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Biocidal Coatings against Gram-Positive Bacteria from Linear and Branched Polycations: The Decisive Role of the Diffusion Coefficients of Macromolecules

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Abstract: Positively charged polyelectrolytes hold significant potential as materials for creating antibacterial coatings. We examined the physicochemical and mechanical properties of the macromolecules in water solutions and in coatings for the series of branched polyethyleneimine (PEI) and linear polydiallyldimethylammonium chloride (PDADMAC) with different molecular weights. The microbiological study was conducted to analyze the biocidal activity of the polycation solutions and coatings towards foodborne bacteria. While the moisture saturation of the polycationic coatings and biocidal activity did not significantly depend on the chemical nature of charged groups or the molecular weight or architecture of macromolecules, the lowering of the molecular weight of polymers resulted in the loss of cohesive forces in the coatings and to a dramatic loss of stability when being washed off with water. The diffusion coefficient (D_0) of macromolecules was identified as a key parameter for the wash-off mechanism. Films formed by molecules with a D_0 below 1×10^{-7} cm^2/s demonstrated a high resistance to wash-off procedures. We demonstrated that PEI and PDADMAC samples with high molecular weights showed high antimicrobial activity towards *L. monocytogenes*. Our results highlight the importance of macromolecule characteristics in the development of new biocidal coatings based on polycations.

Keywords: polycation; polyethyleneimine; polydiallyldimethylammonium chloride; polymer coating; antibacterial coating; foodborne infections; Gram-positive bacteria; biocidal coating; biocide coating

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1. Introduction

The United Nations General Assembly set a goal for sustainable development in 2015 to fight against hunger, which includes ensuring food security [1,2]. However, one of the major problems that threaten food safety is the formation of biofilms and the spread of pathogenic bacteria in the premises for the production and storage of food products, as well as on equipment for its transportation [3–7]. Biocidal coatings are coatings made to stop the growth of bacteria or eliminate them [8,9]. The use of biocidal coatings on food contact surfaces, equipment, and packaging is an effective way to prevent the growth and spread of harmful microorganisms [8]. These coatings contain antimicrobial agents, such as silver ions, organic antibiotics such as chlorohexidine, etc., which work by attacking the cell walls of microorganisms and preventing their growth [8,10–13].

Salmonella, Listeria and *Escherichia coli* are just a few of the major foodborne pathogens that biocidal coatings have been proven to be effective against [14,15]. Overall, the use of

biocidal coatings in the food industry is an effective and important measure to reduce the risk of foodborne infections. It can help protect the health and well-being of consumers and also reduce the economic impact of foodborne illnesses.

Although it is normal procedure to treat production facilities with formulations based on conventional antibiotics, it may not always lead to the desired result due to the low adhesion of the biocides to the surfaces being treated and to the rapid development of bacterial resistance [16,17]. Therefore, there is a need for new, affordable and cheap antibacterial compositions that can be effectively used in food factories and shops. To tackle this issue, polymers have been used as new antibacterial functional coatings. In general, polymers are usually the matrix for low-molecular-weight biocides, which provide durability but do not have an antibacterial effect by themselves [18,19]. One specific class of polymers that have the potential to be used as biocidal coatings are the so-called biocidal polymers, which are macromolecules with functional groups that provide antibacterial action in each monomer unit [20–22]. Among this class of polymers, polycations are of particular interest. Polymers with quaternized amino groups were reported to be effective non-specific biocides with serious benefits compared to conventional low-molecular-weight antibacterial agents. Polycations do not cause the development of induced tolerance of the bacteria and do not give rise to mutant species [23]. Among commercially available polycations, polydiallyldimethylammonium chloride (PDADMAC) is of great potential [24,25]. One notable advantage of using PDADMAC as a biocide is its relatively low toxicity to humans and the environment. Quaternized polyethyleneimine (q-PEI) is the product of alkylation of a widespread polymer, polyethyleneimine (PEI), which was also admitted as effective biocide [26]. These both polymers are completely charged in a wide range of pH that supports their high antibacterial activity independently of the pH of surrounding media. Nevertheless, the initial PEI with primary and ternary amino groups was also reported to demonstrate antimicrobial activity [27,28]. The mechanism of the biocidal action of the polycations is still under discussion. The antibacterial activity of polycations is primarily due to their ability to disrupt the bacterial cell membrane and cause cell death [29]. Polycations are believed to interact with bacterial membranes through electrostatic interactions, forming a complex with the bacterial cell wall or membrane, leading to membrane destabilization. Once the bacterial membrane is disrupted, polycations can enter the bacterial cell and bind to intracellular molecules such as DNA and proteins, leading to further cell damage and eventually cell death.

PEI and PDADMAC have been shown to be effective against number of pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. They have also been found to be effective against antibiotic-resistant strains of these bacteria.

Overall, the antibacterial activity of PDADMAC and PEI makes them promising candidates for use in a variety of applications, including food processing and packaging.

