**Summary of the main results and scientific contributions of**

**assoc. prof. Dr. eng. Rayna Georgieva Bryaskova,**

**Submitted for participation in the competition for professor of**

**4.2 Chemical Sciences (Macromolecular chemistry) at UCTM**

Associate Professor Dr. Raina Bryaskova's publication activity, as part of her application for the position of full professor, comprises **19** articles, including **12** in international journals with impact factors (cumulative impact factor Σ=38.376) and **7** in indexed journals without impact factors, with SJR ratings. Among them, **5** publications are in **Q1** journals, **7** in **Q2** journals, **5** in **Q3** journals, and **2** in **Q4** journals. The scientific output in which assoc. prof. Rayna Bryaskova has participated has been cited **107** times, indicating the significant impact of her research within the academic community. Furthermore, she has actively participated in **20** international, national, and university scientific forums. Moreover, she has led **7** contracts with the SRC at UCTM and has served as leader, coordinator, and participant in **6** contracts with the Fund for Scientific Research, The Operational Program "Science and Education for Intelligent Growth," The Operational Program "Innovation and Competitiveness," and The National Program "European Scientific Networks." Under her guidance during the period 2016-2024, assoc. prof. Dr. Rayna Bryaskova successfully supervised the defense of 18 students, including 10 for the Bachelor's degree and 8 for the Master's degree. Her research focuses on the synthesis and application of hybrid materials using the sol-gel method, the synthesis and applications of metal nanoparticles embedded in polymer materials, the synthesis, characterization, and application of fluorescent polymer materials for visualization and treatment of cancerous diseases, as well as the development of innovative antibacterial coatings based on novel photoactive nanogels.

As a co-author, she has contributed to total of **53** articles, with **49** of them published in journals with impact factors and ranking, according to Scopus. These articles have garnered **931** citations, excluding self-citations from all co-authors (**h**-index = **15**, according to Scopus, without self-citations).

**Table 1**. Minimum required points by groups of indicators for the academic position "professor" under 4.2. Chemical Sciences at UCTM

| Content | Minimal requirements for professor (number of points) | Assoc. prof. R. Bryaskova |
|---|---|---|
| Indicator1 | 50 | **50** |
| Indicator 2 | - | |
| Indicators 3 or 4 | 100 | **115** |
| Sum of points from 5 to 10 | 200 | **215** |
| Sum of points of indicator 11 | 100 | **214** |
| Sum of points of indicator from 12 to the end | 150 | **184** |
| Total points | **600** | **778** |

Scientific and applied contributions of the presented works of assoc. prof. R. Bryaskova can be grouped into several main research groups:

## 1. Synthesis, characterization, and application of polymeric materials with pronounced antibacterial properties for medical applications.

### 1.1. Synthesis, characterization, and application of novel antibacterial hybrid materials with included silver nanoparticles (publications 1-2,7).

