R E P O R T
by

Assoc. Prof. Dr. Georgy Grancharov
Member of the Academic Jury set to render a decision
on the competition for filling the academic position of an **Associate 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 Report 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 Report 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*.

The report consists of two chapters and a conclusion. There is only one candidate in the procedure- Dr. Ivelina Tsankova Tsacheva, Assistant Professor in the Department of Polymers for alternative energy and environmental protection, Institute of Polymers, Bulgarian Academy of Sciences.

1). Assessment of the scientific and research accomplishments of the candidate-

1.1. Analysis of the application materials and documents presented by candidate-

Dr. Ivelina Tsacheva participates in this competition with 19 publications and one book chapter, wherein the last 6 of them have been produced and presented in the last 5 years. It is shown that 5 of the presented publications participate in indicator "B.4": Habilitation work, and the remaining 14 publications and the book chapter are included in indicator „Г.7": Additional publications not included in the habilitation work. In three of the articles at indicator „B.4", Assistant Professor Dr. Ivelina Tsacheva is in the first places of coauthors, and the position in the paper affiliation that she occupies also shows her activity and commitment to the work. Three of the publications at this indicator belong to the most prestigious quartile - Q1, which undoubtedly shows the qualities of those works. The candidate earns 107 points at this indicator with the required 100 points by law. In the case of the indicator „Г.7", three of the publications belong to quartile Q1, one to quartile Q2, and rest of them to quartile Q3 and Q4. In many of the publications she is in the leading places of the coauthors depending on the scientific team. The candidate earns 245 points at this indicator with the required 220 points by law. All presented works are connected with hot current topics and show high scientific level, which is reflected in their citation. Assistant Professor Dr. Ivelina Tsacheva showed 192 citations, which has earned

her 384 points (2 points per citation). It is shown, that minimum citation requirements for the position of Associate Professor are covered many times. Thus, the proposed science metric materials fully meet the requirements of Development of Academic Staff in the Republic of Bulgaria Act (DASRB), the Rules for the Application of the DASRB Act, the Rules of BAS and with the Rules set at the Institute of Polymers, Bulgarian Academy of Sciences (BAS), for applying the Act aforementioned, and allow the candidate to participate in the competition.

1.2. Main scientific contributions - habilitation work (indicator „B.4“) -

A). Synthesis and characterization of lowmolecular weight aminophosphonates with potential antitumor activity (publications № 1 and 2).

A new biologically active Schiff base, 9-anthrylidene-furfurylamine, was obtained and characterized as well as three new α-aminophosphonic acid diesters bearing an anthracene moiety- [N-methyl(dimethoxyphosphonyl)-1-(9-anthryl)]-p-toluidine, [N-methyl(diethoxyphosphonyl)-1-(9-anthryl)]-p-toluidine and [N-methyl(diethoxyphosphonyl)-1-(9-anthryl)]furfurylamine. Kabachnik-Fields reaction was used in and without the presence of CdI_2 as a catalyst. The new compounds obtained in this way have been proven by NMR and IR spectroscopy, elemental analysis and thin-layer chromatography.

As an alternative to the classical preparation method, the microwave assisted synthesis of aminophosphonates was also developed, and for the first time by this method [N-methyl(dimethoxyphosphonyl)-1-(9-anthryl)]furfurylamine was obtained. The method was found to have a number of advantages such as shorter reaction time, milder reaction conditions and obtaining of higher yields.

The *in vitro* antitumor activity of the newly synthesized aminophosphonates was investigated on a panel of 7 human epithelial cancer cell lines. Two of the newly obtained products showed optimal antiproliferative activity towards human tumor cells in 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 other well-known anticancer and cytotoxic agents.

B). Synthesis and characterization of poly(oxyethylene aminophosphonates) as new biologically active drug carriers (publication № 3, 4, and 5).

A new class of biodegradable polymeric carriers has been synthesized and characterized, and it was found that they act as aminophosphonate-based prodrugs. They consist of PEG units (PEG-200 or PEG-600) as well as aminophosphonate fragments linked to the two Schiff bases - N-(4-dimethylaminobenzylidene)-p-toluidine and N-furfurylidene-p-toluidine. The structure of new obtained poly(oxyethylene aminophosphonates) was proven by IR and NMR (^{1}H, ^{13}C and ^{31}P) spectroscopy.

Additionally, the reaction conditions for the preparation of poly(oxyethylene aminophosphonates) were optimized by varying the ratios of the starting reagents, the presence or absence of catalytic amounts of CdI_2. Poly[oxyethylene(aminophosphonate-co-H-phosphonates)] with a higher content of hydrophilic H-phosphonate units than aminophosphonate units were also obtained, which makes them water-soluble and suitable as carriers of biologically active substances.

Again, microwave assisted synthesis was applied as a proven method in the preparation of low molecular weight aminophosphonates, as well as in the synthesis of poly[oxyethylene(aminophosphonate-co-H-phosphonates)]. It was found by the $^{31}P\{1H\}$ NMR spectrum that when using microwave heating, the reaction took place in a shorter time and the content of aminophosphonate units in the final products was 81 mol %, while with conventional heating the reaction was longer and the content of aminophosphonate units was 54 mol %.