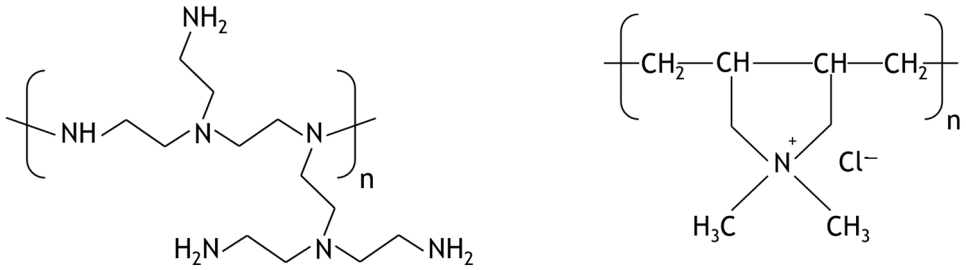
The average molecular weight and molecular weight distributions of polymers are key parameters that determine the physical and mechanical properties of the materials [30–32]. Due to the polymerization technique used to produce them, commercial samples of PEI and PDADMAC have high polydispersity. More accurate and regulated polymerization processes should be used to control the molecular weights and their distribution [33]. However, this could make getting polycations on an industrial scale more difficult and reduce their commercial availability.

Therefore, the key task is to establish the role of the molecular weight of polycations to determine the optimal degrees of polymerization required to create stable and effective antibacterial coatings. In the first part of this paper, we focus on exploring the main properties of coatings based on PDADMAC and PEI with different molecular weights and make a recommendation on the choice of the degree of polymerization for creating stable coatings. The second part is dedicated to the study of the biocide's efficiency against foodborne Gram-positive bacteria of the coatings from the optimal samples of the polycations.

2. Materials and Methods

2.1. Materials

The series of PEI with average molecular weight Mw = 1.3 kDa (PEI-1.3); Mw = 2 kDA (PEI-2); Mw = 25 kDa (PEI-25); Mw = 40 kDa (PEI-40); Mw = 70 kDa (PEI-70); Mw = 750 kDA (PEI-750) and PDADMAC with average molecular weight Mw < 100 kDa (PDADMAC-100); Mw = 300 kDa (PDADMAC-400), and Mw = 500 kDa (PDADMAC-500) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Structure formulas of polycations are presented in Figure 1.



(**a**) (**b**)

Figure 1. Structure formulas of polycations—PEI (**a**) and PDADMAC (**b**).

To study the interaction of coatings with model bacteria particles, the biomimetic lipid membranes were prepared by the procedure described elsewhere [34]. Briefly, 2-micron latex microspheres were completely covered with a lipid bilayer composed of mixtures of anionic and electroneutral lipids to simulate anionic cell surfaces of bacteria. Thus, latexes with supported lipid membranes were obtained.

Freshly cleaned glass cover slips with an area of 3.24 cm^2 were used in the experiments on washing off polymeric coatings. To estimate the adhesive properties of polyelectrolyte coatings and to measure the moisture saturation, freshly cleaned glass slides of 19.76 cm^2 area were used. The cleaning procedure was the following. The glass substrates were dipped in methanol and washed for a minute by vigorous shaking. Then, the glass substrates were treated with 1 M KOH solution and finally washed with bi-distilled water. Cleaned samples were dried in air.

2.2. Methods

2.2.1. Determination of the Diffusion Coefficients

Dynamic light scattering (DLS) was used to determine the diffusion coefficients of macromolecules in water–salt solutions by a procedure described elsewhere [24].

2.2.2. Coatings Resistance to Wash-Off with Water

The preparation of polyelectrolyte coatings on glass substrates and further investigation of their resistance to wash-off with water were carried out by gravimetry analysis in a procedure described elsewhere [24]. Briefly, the coatings with a mass of 4 mg each were formed on the cover slip substrate by deposition of the polyelectrolyte solution on the substrate surface with 100% area coverage. After drying of the coating, the consequent steps of the following procedure were: (a) covering of the modified surface with a thin layer of water (200 μL per cover slip); (b) 2 min of incubation and (c) further removal of liquid. Then, the residual masses of dried coating were analyzed. Relative humidity during mass measurements did not exceed 20%.

2.2.3. Water Content in Coatings Analysis

A glass slide was completely covered with a polycation to form a film with an average mass of 24.4 mg by depositing a 20 mg/mL solution on the total area of the slide and a further 24 h incubation in a chamber with a relative humidity of 5%. Then, the sample was transferred to a chamber with a relative humidity of 13% for 24 h incubation. The

procedure was repeated with the different values of relative humidity in the chambers of up to 90%. The masses of the samples after each incubation were measured to analyze the increase in the mass of the coating by water adsorption. The control of the humidity was made using the Temperature and Humidity Datalogger DT-172 by CEM Test Instrument (Moscow, Russia). Thermogravimetric analysis (TGA) on STA 449 F3 Jupiter by Netzsch (Selb, Germany) was carried out for a series of lyophilized powders of polycations incubated in a chamber with a controlled relative humidity of 40%. The details of the experiment can be found in [35].

High-precision scales VLA-120 M by Gosmetr (Saint Petersburg, Russia) were used in the gravimetric studies.

2.2.4. Polycation Coatings Adhesive Properties Analysis

The glass slide was completely covered with a 20 mg/mL solution of the polycation. After 2 min of incubation, the non-adsorbed polymer was removed by washing of the sample in bi-distilled water. The second glass slide was deposited on the formed coating and was left to dry for 24 h. The resulting "sandwich" structure was analyzed by dynamometry using a tensile testing machine by Metrotest (Moscow, Russia) to estimate the stress required for the separation of two glass slides. Analysis of the experimental data was performed with software provided by the manufacturer.