Among metallic nanoparticles, silver nanoparticles are of great interest due to their unique properties and potential applications in various fields, particularly in medicine as effective antibacterial agents. The use of nanoparticles is a new method that leads to effectively suppressing bacterial growth, especially when silver nanoparticles (AgNps) are used, which are known to possess powerful activity against bacteria, viruses, and fungi. Compared to silver ions, silver nanoparticles are considered environmentally safe. The strong antibacterial activity of silver nanoparticles results from their well-developed surface, which ensures maximum contact with the environment. However, to realize their potential for technological and biological applications, they need to be included into a polymeric matrix capable of forming thin films. Therefore, for the first time, new hybrid materials based on polyvinyl alcohol (PVA) and three types of organosilanes, aminopropyl triethoxysilane (APTEOS), mercaptopropyl triethoxysilane (MPTEOS) and tetraethoxysilane (TEOS) with included silver nanoparticles, were synthesized using the sol-gel method, which is a widely used method for synthesis of hybrid materials. The use of polyvinyl alcohol as a film-forming polymer for including of silver nanoparticles is appropriate because PVA is a hydrophilic, biocompatible, and water-soluble polymer. These characteristics provide PVA with wide applications in medicine. Silver nanoparticles with varying concentrations were obtained through thermal reduction in the presence of PVA. To improve its physicochemical properties, the inclusion of aminopropyl triethoxysilane (APTEOS), mercaptopropyl triethoxysilane (MPTEOS), and tetraethoxysilane (TEOS) in the polymeric matrix through the sol-gel method is necessary. Initially, organoalkoxysilanes are hydrolyzed in an aqueous environment in the presence of an acidic catalyst. During this hydrolysis, the alkoxy groups (OR: hydroxyl, amino, and thiol groups) are converted into hydroxyl (-OH) groups, leading to the formation of silanol groups. After the solution is poured, polycondensation occurs, during which Si-O-Si bonds are formed and water or alcohol is released depending on the degree of hydrolysis. The presence of hydroxyl groups in the repeating units of PVA suggests the formation of strong secondary interactions with the residual silanol groups obtained from acid-catalyzed hydrolysis and polycondensation of organosilanes without the formation of by-products. The obtained hybrid materials are characterized by appropriate analyses such as TEM, SEM, and suitable spectral analyses including infrared spectroscopy (FTIR) and UV-Vis analysis.

The hybrid materials based on PVA/AgNps/APTEOS were tested for fungicidal activity against the following etalon strains: *Candida albicans ATCC 10231*, *Candida krusei ATCC 6258*, *Candida tropicalis B 6030413, and Candida glabrata ATCC 90050,* as well as against clinical strains isolated from patients with urinary tract infections: *Candida glabrata 8–122*, *Candida albicans 8–127, Candida albicans 8–137, Candida krusei 8–126,* exhibiting clear zones of inhibition. Subsequently, the obtained hybrid materials were tested for immobilizing yeast cells, and it was found that they possess high efficiency in removing manganese $Mn^{2+}$ ions even at the lowest concentration of silver nanoparticles in the matrix.

The hybrid materials based on PVA/AgNps/MPTEOS were tested against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* bacteria using the disc diffusion method, which demonstrated strong antibacterial activity, which was enhanced with increasing of silver content in the PVA/MPTEOS matrix. The thermal properties of hybrid materials based on PVA/AgNps/TEOS were also investigated through thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC), revealing that the introduction of TEOS into the polymeric matrix improves the thermal stability of the resulting materials. Furthermore, the inclusion of AgNps in the hybrid PVA/TEOS matrix significantly enhances their thermal resistance. The fungicidal activity of the obtained PVA/AgNps/TEOS films was tested against five clinical strains of Candida: *Candida albicans ATCC 10231*, *Candida krusei ATCC 6258*, *Candida tropicalis B 6030413*, and *Candida glabrata ATCC 90050*. It was found that PVA/AgNps/TEOS films with silver concentrations ranging from 0.9 mg/mL to 3.6 mg/mL possess fungistatic activity against *Candida albicans ATCC 10231*, *Candida krusei ATCC 6258*, *Candida tropicalis B 6030413*, and fungicidal activity against *Candida glabrata ATCC 90050*. With increasing the silver concentration up to 18 mg/mL in the PVA/AgNps/TEOS films, the activity against *Candida albicans ATCC 10231*, *Candida krusei ATCC 6258*, and *Candida tropicalis B 6030413* becomes fungicidal.

From the obtained results, it can be concluded that the synthesized new hybrid materials with pronounced antimicrobial activity can find potential application in various fields of biotechnology and medical engineering.

### 1.2. Synthesis of silver nanoparticles stabilized with polyvinyl alcohol for veterinary applications (publication 4).

Silver nanoparticles stabilized with polyvinyl alcohol (PVA) were synthesized. This was achieved through thermal reduction of silver ions in a PVA solution to silver nanoparticles. The obtained PVA/AgNps complex was investigated for the treatment of external otitis (inflammation of the outer ear and external auditory canal) in dogs as an additional component in the commercial product "Betazon Trio" "Antibiotic-Razgrad". Experimentally, it has been confirmed that the innovative cream product with added PVA/AgNps material with a silver concentration of 600 mg/L in the commercial product "Betazon Trio" improves microbial homeostasis and provides a positive effect in cases of external ear canal infection when applied once daily for a period of 14 days.

