Antibacterial treatment of LDPE with halogen derivatives via cold plasma

A. Popelka^{1*}, I. Novák², M. Lehocký³, F. Bílek³, A. Kleinová², M. Mozetič⁴, M. Špírková⁵, I. Chodák²

¹Center for Advanced Materials, Qatar University, P.O. Box 2713, Doha, Qatar

²Polymer Institute, Slovak Academy of Sciences, Dúbravská cesta 9, 845 41 Bratislava 45, Slovakia

³Centre of Polymer Systems, University Institute, Tomas Bata University in Zlín, Nad Ovčírnou 3685, 760 01 Zlín, Czech Republic

⁴Department of Surface Engineering, Jožef Stefan Institute, Jamova cesta 39, SI-1000 Ljubljana, Slovenia

⁵Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Heyrovsky Sq. 2, 160 06 Prague, Czech Republic

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Abstract. The factor limiting the application of low-density polyethylene (LDPE) in healthcare is its high susceptibility to bacterial growth. For this reason, we here investigated antibacterial treatments of LDPE foils using appropriate antibacterial agents. Benzalkonium chloride and bronopol were selected because of their satisfactory antibacterial effect, which has been confirmed by their application in the medical and cosmetic industries. The aforementioned substances were immobilized by a multistep approach via the grafting of polyacrylic acid (PAA) brushes onto LDPE surfaces pre-treated with low-temperature plasma. Measurements of the surface energy, peel strength of the adhesive joints, X-ray photoelectron spectroscopy (XPS), Fourier-transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR), and atomic force microscopy (AFM) were used to investigate the surface and adhesive properties of the antibacterial-treated LDPE. Moreover, the antibacterial effect was determined via measurements of the inhibition zone of the *Staphylococcus aureus* (*S. aureus*) bacterial strain. The antibacterial activity of benzalkonium chloride was observed to be more pronounced than that of bronopol. Inhibition-zone measurements of *Escherichia coli* (*E. coli*) were also conducted, but an antibacterial effect was not observed.

Keywords: adhesion, benzalkonium chloride, bronopol, plasma treatment, antibacterial activity

1. Introduction

Polyethylene (PE) has found uses in various biomedical applications [1], such as the production of catheters [2]. However, PE often leads to unfavorable infections that represent serious clinical complications [3]. These infections can result in implant failure, which can lead to numerous health problems [4]. To overcome this disadvantage, an anti-infection modification of PE is frequently applied. Anti-infective properties of polymers are achieved by the incorporation of an anti-infection agent into the polymer bulk or by copolymerization of an anti-infection agent with a monomer. Recently, an anti-

bacterial surface treatment was demonstrated to be the preferred choice because it exhibited several advantages: it did not influence the bulk properties of the polymer, antibacterial agents were not released from the polymer volume, and the procedure was relatively simple and effective [5].

In this study, a multistep approach consisting of plasma surface treatment, radical graft polymerization [6, 7] of acrylic acid (AA) and the immobilization of antibacterial agents, was used (Figure 1) [8]. Surface treatment with low-temperature plasma plays a pronounced role in the surface treatment of polymers without influencing their volume properties

*Corresponding author, e-mail: anton.popelka@qu.edu.qa

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Figure 1. Multistep approach of the antibacterial treatment: 1. plasma treatment; 2. radicals generation; 3. AA radical graft polymerization; and 4. immobilization of the antibacterial agents

[9, 10]. Moreover, the use of low-temperature plasma is an environmental friendly, clean and dry process [11]. Low-temperature plasma facilities are often used in the electronic, aerospace, automotive, textile and biomedical industries [12, 13]. The products of the low-temperature plasma discharge, such as electrons, ions and excited atoms, are capable of interacting with the solid polymer surface. These interactions can lead to the functionalization (i.e., the creation of functional groups), etching (i.e., ablation), polymerization, or cross-linking processes [14]. Diffuse coplanar surface barrier discharge (DCSBD) plasma generators are used by facilities to generate the high surface power density of plasma discharges. The main advantage of this plasma system is its ability to operate at atmospheric pressure (i.e., it is suitable for continuous processes) [15]. The lifetime of electrodes is prolonged through the use of electrodes embedded in Al_2O_3 and connected to a cooling system [16]. Moreover, the high surface density of plasma generates visually macroscopically homogeneous low-temperature plasma discharges [17]. Low-temperature plasma can also initiate a radical graft polymerization of acrylic acid (AA) on the polymer surface [18]. The brushes of the resulting polyacrylic acid (PAA) represent an effective interfacial layer for antibacterial immobilization [19]. Benzalkonium chloride (Figure 2a) is known to be a safe, nontoxic biocide and is currently used in human pharmaceuticals [20]. Bronopol (Figure 2b) is another efficient antibacterial agent with a low clinical toxicity and as an effective protecting agent is used in various applications (e.g., water protection, cosmetics) [21]. These antibacterial agents are capable of inhibiting the proliferation of microorganisms on

Figure 2. Scheme of: a) benzalkonium chloride and b) bronopol

the polymer surface [22]. The antibacterial activity of treated LDPE was investigated as a tool for eliminating the bacterial strains *Staphylococcus aureus* (*S. aureus*)—a gram-positive coccal bacterial strain that can lead to, for example, urinary tract infections and *Escherichia coli* (*E. coli*), which is a gram-negative rod-shaped bacteria that causes, for example, intestinal disease [23].

