic: "Polymer radioprotective complexes: design, characterization and performance"
IVELINA TSANKOVA TSACHEVA worked as:
- Microbiology teacher at "Marie Curie" Vocational High School of Chemical Technologies and Biotechnology, Razgrad,
- Doctoral student at Institute of Polymers, BAS-Sofia,
- Biotechnologist at the Institute of Engineering Chemistry, BAS-Sofia,
- Chief assistant at the Institute of Polymers, BAS-Sofia.

The candidate’s total work experience as an expert to date is 19 years.

The total number of scientific publications of the candidate is 28 and 1 book chapter. 19 scientific publications in specialized journals with an impact factor and 1 book chapter are submitted for participation in the competition.
Scientific publications are distributed as follows:
1. Quartile Q1 - 6,
2. Quartile Q2 – 2,
3. Quartile Q3 - 5,
4. Quartile Q4 – 6,
5. Chapter from a book published by Elsevier.

Indicator B.4: 5 scientific publications are published in journals, referenced and indexed in world-famous databases with scientific information (Web of Science and Scopus), which giving a total of 107 points.

Indicator D: total of 245 points, i.e. D.7 14 publications equal to 230 points, together with indicator D.8 - 1 chapter of a book giving 15 points.
The total number of noticed citations (without self-citations for all authors) of the candidate is currently 192. They are equal to 384 points, summarize in indicator D.11. The total number of points, which are obtained according to the indicators from A to D according to the materials presented in the competition, are 786 points with a minimum required of 430 points.

Dr. TSACHEVA is a participant in 12 scientific projects.

She specializes in:
2012- at the Institute of Medical Radiobiology, University Hospital Essen, Germany
2011- at Institute of Medical Radiobiology, University Hospital Essen, Germany
2009- at Institute of Medical Radiobiology, University Hospital Essen, Germany
2009- at the Milestone Learning Center for Microwave Fusion, Sorisole, Italy
2006- in the Biotechnology laboratory at the Department of Pharmacy at Ludwig University Maximilian, Munich, Germany.

He has participated in 19 scientific conferences.
She also received 2 awards, namely:
2011 - DAAD Scholarship for university teachers and scientists
2010-2011 World Federation of Scientists (WFS) National Scholarship, Planetary Emergency –"Medicine & Biotechnology

From 2011-2012 she was a member of the World Organization for Radiation Research, SIT Member inRadiation Research Society
This information presents Dr. IVELINA TSANKOVA TSACHEVA as a fully developed scientist.

Assessment of the scientific and research accomplishments of the candidate

Indicators from group B.4 are related to improvement of synthetic approaches for obtaining new anthracene and furan-containing aminophosphonates, poly(aminophosphonates), as well as proving the structure of new compounds.

The aim of the conducted scientific research is to create new biodegradable phosphoester polymer-drug conjugates with their own biological activity, which are of interest to the pharmaceutical industry.

Synthesis and characterization of low molecular aminophosphonates with potential antitumor activity (Publications 1 and 2).

The scientific contributions to this direction are related to the development of the experimental synthetic approaches aimed at developing strategies for synthesis of new biologically active aminophosphonates. An effective synthetic method for obtaining a new biological has been developed active Schiff base, 9-anthrylidene-furfurylamine and three new diesters of α- aminophosphonic acid bearing an anthracene moiety.
Applying the Kabachnik-Fields reaction, α- aminophosphonates in the presence of a catalyst (CdI2) as well as without a catalyst.

The structure of the obtained new compounds was proved by elemental analysis, thin layer chromatography, IR and NMR spectroscopy. (**publication 1**)

In search of efficient synthetic procedures to obtain these valuable biologically active compounds, microwave synthesis has been applied as an alternative to the classical method. Thus, the Schiff base 9-anthrylidene-furfurylamine and its derivative aminophosphonate N-methyl(dimethoxyphosphonyl)-1-(9-anthryl)]furfurylamine were obtained for the first time.
Microwave synthesis has been found to have a number of advantages as these synthetic procedures take place in a short reaction time, under mild conditions and high yield. (**publication 2**)

The in vitro antitumor activity of the newly synthesized aminophosphonates was investigated on a panel of seven human epithelial cancer cell lines. Two of the compounds combining in their molecules an anthracene residue and a furan ring have shown optimal antiproliferative activity towards human tumor cells of colon carcinoma, malignant tumors of the breast, urinary tract and bladder.In vitro and in vivo safety tests have shown that the resulting compounds have lower toxicity to healthy cells than well-known anticancer and cytotoxic agents. The fluorescent properties of the anthracene ring additionally allow for adequate and precise cellular investigationuptake and intracellular distribution of new compounds in tumor and healthycells. (**publications 1 and 2**)

Synthesis and characterization of poly(oxyethylene aminophosphonate) and as new biologically active drug carriers. (publications 3, 4 and 5)

The scientific contributions to this direction are related to the development of sustainable experimental synthetic procedures for obtaining a new type of polymer carriers with improved properties by attaching aminophosphonate units to biodegradable polymeric carriers such as polyphosphoesters.