## 2. Development of "ghost" vaccines for veterinary applications based on silver nanoparticles stabilized with polyvinyl alcohol (publications 3 and 5).

The development of “ghost vaccines” through the inactivation of various strains of *Salmonella* bacteria or strains of *E. coli O104*, *E. coli O157H7* with a hybrid material based on silver nanoparticles stabilized with polyvinyl alcohol (PVA/AgNps) is an innovative new approach to the application of inactivated vaccines with preserved cell surface integrity. The use of vaccines treated in this way has several advantages, including the preservation of the antigenic range and the creation of a complex protective immunity. Initially, the minimum bactericidal concentration (MBC) for the different strains of *Salmonella* or *E. coli* was determined using the macrodilution method. Minimal non-toxic concentrations of the PVA/AgNp hybrid complex and cell viability ($CD_{50}$) were also established. The specific titer of all obtained after immunization with the "ghost" Salmonella vaccine rabbit antisera was determined by the Gruber reaction, with strains *S. Enteritidis*, *S. Newport-Puerto Rico*, and *S.*

*Typhimurium* selected as suitable candidates for their inclusion, aiming to create a polyvalent Salmonella "ghost" vaccine for veterinary use. Adding more strains to the vaccine will allow expanding of its scope, and their inactivation by the PVA/AgNps complex will preserve a full set of antigenic determinants, thus providing complete protection. The antigens obtained in this way have the potential to be applied in vaccine production.

## 3. Design, synthesis, and characterization of polymer micelles with embedded gold nanoparticles (publications 11, 12, 13).

The use of gold nanoparticles in medicine, biomedicine, and the biological field finds wide applications. They can be applied for diagnosis and therapy, immunoassays, clinical chemistry, detection, photothermal lysis of microorganisms and cancer cells and tissues, targeted drug delivery, and others. The fabrication of nanostructures with embedded gold nanoparticles is widely employed for cancer diagnosis and therapy. They are of significant interest for photothermal therapy due to their strong and tunable linear absorption in the near-infrared region, where tissue penetration can be maximized. On the other hand, it is known that polypyridines such as poly(2-vinylpyridine) and poly(4-vinylpyridine) (P4VP) are insoluble in water at neutral or alkaline pH, but at pH <5 they are protonated and therefore the block copolymers based on polypyridines could react to changes in pH in the environment. Additionally, they are capable of forming complexes with various metal ions such as gold, silver, and others. The use of poly(ethylene glycol) is a suitable choice as a second building block for obtaining block copolymers because it is a hydrophilic polymer, water-soluble, biocompatible, and low in toxicity. This is why series of well-defined diblock and triblock copolymers based on poly(ethylene glycol) and poly(4-vinylpyridine) (PEG-b-P4VP) and (P4VP-b-PEG-b-P4VP) have been synthesized and obtained through atom transfer radical polymerization (ATRP). This was achieved using chlorine-terminated PEG as a macroinitiator. As the second forming block, 4-VP was used in the presence of CuCl/PMDETA as a catalytic system. The successful synthesis of the block copolymers was confirmed through $^{1}$H NMR and FTIR spectroscopies, demonstrating the presence of all characteristic signals originating from PEG and 4-VP units. The results from gel permeation chromatography (GPC) analysis show the formation of well-defined block copolymers with low dispersity, confirming the controlled nature of the radical polymerization. The self-assembly behavior of these block copolymers in aqueous media was also investigated. Subsequently, gold nanoparticles were incorporated into the core of the obtained micelles, which was confirmed by UV-vis, TEM, and DLS analysis. It was found that the diblock copolymers formed well-defined spherical micelles, while the triblock copolymers self-assembled, forming so-called "flower-like" micelles. The cytotoxicity of the micelles with incorporated gold nanoparticles against MDA-MB-231 breast cancer cells and L929 fibroblasts was also determined. The results obtained show that the synthesized micelles with embedded gold nanoparticles could find application for biomedical purposes.