2. Experimental

2.1. Materials

Branched low-density polyethylene LDPE foils BRALEN FB 2-17 ($T_m = 114^\circ\text{C}$, $T_g = -110^\circ\text{C}$, $M_w = 260\,000$ and $\text{PDI} = 22.5$) with a thickness of $20\,\mu\text{m}$ were used in this study. The foils were supplied by Slovnaft-MOL (Slovakia) and contained no additives. These LDPE foils comply with the Food Contact Regulations and are approved for food contact; in addition, the grade is appropriate for use in pharmaceutical packaging products. Acrylic acid (AA, 99.0%, anhydrous, Acros Organics, Belgium) was used for the radical graft polymerization. Sodium metabisulfite (99.0%, Reagent plus, Merck, USA) was used as an inhibitor of acrylic acid homopolymerization. *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDAC, 98.0%, Fluka, USA) was used as a carboxyl-group activator for enabling immobilization of antibacterial agents. Benzalkonium chloride (benzyl-dimethyl-tridecylazanium chloride, Sigma-Aldrich, Denmark) and bronopol (2-bromo-2-nitropropane-1,3-diol, Fluka, Germany) were used as antibacterial agents. Ultrapure water (produced by a Heal Force NW ULTRA pure water system) was used in the experiments. Diiodomethane (99.0%, Reagent plus), formamide (99.5%, molecular biology grade), and glycerol (99%, for molecular biology), which were supplied by Sigma-Aldrich (USA), were used as testing liquids for the surface wettability measurements.

2.2. Plasma treatment

The DCSBD plasma system developed by Černák et al. [15] was used to activate the LDPE foils. The

surface treatment was performed under dynamic conditions (i.e., the samples were in motion above the electrodes) at atmospheric pressure; the surface power density was 1 W/cm^2 , the duration of the plasma treatment was 15 s, and air atmosphere was used as a working gas. Both sides of all samples were treated. A schematic of the DCSBD plasma equipment is shown in Figure 3. Two parallel banded systems consisting of several electrodes made of Ag paste (1 mm wide, $50 \mu\text{m}$ thick, and 0.5 mm spacing between the strips) were used to generate the low-temperature plasma discharges in an effective manner. These electrodes were embedded in 96% Al_2O_3 , which protects the electrodes from direct contact with the plasma discharges and prolongs their durability. Moreover, the plasma panel was connected to a cooling system to prevent its overheating. A high-frequency sinusoidal voltage ($\approx 15 \text{ kHz}$, $U_m \approx 10 \text{ kV}$) was used to provide power to the electrodes led to the generation of

macroscopically homogenous plasma and resulted in a uniform surface treatment.

2.3. PAA grafting

Grafting of PAA onto the surface of the LDPE foil was performed immediately after plasma treatment by immersing the activated foil into a 10 vol% aqueous solution of AA for 24 h at 30°C . The solution also contained 0.1 wt% sodium metabisulfite as an efficient inhibitor of AA homopolymerization. The AA polymerization reaction led to the formation of PAA brushes, such was seen from AFM measurement and rendered it suitable for the effective immobilization of antibacterial agents. Finally, the prepared samples were thoroughly washed in distilled water for 5 min at 30°C to remove weakly bound or unreacted AA. The pre-treated samples were used for the immobilization of the antibacterial agents.

2.4. Benzalkonium chloride and bronopol immobilization

The PAA grafted substrates were subsequently immersed in a 0.1% (w/v) aqueous solution of EDAC at 4°C for 6 hours to activate the carboxyl groups, as shown in Figure 4. *O*-Acylisourea moieties are formed by this activation, and the PAA brushes are subsequently able to react with certain reducing agents. Two procedures were used: pre-treated LDPE samples were immersed in a benzalkonium chloride 2% (w/v) solution in distilled water for 24 hours at 30°C , and, alternatively, pre-treated LDPE samples were immersed in a bronopol 2% (w/v) solution in absolute ethanol for 24 hours at 30°C . Finally, samples with an immobilized antibacterial agent were washed in water and dried for 24 h at room temperature.