The newly synthesized poly(aminophosphonate)s consist of aminophosphonate (biologically active) and non-toxic poly(ethylene glycol) (PEG) structural components and are interesting as polymers with potential intrinsic biological activity. They have the coordination centers in their repeating units and can be used as biodegradable polymer carriers for immobilization of biologically active substances.
The optimal conditions for the polymer to undergo the analogous reaction upon joining the azomethine bond to the P-H groups of the polymer have been established.
A new class of biodegradables has been synthesized and characterized for the first time polymeric carriers, and biological studies have shown that they act as aminophosphonate prodrugs.
The structure of the obtained poly(aminophosphonates) was proved by IR and NMR (1H, 13C and 31P) spectroscopy. (**publication 3**)

The synthetic approaches for obtaining new biologically active carriers containing an anthracene moiety and a furan ring were improved by optimizing the reaction conditions by varying different ratios of the starting reagents, in the presence of a catalytic amount of CdI2 or without a catalyst.
The obtained new poly[oxyethylene(aminophosphonate-co-H-phosphonates)] have a content of hydrophilic H-phosphonate units greater than aminophosphonate units, which makes them water-soluble and suitable as carriers of biologically active substances. (**publication 4**)

Two approaches have been developed to prepare these polymeric supports, namely by conventional and by microwave heating. It was found that when using microwave heating, the reaction takes place in a shorter time as the content of aminophosphonate units in the final products, under conventional heating is 54 mol % and upon microwave heating is 81 mol % calculated from 31P{1H} NMR the spectral data. The structure of the obtained polymer supports was confirmed by IR and NMR (1H, 13C, and 31P) spectroscopy. (**publication 5**)

The established cytotoxicity of the obtained poly(aminophosphonates) is similar or comparable to that of the control and can be investigated as candidates for a new class of cytotoxic compounds.(publication 3) The results of the studies on the in vitro antitumor activity on panel of seven human epithelial carcinoma cell lines show that both tested copolymers belong to the low-toxic group of DNA intercalators and are promising for the development of active antineoplastic agents for chemotherapy of malignant diseases of the breast and liver. (**publication 4**)
The main scientific contributions of the candidate in the conducted research for development of new polymeric drug carriers with intrinsic biological activity can be summarized as follows:

- A new biologically active Schiff base containing an anthracene moiety and a furan ring, 9-anthrylidene-furfurylamine, was synthesized and characterized.

-A synthetic strategy was developed and three new diesters of α- aminophosphonic acid bearing an anthracene moiety

- An efficient synthetic procedure was proposed and a new class of biodegradables was obtained polymeric carriers consisting of aminophosphonate and poly(ethylene glycol) units.

-Four new poly(oxyethylene) were synthesized and characterized for the first time aminophosphonates) based on N-(4-dimethylaminobenzylidene)-p-toluidine or N-furfurylidene-p-toluidine and two new poly[oxyethylene(aminophosphonate-co-H-phosphonates)].

- The optimal conditions for the synthesis of biologically active substances have been established compounds as an efficient microwave synthetic procedure is also proposed heating.

- The new low- and high-molecular aminophosphonates possess in vitro antitumor activity against all tested cancer cell lines, which gives reason to consider them as a new class of cytotoxic agents.

The results obtained emphasize the importance of the simultaneous presence of three pharmacophoric fragments (anthracene part, furan ring and aminophosphonate group) in the same molecule, which is an important prerequisite for higher antitumor activity.

Part of the publications referred to group D.7. are thematically related to the publications referred to group B.4.

Development of experimental synthetic methods for obtaining low molecular weight aminophosphonates. Study of their structure and biology activity. (publications 6, 7, 8, 9, 10, 11, 12 and 13)

A synthetic strategy was developed to obtain new diesters of α- aminophosphonic acid, N,N-dimethyl-[N'-methyl(diethoxyphosphonyl)-(2-furyl)]-1,3- diaminopropane, p-[N-methyl(diethoxyphosphonyl)-(2-furyl)toluidine and p-[N- methyl(diethoxyphosphonyl)-(4-dimethylamino phenyl)] toluidine. The conditions were investigated for the progress of addition reactions of dimethyl or diethyl phosphite to the corresponding Schiff base. The reaction was studied in the presence of catalysts (C2H5ONa or CdI2) and no catalyst. CdI2 was found to be comparatively more effective catalyst for the preparation of these aminophosphonates. The structure of the newly acquired compounds has been demonstrated by elemental analysis, IR and NMR spectroscopy. (**publications 6, 7, 10 and 13**)
The newly obtained compounds have moderate genotoxic and in vivo antiproliferative activity and are suitable candidates in the development of new anticancer drugs for the treatment of hepatocellular carcinoma. (**publication 7**) In search of new compounds with potential antitumor activity, the possibility of obtaining new anthracene-containing bisaminophosphonates was investigated.