## 4. Design, synthesis, and characterization of novel fluorescent polymer micelles for biomedical and medical applications (publications 9, 10, 14, 16, 19).

### 4.1. Synthesis and characterization of pH-sensitive polymer micelles with included fluorescent compounds for visualization and diagnostics (publications 9, 10, 14, 16).

Over the past two decades, there has been a tremendous interest in polymer materials that can reversibly or irreversibly alter their physical and chemical properties under the influence of external stimuli, such as pH, temperature, the presence of specific ions, light irradiation, mechanical force, magnetic field, electric field, and bioactive molecules. Special attention, however, is given to well-defined pH-sensitive block copolymers, which are stable at physiological pH (7.1-7.3) and can self-organize into micellar structures. Upon acidification of the environment, they can selectively release the drug by micelle dissociation. Incorporating fluorescent compounds into their structure allows the design of these micelles to be utilized as "multifunctional" micelles, possessing both diagnostic and therapeutic properties. They can visualize in real-time while delivering drugs or biological compounds simultaneously depending on the pH of the environment.

Therefore, a pH-sensitive nanostructure was synthesized and its sensing activity was trased, designed as water-soluble fluorescent micelles using a well-defined amphiphilic diblock copolymer based on poly(methyl methacrylate)-block-poly(methacrylic acid) (PMMA-b-PMAA) by grafting a 1,8-naphthalimide-rhodamine bichromophoric FRET system (RNI) to the PMMA block. The RNI-PMMA-b-PMAA adduct is capable to self-association into micelles with a hydrophobic core of PMMA containing the covalently attached fluorescent compound and a hydrophilic shell composed of the PMAA block. The new fluorescent micelles are able to serve as highly sensitive pH indicators in water and for successful internalization into HeLa and HEK cells. Furthermore, they exhibit cellular specificity and significantly higher photostability than those labeled with pure organic dye such as BODIPY. The valuable properties of the newly obtained fluorescent micelles demonstrate the high potential for future biological and biomedical applications.

To obtain pH-sensitive fluorescent polymer micelles, well-defined block copolymers based on poly(tert-butyl acrylate) and poly(n-butyl acrylate) (PtBA-b-PnBA) were synthesized through atom transfer radical polymerization (ATRP), using PtBA-Br as a macroinitiator. Optimal conditions and ratios between the individual components in the system were determined, leading to the production of well-defined polymers based on PtBA and PnBA. The obtained copolymers were characterized and proved through various spectral methods such as infrared spectroscopy (FTIR) and nuclear magnetic resonance ($^1$H NMR). The composition of the resulting block copolymers was determined through conducted $^1$H NMR, and SEC analysis demonstrated the preparation of block copolymers with relatively low dispersity. Selective hydrolysis of PtBA-b-PnBA block copolymers has resulted in the formation of amphiphilic pH-sensitive block copolymers based on polyacrylic acid (PAA) and n-butyl acrylate (PnBA), which were proved using appropriate spectral methods. Modification of the obtained amphiphilic copolymer with a suitable fluorescent compound, 4-amino(2-aminoethyl)-dihydroimidazo[4,5-c]naphthalimide (DHI), was successfully conducted under specific molar ratios and reaction conditions, leading to the production of fluorescent copolymers with covalently attached fluorescent compound to the PAA block. Subsequently, the self-assembly ability of the obtained pH-sensitive fluorescent amphiphilic diblock copolymers in aqueous media was investigated, resulting in pH-sensitive micelles with a modified shell composed of a fluorescent compound and a core consisting of the hydrophobic PnBA block. The obtained nanoscale micelles were characterized and confirmed using dynamic light scattering (DLS) and transmission electron microscopy (TEM). The photophysical properties of the resulting fluorescent micelles were also examined, revealing a stable fluorescent signal in the pH range of 5-8, which rapidly decreases upon lowering the pH from pH=5 to pH=2.