2.2.5. Antibacterial Action of Polycations

The minimal inhibitory concentration (MIC) for the polycations was determined by the procedure of broth microdilution assay described in [36]. The Tryptic Soy Broth (TSB) medium was chosen, as it ensures the formation of biofilms.

The optical densities at 590 nm in 96-well microplates were analyzed with the VICTOR X5 Light Plate Reader by PerkinElmer (Waltham, MA, USA). The MIC was defined as the lowest concentration of the tested compound that resulted in no growth after 16–20 h.

The minimum bactericidal concentration (MBC) was defined as the lowest concentration of each of the tested polymers that results in the destruction of 99.9% of the tested bacteria [37].

For the cytometric experiments, *Pseudomonas aeruginosa* bacteria were stained using the LIVE/DEAD BacLight Bacterial Viability and Counting Kit (Thermo, Waltham, MA, USA). First, 987 μL of 0.9 wt% aqueous sodium chloride solution was mixed with 1.5 μL of a ready-made SYTO 9 solution, 1.5 μL of a ready-made propidium iodide solution and 10 μL of the bacterial culture. The mixture was thoroughly stirred and incubated in light-protective Eppendorf tubes for 15 min. Quantification of live and dead cell populations was done with a Guava EasyCyte flow cytometer (Merk Millipore, Darmstadt, Germany), while living cells turned green and dead cells turned red.

For the microbiological assessment of bacterial survival in solution, the daily broth cell culture was diluted 100 times with a nutrient medium, and the polymers were added at a 1:1 ratio. The mixtures were incubated for 18 h at 37 °C, then diluted 10 times with sterile distilled water and used for determination of colony-forming units (CFU) using a standard protocol. Bacterial samples without polymers were used as controls.

In order to prepare polymer films, glass slides were washed successively with potassium bichromate/sulfuric acid mixture, potassium hydroxide/methanol mixture and bi-distilled water and finally air-dried at RT. Then, 200 μL of a 2 wt% aqueous cationic polymer solution was applied to a freshly cleaned glass slide; the sample was air-dried at RT, resulting in a polymer film with a thickness of 0.15 mm. The glasses with deposited polymer films were put into the broth cell culture and incubated for 18 h at 37 °C. After that, the glasses were washed three times with distilled water and transferred to test tubes with saline solution and shaken intensively. In the resulting washes, CFU were determined using a standard protocol.

2.2.6. Measurements of Morphology of Coatings

Atomic-force microscopy (AFM) imaging was performed using a scanning probe microscope Nanoscope IIIa (Nanoscope, Dallas, TX, USA) operating in a tapping mode in air. Cantilevers from silicon with resonance frequencies of 140–150 KHz from TipsNano (Zelenograd, Russia) were used. A 15 mm × 15 mm cover glass was put in a 1 wt% polycation solution for 5 min. After that, the glass was transferred into bi-distilled water and rinsed for 1 min, resulting in a removal of excess polymer, and the sample was left to dry in air.

3. Results

3.1. Samples Characterization

The diffusion coefficients of the samples of PDADMAC and PEI were studied by means of DLS. The dependencies of the diffusion coefficients (D) of polycations upon their concentration were measured in 0.15 M of NaCl solution in Tris buffer with pH 7 to avoid a polyelectrolyte swelling effect. Extrapolation of the concentration dependencies of D to zero concentration allowed us to estimate the resulting D_0 values, which are presented in Table 1.

Table 1. Characteristics of polycations.

Polycation	Diffusion Coefficient, cm^2/s *	Pw
PDADMAC-100	4.2×10^{-7}	620
PDADMAC-300	3.0×10^{-8}	1860
PDADMAC-500	8.0×10^{-8}	3100
PEI-1.3	2.5×10^{-5}	30
PEI-2	6.0×10^{-7}	50
PEI-25	2.5×10^{-7}	580
PEI-40	1.3×10^{-7}	930
PEI-70	5.8×10^{-8}	1630
PEI-750	N/A [1]	17,440

* The diffusion coefficient of the largest fraction in multi-peak distributions.

3.2. Screening of the Antibacterial Activity of PEI and PDADMAC

MICs of bacterial growth of *B. subtilis* were measured for several samples for brief screening. The results are presented in Table 2. Antibacterial activity of polycations under investigation was found be similar. No difference in biocidal activity between PEI and PDADMAC of high molecular weights was observed. However, a decrease in MIC values was detected for oligomeric fractions of PEI.

Table 2. *B. subtilis* MICs for PDADMAC and PEI.

Polycation	MIC ($\mu g\ mL^{-1}$)
PDADMAC-100	0.025
PDADMAC-200	0.025
PDADMAC-500	0.025
PEI-1.3	0.2
PEI-2	0.2
PEI-25	0.05
PEI-70	0.0125
PEI-750	0.025

3.3. Estimation of Moisture Saturation of Coatings

The absorbance of water by coatings of polycations was studied at different environmental humidity. The results are presented as the dependencies of an increase in coating weight upon the relative humidity. For the PEI coatings at 5% relative humidity, the mass of the absorbed water was 3% of the total mass of dry film. Further increases in humidity

up to 60% resulted in gradual increases in the weight of the coatings of up to 35% (see Figure 2a). For the whole range of PEI samples, no impact of molecular weight on the amount of absorbed water at a certain humidity of the surrounding media was detected. Similar dependencies were observed for coatings made from PDADMAC. At a relative humidity of 5%, the mass of the absorbed water was 5% of the total mass of the dry film. Further increases in humidity up to 60% resulted in gradual increases in the weight of the coating of up to 31% (see Figure 2a). For the whole range of PDADMAC samples, no impact of molecular weight on the amount of absorbed water at a certain humidity of the surrounding media was detected. It is important to stress that the polycationic coatings on the glass substrates did not change their visually observed shapes during the experiment at the values of humidity of less than 65%. Further increases of the humidity resulted in the formation of water droplets on the film surfaces.