It was found in the cytotoxic tests against a panel of 4 human tumor cell lines that the poly(aminophosphonate) with included in its structure poly(oxyethylene H-phosphonate) based on PEG-600 and 2-furyl-p-toluidine fragment has structural prerequisites, leading to greater antitumor activity compared to other studied Schiff bases analogs- N-(4-dimethylaminobenzylidene)-p-toluidine and N,N-dimethyl-N'-furfurylidene-1,3-diaminopropane. The cytotoxicity of the resulting poly(aminophosphonates) was similar or comparable to that of the reference drug cisplatin.

1.3. Scientific contributions not included in habilitation work (indicator "Г.7") -

From the attached literature sources, three additional topics stand out which are summarized in the scientific contributions outside the habilitation work, while the book chapter shows the diversity of nanomaterials based on phosphorus-containing polymers. Part of this research is thematically related to the publications referred to group B.4. and can be tentatively referred to the following areas:

A). Development of experimental synthetic methods for obtaining low molecular weight aminophosphonates. Study of their structure and biological activity (publications № 6, 7, 8, 9, 10, 11, 12, and 13).

Here, attention is drawn to anthracene and furan-containing aminophosphonates which have been designed as novel antitumor therapeutics. 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 were synthesized in or without the presence of catalysts. The newly obtained compounds have moderate genotoxic and *in vivo* antiproliferative activity and are suitable candidates for the development of drugs for the treatment of hepatocellular carcinoma.

New anthracene-containing bis-aminophosphonates- 4,4'-bis[N-methyl(diethoxyphosphonyl)-1-(9-anthryl)]diamino-diphenylmethane, bis[N-methyl(diethoxyphosphonyl)-1-(9-anthryl))]benzidine and 1,3-bis[N-ethyl(diethoxyphosphonyl)-1-(9-anthryl)]diaminobenzene were obtained. *In vitro* antitumor activity suggests that the first two bis-aminophosphonates may 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 they also have moderate clastogenic and antiproliferative effects.

B). Design of new polymeric drug carriers with improved properties and study of their biological activity (publications № 14, 15, and 16).

Here of interest is the comparison of the radiation protector WR 2721 (a cysteamine analog) and its complex with a polyphosphoester to which it is ionically linked. It has been proven that the polymer complex has a significantly better anti-radiation effect than the original radioprotective compound.

For *in vitro* antitumor activity against a panel of 6 human epithelial cancer cell lines, for cytotoxicity to mouse fibroblast cells, and *in vivo* for clastogenic and antiproliferative effects were analyzed the previously prepared poly(oxyethylene aminophosphonates) with Schiff bases of N,N-dimethyl-N'-furfurylidene-1,3-diaminopropane and N-(4-dimethylaminobenzylidene)-p-toluidine. The synthesized polymers showed lower cytotoxicity, both *in vivo* and *in vitro*, possessing also lower clastogenicity *in vivo* than the corresponding low molecular weight aminophosphonates.

In vitro antitumor cell culture assays of the two polyphosphoesters consisting of anthracene-containing aminophosphonate incorporated into poly[oxyethylene (aminophosphonate-co-H-phosphonate)] based on PEG-200 and PEG-600 showed that the obtained polyphosphoesters at physiological conditions act as prodrugs.

C). Modification of mesoporous nanoparticles with polymer complexes as carriers for controlled drug release (publications No. 17, 18, and 19).

Silicon mesoporous nanoparticles pre-loaded with the medicinal substances quercetin or curcumin were surface modified with a polyelectrolyte complex. The polyelectrolyte complex was applied to the mesoporous particles by sequential deposition (layer-by-layer) of κ-carrageenan/chitosan/κ-carrageenan layers. From the obtained research, it was established that the release of the encapsulated quercetin or curcumin from the mesoporous nanoparticles can be controlled by the applied surface polyelectrolyte complex.

2. Opinions, notes and recommendations (optional)

The author has tried to comprehensively present her main scientific contributions, both in her habilitation work and works outside habilitation work. In addition to the scientific publications, her participation in numerous scientific projects and scientific forums is impressive. I have no significant remarks but would rather recommend to the candidate for more independence in the preparation of further scientific works and projects. This will further highlight her contributions regarding the indisputable relevance of the conducted research and its scientific significance.

3. Conclusion

The active research activity outlines Dr. Ivelina Tsacheva as an ambitious scientist in the field of phosphorus-containing monomers and polymers, biodegradable carriers of drug substances and modified mesoporous nanoparticles with a flair for solving real and current scientific problems- the creation of new drug substances, new biodegradable polymers, and new drug carriers for targeted delivery for pharmacy and medicine. The candidate fully meets the minimum requirements of the law according to DASRB Act, the Rules for the Application of the DASRB Act, the Rules of BAS and with the Rules set at the Institute of Polymers, BAS for applying the Act aforementioned. On the grounds of the documentations presented by the candidate, and the above assessment, I confidently recommend the Honorable Academic Jury to render a positive decision on Assistant Professor Dr. Ivelina Tsacheva filling the position of an **Associate Professor** at the Institute of Polymers, Bulgarian Academy of Sciences in the professional field **4.2 Chemical Sciences (Scientific Specialty "Polymers and Polymer Materials")**.

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