The ability to obtain PAA-b-PnBA micelles with included fluorescent compound based on 1,8-naphthalimide (NI) in the hydrophobic core of the micelle was also investigated. It was found that nanoscale fluorescent micelles were obtained, as confirmed by dynamic light scattering (DLS) and transmission electron microscopy (TEM). The photophysical properties of the resulting fluorescent micelles were also examined. In alkaline or neutral environments, the pH-sensitive micelles remained stable, and the fluorescent compounds in their cores exhibited green emission, attributed to their aggregation within the micellar core. In acidic environments, due to the destabilization of the micelles, individual fluorescent compounds were released into the system, resulting in observed blue monomeric 1,8-naphthalimide emission. The cytotoxicity tests conducted on the obtained fluorescent micelles showed low cytotoxicity at certain concentrations. It was observed that the obtained micelles penetrated into the cell cytoplasm and exhibited their characteristic properties depending on the environment, suggesting their potential for biomedical applications.

New biocompatible water-soluble fluorescent micelles containing perylene diimide (PDI)-based fluorophores in the hydrophobic core of the micelle were also obtained for intracellular applications through the self-assembly of amphiphilic poly(vinyl alcohol)-block-poly(acrylonitrile) (PVA-b-PAN) copolymers in the presence of the synthesized fluorophores. The amphiphilic PVA-b-PAN copolymers were obtained through selective hydrolysis of well-defined poly(vinyl acetate)-block-poly(acrylonitrile) (PVAc-b-PAN) copolymer. The successful preparation of the new fluorescent micelles, consisting of a PVA hydrophilic shell and PAN hydrophobic core with included PDI fluorophores, was confirmed by DLS and TEM analyses. The cytotoxicity of the water-soluble fluorescent micelles was tested, along with their internalization into living cells depending on the micellar concentration. It was demonstrated that they can efficiently penetrate living cells without disrupting their morphology.

The obtained results indicate that the new water-soluble fluorescent micelles with included fluorophores would be suitable for potential applications in biomedicine and medicine.

### 4.2. Synthesis and characterization of novel fluorescent polymeric micelles with included biologically active drug for cancer treatment (Publication 19)

Fluorescent micellar nanocarriers with controlled release of a novel anticancer drug have been successfully developed to enable simultaneous intracellular imaging and treatment of cancer. These nanosized fluorescent micellar systems with incorporated new anticancer drug were obtained through the self-assembly of well-defined block copolymers based on poly(acrylic acid)-block-poly(n-butyl acrylate) (PAA-b-PnBA) copolymers via atom transfer radical polymerization (ATRP) and the hydrophobic anticancer benzimidazole-hydrazone drug (BzH). Through this method, well-defined nanosized fluorescent micelles consisting of a hydrophilic PAA shell and a hydrophobic PnBA core with included BzH drug have been obtained, resulting from hydrophobic interactions, thereby achieving very high encapsulation efficiency. The size, morphology, and fluorescent properties of the drug-loaded micelles were investigated using dynamic light scattering (DLS), transmission electron microscopy (TEM), and fluorescence spectroscopy. Spectrophotometric analysis determined that after 72 hours of incubation, the drug-loaded micelles released 3.25 μM BzH. It has been found that micelles loaded with the BzH drug exhibit enhanced anti-proliferative and cytotoxic effects on MDA-MB-231 cells, with long-lasting effects on microtubule organization, apoptotic changes, and preferential localization in the perinuclear space of cancer cells. Conversely, the anti-tumor effect of BzH alone or included in micelles on non-cancerous MCF-10A cells is relatively weak. The obtained results provide evidence that the novel micelles with incorporated benzimidazole-hydrazone represent a new drug delivery system with high potential for application in anticancer therapy.

## 5. Preparation of innovative antibacterial coatings based on new photoactive nanogels (publications 6, 8, 15, 17, 18).