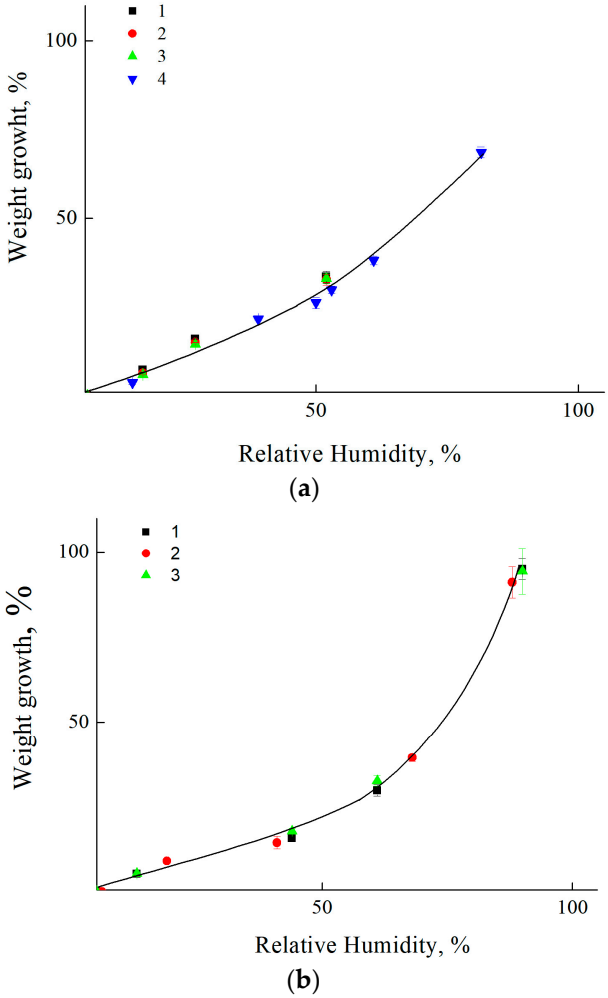


Figure 2. (**a**) Relative mass of the films of PEI versus environmental humidity. PEI-1.3 (1); PEI-25 (2); PEI-70 (3); PEI-750 (4); (**b**) Relative mass of the films of PDADMAC versus environmental humidity. PDADMAC-100 (1); PDADMAC-300 (2); PDADMAC-500 (3).

The saturation of the polycationic films with water was controlled by TGA. The powders of polycations were kept in a chamber with a relative humidity of 40% prior to the experiment. The TGA curves of water-saturated powders of PDADMAC-500 and PEI-750 are presented in Figure 3. The loss of the mass on the curves corresponds to the loss of the absorbed water. The detected loss of weights for polycations was found to be about 27 ± 1%. This result is in good agreement with the gravimetric data presented in Figure 2.

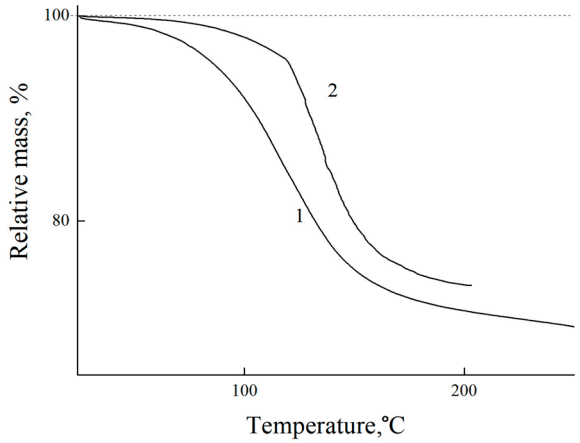


Figure 3. TGA curves of polycation powders. PDADMAC-500 (1); PEI-750 (2), scanning rate 200 K/min; relative humidity of the environment 40%.

3.4. Wash-Off Resistance of Polycation Coatings

The resistance of the polymer films towards wash-off with water was controlled by the weight loss of the sample. The results are presented in Figure 4 as the dependencies of the residual mass of the film upon the number of the wash-off cycles. For PEI-750 and PEI-70, a weight loss of about 50% was observed after the first wash-off cycle, while for the samples of PEI-40, PEI-25, PEI-3 and PEI-1.3 the observed weigh loss was from 72% to 83%, corresponding to a decrease in molecular weight (see Figure 4a). With the increase in the number of wash-off cycles, a linear tendency could be observed: coatings from PEI with high molecular weights—PEI-70 and PEI-750—have similarly high tolerances towards wash-off, while for the molecules with weights of PEI-40 and lower the process of the film weight loss goes faster, with a lowering of the number of cationic units in the macromolecule. Almost all PEI coatings were removed after six cycles of wash-off. The similar behavior of the coatings was observed for PDADMAC molecules (see Figure 4b). For PDADMAC-500 and PDADMAC-300, about 65% of the weight loss was observed after the first wash-off cycle, and almost all of the polycation was removed after four wash-off cycles. At the same time, PDADMAC100 lost about 80% of its coating mass after the first wash-off cycle.