Figure 3. Schematic of the DCSBD plasma system

Figure 4. Carboxyl-group activation by EDAC

2.5. Surface wettability investigation

The surface wettability of the samples was investigated via sessile drop contact angle measurements. A surface energy evaluation system (SEE system, Advex-Instruments, Czech Republic) was used in this study. The CCD camera resolution was set to 1280×960 pixels to ensure that high-quality images of the drops were recorded. The contact angle refers to the angle between the solid/liquid interface and the liquid/vapor interface. Ultra-pure water, ethylene glycol, glycerol, formamide, and diiodomethane were used as test liquids. The contact angle was recorded after 3 s to allow thermodynamic equilibrium to be established between the solid, liquid, and gas phases. Average values of contact angles were used to calculate the surface free energy (γ^{tot}), and its polar (γ^{p}) and dispersive (γ^{d}) components were evaluated using the Owens-Wendt-Rabel-Kaelble regression model [24].

Moreover, changes in the graft yield of LDPE samples obtained by gravimetric measurements are also included. The graft yield (*GY*) was calculated by Equation (1):

$$GY = \frac{W_2 - W_1}{W_1} \cdot 100 [\%] \quad (1)$$

where W_1 and W_2 are the weights of the samples before and after the surface treatment [25].

2.6. Adhesive properties assessment

The adhesion properties between two materials are characterized by peel strength (force per unit width). The adhesive joint created from LDPE samples and poly(2-ethylhexyl acrylate) deposited on 15 mm polypropylene foil were used for the peel-strength measurements. Steel roller (3 kg) was used for the preparation of adhesive joint with constant distribution of adhesive forces. We performed these measurements by conducting 90° peel tests at a peel rate 10 mm/min using a universal INSTRON 4301 (UK) dynamometer equipped with 100 N load cell. The end parts of the LDPE samples were firmly fixed in clamps in a circular arrangement to achieve an even distribution of tension across the entire width. PP foil with an adhesive as a second part of the adhesive joint was fixed in the clamp connected to the load cell.

2.7. Surface morphology analysis

Atomic force microscopy (AFM) investigations of the surface topography were performed on a com-

mercial atomic force microscope (Dimension Icon, Bruker) equipped with an SSS-NCL probe, Super Sharp Silicon™-SPM-Sensor (NanoSensors™ Switzerland; spring constant 35 N/m, resonant frequency: 170 kHz). The areas for individual AFM measurements were carefully chosen after previous screening of the surface regularity. Measurements were performed under ambient conditions and in tapping mode. The scans covered the sizes from 0.5×0.5 to 10×10 μm^2 . Characteristic AFM images for each sample (area 10×10 μm^2) are represented in this paper.

2.8. Surface chemistry investigation

Fourier-transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) was used to determine the chemical composition of LDPE samples. The individual measurements were performed with a FTIR NICOLET 8700 spectrometer (Thermo Scientific, USA) using a single-bounce ATR accessory equipped with a Ge crystal at an angle of incidence of 45°. The spectral resolution and the number of scans were 2 cm^{-1} and 64, respectively, for each measurement. A pressure clamp was used to achieve sufficient spectral quality, which depends on a good contact between the crystal and investigated sample. The acquired spectra were analyzed using the OMNIC™ v8.1 spectroscopic software.

X-ray photoelectron spectroscopy (XPS) was used to quantify the chemical composition of LDPE samples. For this purpose, a TFA XPS Physical Electronics (USA) XPS instrument was used. The pressure in the chamber was approximately $6 \cdot 10^{-8}$ Pa. The LDPE samples were irradiated with X-rays over a 400 μm spot area using monochromatic Al $K_{\alpha 1,2}$ radiation at 1486.6 eV. The photoelectrons were detected with a hemispherical analyzer placed at an angle of 45° with respect to the normal to the surface of the LDPE samples. Each survey-scan spectrum was collected at a pass energy of 187.85 eV and at an energy step of 0.4 eV. An electron gun was used for surface neutralization. The concentration of individual elements was determined using the MultiPak v7.3.1 software (Physical Electronics).

2.9. Antibacterial efficacy tests

The antibacterial effect of treated LDPE samples was determined against the *S. aureus* (CCM 4516) bacterial strain via measurement of an inhibition zone diameter on agar. Nutrient agar No. 2 M1269

– 500 g (HiMedia Laboratories PII, Ltd.) was used in this study. Circle-shaped LDPE samples (diameter ≈ 8 mm) were washed with ethanol, dried and placed on an agar plate inoculated with the bacterial suspension (volume = 100 μL , concentration = 10^7 units mL^{-1}). The samples were incubated for 24 h at 37°C. After 24 h, the diameters of the inhibition zone were measured in 5 directions to obtain average values; the test was repeated three times. The inhibition zone area was calculated as the area of annulus between the inhibition zone borders and the LDPE samples.