Due to the increasing number of infectious diseases caused by pathogenic or conditionally pathogenic microorganisms with multidrug resistance to antibiotics from various groups, numerous research groups are directing their efforts towards discovering alternative and more effective products that possess "persistent" antibacterial activity without the risk of developing resistance to them. In recent years, light-activated antimicrobial coatings containing organic and/or inorganic photosensitizers have been considered as promising products that possess continuous antibacterial activity against bacteria, viruses, and fungi. This is due to the property of photosensitizers to absorb light energy of suitable wavelength, which they then transfer to surrounding molecules (primarily oxygen), thereby generating reactive oxygen species (ROS) (radicals and singlet oxygen). These highly reactive species are responsible for destroying the targeted cells. It is of extremely importance for the obtained coatings to possess the necessary set of requirements in order to effectively perform their function. They must have good adhesion to the surface, exhibit good mechanical durability, and at the same time provide the opportunity for suitable functionalization with compounds of various natures to fulfill their designated function. Therefore, our main goal was focused on creating a new type of highly effective self-disinfecting coatings that could be successfully used in various areas of public life, having a significant positive impact on health and quality of life and holding substantial social importance, both in societal and economic aspects.

### 4.1. Preparation of polymer coatings on stainless steel substrate and testing their chemical and physico-mechanical properties. (Publication 6)

To obtain suitable polymer coatings, the polycationic copolymer based on (P(mDOPA)-co-P(DMAEMA$^+$)) copolymer was used. This copolymer consists of a P(mDOPA) block containing 3,4-dihydroxy-L-phenylalanine groups (DOPA), which are the main component of naturally produced proteins secreted by mussels (MAPs), known for their excellent adhesive properties. Thus, there is no need for additional functionalization of the main polymer chain with DOPA units, which is of great importance for industrial applications. The structure of the obtained copolymer was confirmed through various spectroscopic analyses such as Fourier-transform infrared spectroscopy (ATR-FTIR) and proton nuclear magnetic resonance ($^1$H NMR), showing the characteristic signals for both blocks of the copolymer. Subsequently, nanogels based on poly(methacrylamide) containing 3,4-dihydroxy-L-phenylalanine (DOPA) groups (P(mDOPA) were obtained. The conditions to obtain nanogels are critical for their successful formation. It is necessary for the DOPA groups from the main P(mDOPA) chain to be in an oxidized state in order to form water-soluble Pox(mDOPA) polymer. This is achieved through oxidation under basic conditions, followed by the addition of polyallylamine (PAH) containing amino groups that interact with the quinone groups of P(mDOPA) through well-known amine-quinone interactions. In acidic conditions, this reaction is impossible due to the protonation of the amino groups of polyallylamine, which prevents the film from crosslinking.

To confirm the formation of nanosized particles, dynamic light scattering (DLS) and transmission electron microscopy (TEM) were used, which demonstrated the formation of well-defined nanogels. Subsequently, polymer coatings were obtained on a selected stainless steel (SS) substrate through a "deep coating" procedure, involving the sequential immersion of the substrate into an aqueous solution of the polycationic (P(mDOPA)-co-P(DMAEMA$^+$)) copolymer (2 g/L, pH 7) and in a (Pox(mDOPA)/PAH) (1 g/L, pH 9) aqueous solution of the nanogel for a certain period of time. It was found that the appropriate deposition time for the two sequential layers is 15 minutes, with a rinsing step with distilled water between the two depositions, which was confirmed by analysis conducted via quartz crystal microbalance with dissipation (QCM-D) in real-time, by measuring the change in resonance frequency ($\Delta f$) over time, which tracks the online deposition of products, based on which an assessment can be made whether the coating remains on the surface after rinsing. The decrease in $\Delta f$ over time demonstrated the successful deposition of each layer. The conducted ATR-FTIR analysis also proved the formation of the polymer coating on the SS substrate with the appearance of all characteristic signals for the Pox(mDOPA)/PAH nanogel. The surface and mechanical properties of the obtained coatings were determined by atomic force microscopy (AFM), microscratch resistance testing, scanning electron microscopy (SEM) and nanoindentation. Atomic force microscopy (AFM) was used to assess surface roughness, revealing a decrease in roughness with each deposited layer, with epitaxial deposition of each layer following the substrate morphology. Scratch resistance testing is one of the most widely used and well-known methods for assessing the strength of adhesion in the coating-substrate system. As a result of the conducted analyses, it was found that the obtained coatings exhibit good scratch resistance and adhesive strength on the SS substrate, which is confirmed by SEM analysis of the resulting scratches, showing linearly increasing scratches on the polymer coatings deposited on the SS substrate, consistent with the observed changes in the acoustic emission signal from the microscratch test. Nanoindentation tests confirm the production of coatings with good mechanical properties.