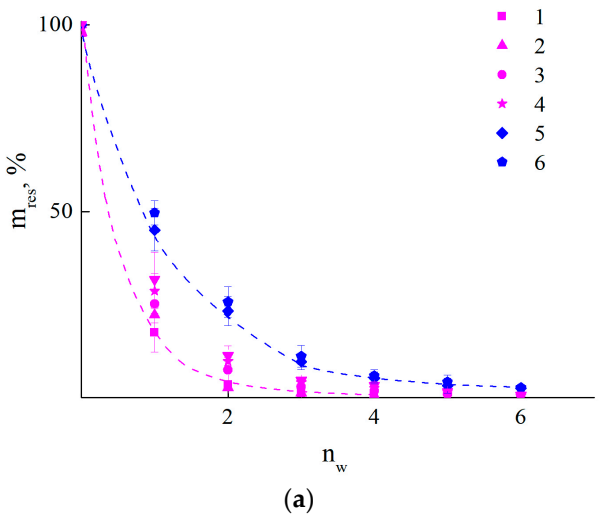


Figure 4. *Cont.*

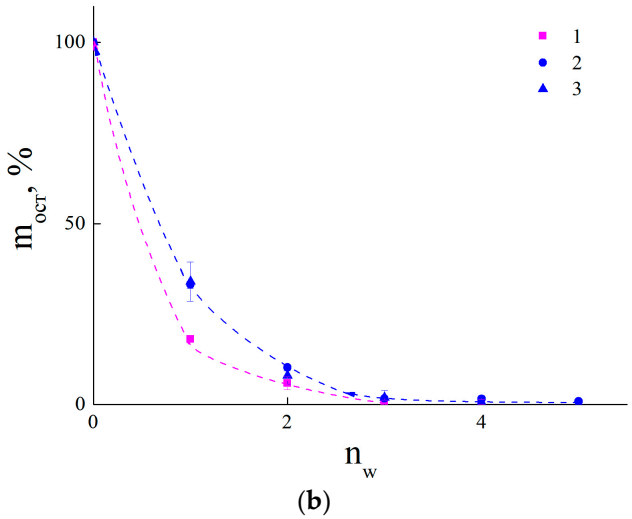


Figure 4. (**a**) Dependence of the percentage of residual mass of coatings (m_{res}) formed from PEI on the number of wash-off cycles (n_w); PEI-1.3 (1); PEI-2 (2); PEI-25 (3); PEI-40 (4); PEI-70 (5); PEI-750 (6); (**b**) Dependence of the percentage of residual mass of coatings (m_{res}) formed from PDADMAC on the number of wash-off cycles (n_w); PDADMAC-100 (1); PDADMAC-300 (2); PDADMAC-500 (3).

3.5. Adhesive Properties of Polycation Coatings

Dynamometry was used to analyze the adhesion of PEI and PDADMAC coatings on the glass substrate. For the different samples of each polycation, the curve stress versus time was obtained. The typical experimental curves are presented in Figure 5. The peak value of the applied stress was taken as a parameter characterizing the adhesive properties of the polyelectrolyte coating. The results of the dynamometry tests for the whole range of molecular weights of PEI and PDADMAC are presented in the Table 3. While PDADMAC coatings have demonstrated higher peak stress values than PEI coatings, no considerable difference for the peak values was detected in the line-up of polycations of identical structure with high molecular weights. Coatings made of oligomers demonstrated a lowering of the peak stress, reflecting the formation of films with lower mechanical properties.

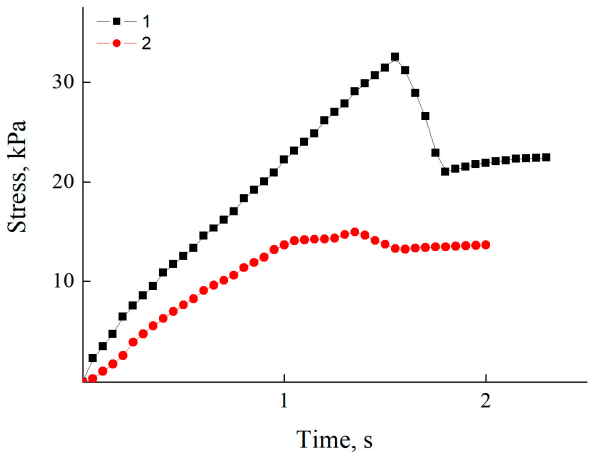


Figure 5. Typical stress versus time curves for PEI-70 (1) and PDADMAC-300 (2). Traverse rate 5 mm/min. Relative humidity 18 ± 2%.

Table 3. Maximal stress values of the polycationic films.

Polycation	Stress, MPa
PDADMAC-100	26,600
PDADMAC-300	30,900
PDADMAC-500	31,500
PEI-1.3	11,600
PEI-70	18,000
PEI-750	19,000

Further experiments were performed with PEI-750 and PDADMAC-500.