The performed biological studies of in vitro antitumor activity show that the first two bis aminophosphonates can be potent cytotoxic agents against the colon carcinoma cell line HT-29, compared to the effect of the control sample doxorubicin. In vivo studies have shown that anthracene-containing compounds have moderate clastogenic and antiproliferative effects. (**publications 11 and 12**)

The candidate's scientific contributions to this direction are:

- A synthetic methodology was developed for obtaining new anthracene and furan-containing aminophosphonates.

- A synthetic method for obtaining new anthracene-containing compounds was developed bisaminophosphonates as potential cytotoxic agents.

Design of new polymeric drug carriers with improved properties and study of their biological activity. (publications 14, 15 and 16)

A polymer complex of the radioprotector WR 2721 – a cysteamine analogue and polyphosphoester was synthesized through ionic bonds, and the radioprotective efficacy of the complex was studied on laboratory animals irradiated with gamma rays. The radioprotective factors for the radioprotector WR 2721 and the polymer complex were evaluated.
It was found that the polymer complex has significantly better radiation effect of parent compound. (**publication 14**)

Poly(oxyethylene aminophosphonates) based on biodegradable polyphosphoester and previously synthesized Schiff bases were obtained.
The newly synthesized poly(oxyethylene aminophosphonates) were tested in vitro for antitumor activity against a group of six human epithelial cancer cell lines, for cytotoxicity to mouse fibroblast cells, and in vivo for clastogenicity and antiproliferative effects.
The synthesized polymers showed lower cytotoxicity, both in vivo and in vitro, and lower clastogenicity in vivo than the corresponding low molecular weight aminophosphonates. (**publication 15**)

The two polyphosphoesters consisting of anthracene-containing were investigated aminophosphonate and hydrophilic H-phosphonate repeating groups and poly[oxyethylene (aminophosphonate-co-H-phosphonate)] for in vitro antitumor activity on cellular cultures.

The results of the tests show that the polyphosphoesters obtained by us at physiological conditions act as prodrugs. The fluorescent properties of the anthracene ring allows precise studies of cellular uptake and the intracellular distribution of these substances in tumor and healthy cells.
(**publication 16**)
The candidate's scientific contributions to this direction are related to:

- Development of sustainable experimental synthetic approaches aimed at development of new phosphorus-containing polymeric carriers of biologically active substances.

Modification of mesoporous nanoparticles with polymer complexes such as controlled release carriers. (publications 17, 18 and 19)

From the research, it was found that the release of the encapsulated quercetin or curcumin from the mesoporous nanoparticles can be further controlled by the applied surface polyelectrolyte complex. It was established that the encapsulated quercetin or curcumin, respectively, in the surface modified with polyelectrolyte complex silicon carriers (KIT-6 and KIL-2NH2), have similar cytotoxic potential for HUT-78 cell line as the free biologically active substances.
Modified silicon particles can be used as carriers for controlled delivery of quercetin or curcumin respectively. (**publications 17, 18 and 19**).
The candidate's scientific contributions to this direction are superficial modification of pre-loaded with quercetin or curcumin respectively mesoporous particles.

Book Chapter: Phosphorus-Containing Polymeric Nanomaterials.

The variety of synthetic methods for the synthesis, effective strategies for modification, the development of new phosphorus-containing macromolecules. Accumulated knowledge and understanding of nano- and bio-interactions significantly influences the design of drug delivery systems.
Results have been published regarding the application of phosphorous nanobiomaterials, which confirms their contribution to the development of nanomedicine.
Drug delivery systems derived from phosphorus-containing polymers show distinguishing features of nanotherapeutics, such as improving pharmac properties of active molecules, targeted delivery, sustained or triggered from stimulated drug release, simultaneous delivery of multiple drugs, visualization of drug delivery sites by combining the therapeutic and imaging agents, etc. (**publication 20**)

Conclusion

Based on the documentation presented to me about the scientific achievements and scientific work of Dr. IVELINA TSANKOVA TSACHEVA, my opinion is that based on ZRASB, PPZRASB, she meets the regulatory requirements for holding the position of “associate professor” in the Institute of Polymers - BULGARIAN ACADEMY OF SCIENCES in field of higher education 4. Natural sciences, mathematics and informatics, 4.2. Chemical Sciences (Polymers and Polymeric Materials)

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Signature:.....

(Prof. D.Sc. Dobromir Enchev)