### 5.2. Synthesis of photosensitizers and modification of polymer coatings (Publication 15)

To create photoactive polymer coatings and their successfully modification, a photosensitizer, 9-aminoacridine, was chosen, which possesses good antibacterial and photobactericidal activity using visible or UV light. To carry out the modification and obtain photoactive antibacterial coatings, the selected photosensitizer was appropriately modified to create functional amino groups, through which covalent attachment to the obtained polymer coatings could be achieved via amine/quinone interactions. The conducted ATR-FTIR and NMR analyses confirm the successful modification of 9-aminoacridine. The photo-physical behavior of the obtained amino-modified 9-aminoacridine was also investigated, and the analyses confirmed the successful modification, indicating that the amino group in 9-aminoacridine performed a successful transformation to an amide group. The preparation of photoactive polymer coating on a stainless steel substrate was carried out by sequentially depositing the three main components: 1) the polycationic solution of the (P(mDOPA)-co-P(DMAEMA$^+$)) copolymer (2g/L, pH 7); 2) a water solution of (Pox(mDOPA)/PAH) (1 g/L, pH 9) nanogel; and 3) a solution of amino-modified 9-aminoacridine, each followed by a step of rinsing with distilled water, necessary to remove the unreacted components from the system, using the previously described procedure. The results from the QCM-D experiment demonstrate that all three components are successfully deposited onto the substrate and remain on it even after rinsing with water. The surface topography was determined using atomic force microscopy (AFM), revealing that each layer is deposited epitaxially following the morphology of the sample. To assess the mechanical properties of the resulting photoactive coatings, surface scratch resistance tests and nanoindentation tests were conducted to determine hardness and elasticity modulus. The scratching process was monitored through changes in the emission of the acoustic signal, revealing that the obtained coatings exhibit good adhesion at low loads up to 15mN. The SEM analyses conducted on the scratches resulting from the scratch resistance tests demonstrate a linear increase in scratches on the photoactive polymer nanogel coatings, without any observed cracking or delamination of the coatings during scratching. The mechanical properties of the obtained photoactive polymer coatings, applied onto the SS substrate, were tested using nanoindentation with a Berkovich indenter at a maximum load of 20 mN. As a result of nanoindentation, better mechanical properties of the obtained polymer coatings on SS were observed compared to the nanogel-substrate coatings.

### 5.3. Design and synthesis of photoactive polymer coatings with embedded photosensitizers and metal nanoparticles (Publications 8, 15, 17, 18)