3.6. The Structure of the Polycationic Coatings

Figure 6 demonstrates AFM images of the coatings from PEI-750 and PDADMAC-500 on a glass surface. Almost-smooth continuous films were obtained for each studied polycation.

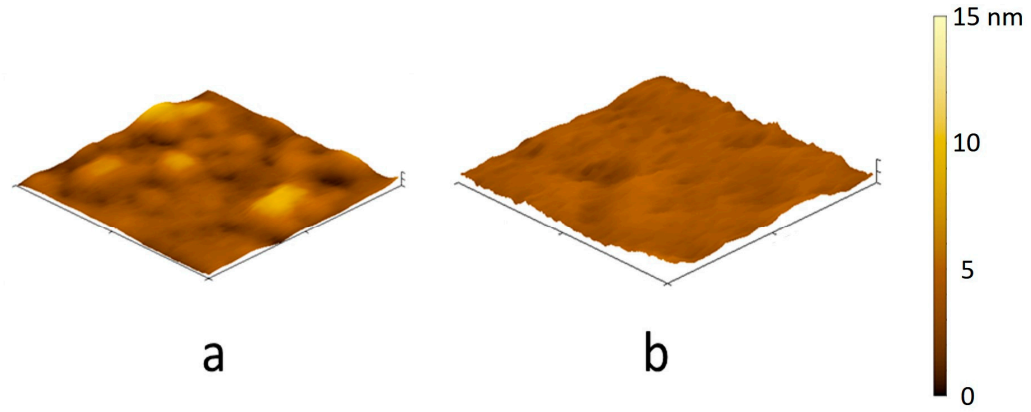


Figure 6. AFM images of four polycationic coatings from PEI-750 (**a**) and PDADMAC-500 (**b**) on a glass surface. Scanning area 500 nm × 500 nm.

3.7. Interaction of the Polycationic Coatings with Model Cell Membranes

To evaluate the ability of the coatings from the PEI and PDADMAC to immobilize bacteria, an experiment with a latex model decorated with a lipid bilayer simulating a bacterial membrane was performed. The optical images of the pure glass, glass coated with PEI and glass coated with PDADMAC after contact with the latex suspension are presented in Figure 7. Almost no adsorption of latex on the pure glass surface was observed. Several latex particles per significant large areas of the glass could be attributed to van der Waals interactions-driven adsorption. For the coatings of polycations, numerous latex particles could be found on the surface, reflecting electrostatic adsorption of anionic microparticles on the surface with cationic groups.

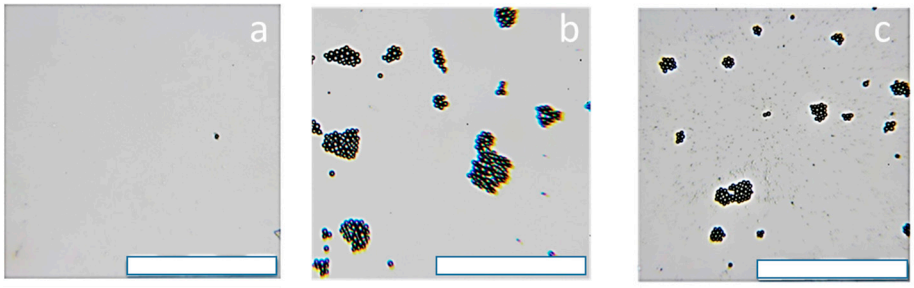


Figure 7. Optical microscope images of glass surface (**a**); glass covered with PEI-750 (**b**) and glass covered with PDADMAC-500 (**c**) after contact with latex. White scale-bar 50 μm.

3.8. Biocidal Properties of the Polycationic Coatings against Food-Born Bacteria

At first, the biocidal activity of polycations is measured by the microbiological assessment of bacterial survival of food-borne bacteria *L. monocytogenes*. The results are presented in Table 4.

Table 4. Microbiological method for testing activity of polymers towards *L. monocytogenes* in solutions.

Sample	CFU/mL
Control	5.0×10^7
PEI-750	0
PDADMAC-500	0

Both PEI and PDADMAC showed an absolute antibacterial effect, killing 100% of *L. monocytogenes*.

The growth-inhibitory effect at the lowest exposure concentration was PDADMAC-500. The concentrations of 2.5 mg/mL turned out to be the minimum inhibitory level at which visual growth was not detected in the liquid medium, and the concentration of 5 mg/mL was the minimum bactericidal concentration at which less than 99.9% of cells grew on agar plates. For PEI-750, the value of MIC of 5 mg/mL did not differ significantly, reflecting high antibacterial activity (Table 5).

Table 5. MIC and MBC method for testing activity of polymers towards *L. monocytogenes* solutions.

Polymers	*L. monocytogenes*	
	MIC, mg/mL	MBC, mg/mL
PEI-750	5	5
PDADMAC-500	2.5	5

Then, antibacterial properties of films, prepared via drying of the polycation aqueous solutions, were examined. Polymer layers were formed on glass slide pieces, which were then inoculated with *L. monocytogenes*. As follows from the data of Table 6, all polymers quantitatively suppressed the growth of bacterial cells.

Table 6. Inhibition of *L. monocytogenes* bacterial film formation by polymers.

Sample	Control	PEI-750	PDADMAC-500
CFU/mL	4.0×10^7	0	0

The cells on the glass surfaces were visualized using the Live/Dead Kit. Figure 8 reflects fluorescence of cells treated with the Live/Dead Kit and observed through a fluorescent microscope. SYTO-9 from the kit only stained living bacteria green, while propidium iodide entered dead bacteria through defects in the cell walls and colored the bacteria from light yellow to dark brown. A control glass with no polymer covered by the *L. monocytogenes* cells (photo 1) demonstrates a bright green color, which definitely indicates the intact structure of the adsorbed cells. Contrastingly, photos 2–3 for cells on the glasses, covered with the cationic polymers, have colors from yellow to very brown, which proves the death of cells after their deposition over films from the cationic polymers.

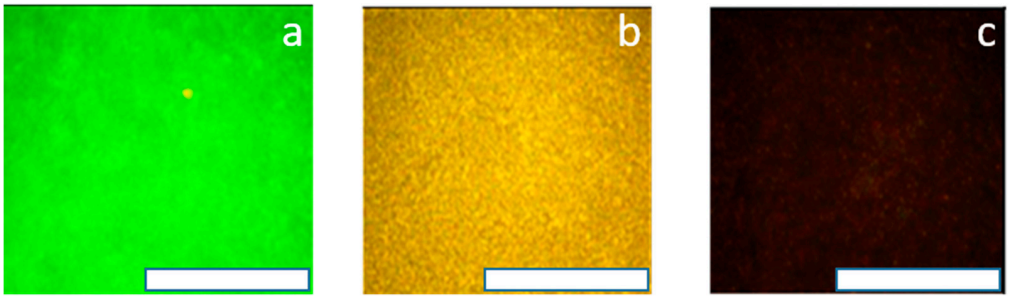


Figure 8. Fluorescent microscope images of *L. monocytogenes* cells attached to the glass surfaces with polycations. The cells were treated with the Live/Dead Biofilm Viability Kit. Control with no polymer (**a**); PEI-750 (**b**); PDADMAC-500 (**c**). White scale-bar 50 μm.

4. Discussion

Polymer coatings are widely used as protective layers to prevent formation of biofilms on different surfaces [38–40]. In general, these protective polymers could be divided in classes of anti-fouling coatings, bacteria-killing coatings and matrixes for distribution of low-molecular-weight biocides. The first ones prevent the adsorption of the bacteria on the surface of the treated material, while the second interact with microorganisms, causing disorders in their functionalization. Polycations commonly act as biocidal molecules. One of the proposed mechanisms of action of polycations is their interaction with the negatively charged membrane of the bacterial cell due to their own positive charge [40,41]. As a result, this leads to structural rearrangements in the lipid bilayer and disruption of the vital activity of bacteria or their deaths [42]. L.D. Melo et al. report that PDADMAC tends to cause membrane stress in bacterial cells, which can ultimately lead to the destruction of cellular structures [43]. PEI is known to contain a sufficient fraction of protonated amines that can bind to negatively charged bacterial cell surface components, which can lead to cell depolarization, cell wall/membrane disruption, and cell lysis [44]. It was found by lkka M. Helander that when PEI interacts with a culture of gram-negative bacteria in solution, it permeates their outer membrane, making the bacteria more susceptible to the environment, including additionally added antibiotics [45]. There is also evidence that polycations can affect the stage of protein biosynthesis (translation) [46]. However, the complete mechanism of the antibacterial action of polycations is still under investigation. The presence of a positive charge on the coating cannot ensure a biocidal effect, as was demonstrated by Sorzabal-Bellido and colleagues: polydimethylsiloxane with a surface layer modified with primary amino groups did not cause a significant antibacterial effect [47]. It seems that not only a charged layer but a number of cationic units on a flexible chain should exist on a biocidal coating. Enhancement of the antibacterial action of polyelectrolyte coatings could be achieved with the use of additional biocides of non-polymer nature and anti-fouling macromolecules. When it comes to biocides, the question of the impact of such substances on humans is important. PDADMAC is one of the brightest representatives of biocidal polycations which are allowed for direct contact with humans. It has also found its medical uses, as a biocidal additive in dental materials, and as a component of wound dressings [48,49]. At the same time, PEI is approved by the FDA for use as an indirect food additive [50].

Both polycations, PDADMAC and PEI, were shown to possess antimicrobial activity towards *B. subtilis* in solutions. Two important observations should be pointed out. The nature of an amino group in a polycation and the degree of polymerization do not play essential roles in the antibacterial activity of the studied polymers in solutions. Taking into account previously reported data on MIC of a hyperbranched copolymer of epichlorohydrin and ethylenediamine towards *B. subtilis* with values of a similar order, we may state that a linear or hyperbranched structure of the macromolecule cannot be considered as a key parameter affecting the biocidal activity of polycations [46].

The application of an aqueous solution of polycations to the surface of a hydrophilic glass with further drying in the air leads to the formation of a polymer coating. The adhesion of the macromolecules on the glass is driven by electrostatic forces between negatively charged silanol groups and amino groups. Most of the macromolecules in an adsorbed layer form a film of interpenetrated chains. Polycations are known to be hygroscopic. Therefore, it is reasonable to expect that polycation films will absorb water from the environment. In solutions, solvation of the ammonia salts depends on the nature of the amino groups, but for the studied films of polyelectrolytes no significant influence of the primary, ternary and quaternary amino groups on swelling in an environment with controlled humidity was found.