Silver nanoparticles have been synthesized and successfully included into a polymer nanogel based on poly(methacrylamide) containing 3,4-dihydroxy-L-phenylalanine (DOPA) groups (P(mDOPA) and polyallylamine (PAH) containing primary amino groups (Pox(mDOPA)-$Ag^0$/PAH). The obtained Pox(mDOPA)-$Ag^0$/PAH nanogel was characterized using appropriate techniques such as transmission electron microscopy (TEM), dynamic light scattering (DLS), and selected area electron diffraction (SAED), which clearly demonstrate the formation of silver nanoparticles embedded in the polymer nanogel. The deposition of the polymer coating onto a stainless steel (SS) substrate through a "deep coating" procedure using the poly(cationic) (P(mDOPA)-co-P($DMAEMA^+$)) copolymer and Pox(mDOPA)-$Ag^0$/PAH nanogel was confirmed by energy-dispersive X-ray analysis and scanning electron microscopy (EDX-SEM). The presence of silver, carbon, oxygen, and nitrogen on the substrate, originating from the Pox(mDOPA)-$Ag^0$/PAH nanogel, has been confirmed. The mechanical properties of the resulting coating were determined through micro-scratch testing, scanning electron microscopy, nanoindentation, and high-speed nanoindentation. The analyses revealed that the obtained coatings exhibit good adhesion, scratch resistance, and mechanical properties.

To obtain photoactive polymer coatings with embedded silver nanoparticles, functionalization of the polymer coating with a photosensitizer based on protoporphyrin was conducted. The photosensitizer was pre-modified to introduce amino groups into its structure. The synthesis of amino-modified protoporphyrin (PPIX-ED) was confirmed through various spectroscopic analyses such as ATR-FTIR, $^1$H NMR, UV-Vis, and fluorescence spectroscopy.

The surface and mechanical properties of the obtained photoactive antibacterial coatings were investigated using energy-dispersive X-ray analysis and scanning electron microscopy (EDX-SEM), micro-scratch testing and scanning electron microscopy, nanoindentation, and high-speed nanoindentation (XPM). The EDX-SEM analysis revealed the presence of carbon (C), oxygen (O), and nitrogen (N), with a less pronounced presence of silver. The micro-scratch test showed higher scratch resistance and better adhesion of the coating to the SS substrate compared to the polymer coating with embedded silver nanoparticles. Confirmation of this was also provided by the improved mechanical properties obtained from nanoindentation and XPM analysis.

To compare the properties of coatings with and without the presence of silver nanoparticles in the second polymer layer, coatings were obtained where the second polymer layer consisted of cross-linked Pox(mDOPA)/PAH nanogel, and the latter of amino-modified protoporphyrin. From the conducted mechanical tests, it was concluded that the coatings consisting of Pox(mDOPA)/PAH nanogel with included silver nanoparticles and a final layer consisting of amino-modified protoporphyrin exhibit the best adhesive and mechanical properties.

Photoactive coatings were also obtained, where the final layer consisted of protoporphyrin with included silver nanoparticles. For this purpose, a complex of amino-modified protoporphyrin IX with included silver nanoparticles was synthesized, which was confirmed by fluorescence spectroscopy, ATR-FTIR, TEM, and SAED analyses. The mechanical properties of the obtained coatings determined by nanoindentation show values close, but not as good as those of the coatings consisting of Pox(mDOPA)/PAH nanogel with included silver nanoparticles and a final layer consisting of amino-modified protoporphyrin.

### 5.4. Testing the Antibacterial Properties of Modified Polymer Coatings (Publications 8, 15, 17, 18)

The antibacterial properties of the polymer coatings modified with photosensitizers were determined against Gram-positive and Gram-negative bacteria using the disc diffusion method (DDM). The results show that all coatings exhibit strong antibacterial activity, which is more pronounced against Gram-negative bacteria due to differences in their membrane structure, notably the thickness of the peptidoglycan layer. The coatings consisting of Pox(mDOPA)/PAH nanogel with incorporated silver nanoparticles and a porphyrin-based photosensitizer, as well as those where the top layer is composed of a complex of amino porphyrin/silver nanoparticles, demonstrate the most prominent antibacterial properties. The superior results obtained for these coatings suggest that their antibacterial activity is a result of a combined effect of the photosensitizer's photodynamic activity and its antibacterial activity, as well as the antibacterial activity of the silver nanoparticles, attributed to an alternative mechanism of action.

The obtained photoactive antibacterial coatings and demonstrated antibacterial activity against Gram-negative and Gram-positive bacteria are of exceptional societal importance given the increasing resistance of many microbes to traditionally used agents.