It is obvious that degree of polymerization of macromolecule should affect the strength of adhesion on a glass surface. The dynamometric experiments have shown that the mechanical break of the polyelectrolyte films is predominantly governed by cohesion forces. The shape of the stress curves in Figure 5 reflects some correspondence with a cohesion break mechanism. For the PEI samples with high molecular weights, the peak stresses were almost identical, with a mean value of 18,500 Pa, and a decrease in the mechanical properties of films was observed for an oligomer fraction with a mean value of peak stress of 11,600 Pa. The same behavior of the mechanical properties was observed in the films from different samples of PDADMACs. For the samples with high molecular weights, the peak stresses were almost identical, with a mean value of 30,800 Pa, and the decrease in the mechanical properties of films was observed for an oligomer fraction with a mean value of peak stress of 26,600 Pa. These results are in good agreement with the cohesion-governed mechanism of the film break. Moreover, the differences in absolute values of peak stresses between PEI and PDADMAC macromolecules could be attributed to differences in macromolecule architectures. Linear PDADMAC macromolecules could penetrate between many lateral layers inside the film, while for the branched PEI molecules this possibility is restricted.

The vanishing of the polyelectrolyte coating with water depends on two major parameters. First, the nature of the amino group: for the quaternary amino groups in PDADMAC, the process of the dissolving of the film takes place faster than for the PEI with primary and ternary groups. Second, the molecular weight of macromolecules: with the reaching of critical values of molecular weights of polycations, the films undergo fast mass loss under the wash-off procedure. For the PDADMAC molecules, this critical weight was 100 kDa, while for the PEI this value was 40 kDa. Both polycations have different masses of monomer units and different architectures. Therefore, such parameters as "degree of polymerization" and "average molecular weight" could not be used for direct comparison of the results for these polycations. Nevertheless, we have demonstrated that the diffusion coefficient of the polycation is the parameter that allows us to describe correctly the behavior of the films in the wash-off procedure. The critical value of the $D_0 = 1 \times 10^{-7}$ cm^2/s determines the resistance of the polyelectrolyte film towards fast wash-off for both PEI and PDADMAC.

Despite it being reported that an increase in the molecular weight of the polymers of the same structure could decrease or increase their biocidal activity [51–53], we have demonstrated that the molecular weight of the polycation has a greater effect on the mechanical properties of the films and their behavior under watering conditions. Only oligomers of PEI with a mass of 2 kDa and less demonstrated a decrease in biocidal activity. Therefore, polycations with high molecular weights have a higher potential for the preparation of effective biocidal coatings.

Thus, the efficiency of polycationic coatings towards foodborne bacteria *L. monocytogenes* was analyzed using the samples with higher values of Mw: PEI-750 and PDADMAC-500. The morphology of the coatings obtained by AFM were confirmed to be above the coating. Hence, bacteria with negatively charged membranes will adsorb on the film and undergo the action of the polycations. At the same time, the macromolecules that leave the surface of the film during the watering process could act as biocides in suspension of the bacteria that were not adsorbed on the film. Both PEI and PDADMAC were demonstrated

to have antibacterial activity in solution and on the surface of films against *L. monocytogenes*. Therefore, the choice of the polycation and its molecular weight for the formation of the biocidal coatings should be determined more by requirements than by the mechanical properties of the supposed coating.

5. Conclusions

Branched PEI and linear PDADMAC of different molecular weights were studied to estimate the roles of the chemical nature of the charged groups, the architecture and the degree of polymerization of macromolecules in the physical–mechanical and antibacterial properties of the coatings from these polymers on glass surfaces. Surprisingly, the values of MIC for polyelectrolytes did not depend on molecular weights of the polymer samples in a wide range of the masses. The chemical nature of the amino group (primary, ternary or quaternary) was not found to possess a significant impact on the biocidal properties of macromolecules. For the coatings from the different samples of PEI and PDADMAC, it was demonstrated that saturation of the films with water depends on the humidity of the surrounding media but not on the structural and chemical characteristics of the polycations. The mechanical properties of the coatings from polycations were demonstrated to have a determining cohesive nature between macromolecules over adhesion forces between surface and polymer film. Linear PDADMAC ensures a higher cohesive strength due to the possibility of penetrating between more layers in films than branched PEI. With the decrease in molecular weight, the mechanical stress required to break the coating is reduced for both series of PEI and PDADMAC. The ability of the polyelectrolyte film to resist wash-off with water strongly depends on the diffusion coefficient of the macromolecules that form the coatings. This parameter is more suitable for describing the decisive characteristics of macromolecules that allows one to compare polyelectrolytes with different architectures and chemical structures. Thus, the polycations with high molecular weights have higher potential for utilization in formation of coatings. Concerning the biocidal activity of the polycationic films, the antibacterial effect towards *L. monocytogenes* was demonstrated for both PEI and PDADMAC with no significant difference.

Therefore, the choice of the polycation for the effective biocidal coatings should be determined by the requirements for the mechanical properties of the films. These properties are affected by the diffusion coefficients, architectures and chemistry of macromolecules. At the same time, the antimicrobial activity of the coatings is determined by the polycationic nature of the coatings without a significant role being played by the nature of the amino group.

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