3. Results and discussion

3.1. Surface wettability

The ability of liquid to remain in contact with the solid surface is expressed by the surface's wettability. The wettability belongs among a very important factor supporting the antibacterial behavior. Hydrophilic brush layer can be responsible for attraction of water and form a repellent layer on the surface. There is steric hindrance for proteins or microorganisms to adsorb to the surface and the adsorption is reduced by several orders of magnitude [26]. For the above mentioned reason, the wettability measurement has very high importance for a characterization of the antibacterial surfaces. The wettability denotes the angle between the liquid drop and the solid surface. The contact-angle measurements were used to determine the wettability. The contact angle of several testing liquids was used to evaluate the surface free energy. The surface free energy indicates reversible work done for a unit surface area creation. The parameters related to wettability, such as the contact angle of testing liquids, the total surface free energy (γ^{tot}) and the dispersive (γ^{d}) and polar (γ^{p}) components of the free energy and GY are summarized in Table 1.

Given the hydrophobic and chemically inert nature of the surface of LDPE, the contact angles of the

investigated testing liquids were relatively high. A significant decrease in contact angles was observed after plasma treatment of LDPE samples because of the incorporation of characteristic polar functional groups such as C=O , $-\text{OH}$, COOH , COO- , and C-O-C into the surface. The next step, which consisted of the radical graft polymerization of AA, resulted in a further decrease in the contact angles due to the polar character of PAA. The presence of bronopol and benzalkonium chloride also led to decreased contact angles in comparison to those of untreated LDPE. On the basis of contact angle measurements, we calculated the values of the surface energy and its components. Untreated LPDE is characterized by a very low surface energy, consistent with its hydrophobic character. The plasma treatment of LDPE resulted in an increase in the surface free energy due to the increased surface polarity. LDPE modified with polar PAA, bronopol and benzalkonium chloride exhibited very high surface free energy values. The graft yield of LDPE samples modified with antibacterial agent increased in compare with LDPE modified by PAA as result of introduction of benzalkonium chloride and bronopol on PAA brushes. The value of GY in case of benzalkonium chloride introduction was higher than in case of bronopol probably because of higher immobilization efficiency of benzalkonium chloride on PAA brushes.

3.2. Adhesive properties

The wettability closely relates to adhesion, which can be characterized by peel-test measurements. The results obtained from these measurements are presented in Figure 5. The adhesion of polymers correlates to their surface energy and to their roughness. Increased wettability of the LDPE surfaces results in increased adhesion and vice versa. Moreover, the increase in surface roughness is correlated with the adhesion increase due to the increase of surface con-

Table 1. Surface properties of LDPE modified via a multistep process (θ – contact angle, γ^{tot} – total surface free energy, γ^{d} , γ^{p} – dispersive and polar components of the surface free energy, respectively; GY – graft yield)

LDPE sample	θ_w [°]	θ_e [°]	θ_g [°]	θ_d [°]	θ_f [°]	γ^{tot} [mJ/m ²]	γ^{d} [mJ/m ²]	γ^{p} [mJ/m ²]	GY [%]
Untreated	99.2 (± 0.6)	70.9 (± 1.2)	85.3 (± 0.9)	48.4 (± 1.2)	80.7 (± 0.9)	31.7	31.5	0.2	
Plasma-treated (A)	77.5 (± 1.1)	51.0 (± 2.8)	67.1 (± 2.8)	36.0 (± 1.2)	52.8 (± 1.5)	42.6	41.4	1.1	0.0
A + PAA grafted (B)	66.9 (± 0.7)	32.1 (± 2.4)	57.2 (± 2.7)	32.5 (± 1.6)	37.0 (± 2.0)	48.1	43.7	4.5	0.5
B + benzalkonium chloride coated	70.7 (± 0.4)	52.9 (± 2.2)	58.9 (± 2.0)	40.3 (± 0.8)	37.2 (± 2.7)	43.1	36.4	6.7	3.4
B + bronopol coated	73.1 (± 1.2)	31.8 (± 3.0)	65.6 (± 2.5)	37.5 (± 1.0)	36.8 (± 2.5)	45.6	40.4	5.2	0.9

w = deionized water, e = ethylene glycol, g = glycerol, d = diiodomethane, f = formamide

Figure 5. Changes in peel strength after each particular step of the LDPE antibacterial treatment

tact area. Adhesion is a complex phenomenon consisting of several chemical and physicochemical factors, as demonstrated in aforementioned results. A relatively low value of peel strength for the untreated LDPE was observed as result of the hydrophobic and chemically inert nature of the polymer. Moreover, the roughness of untreated LDPE was relatively low. The plasma treatment led to a more than twofold increase in the peel strength, caused by changes in the polarity and surface morphology. The grafting of PAA onto LDPE resulted in increased peel strength. The peel strength also increased in the cases of benzalkonium-chloride- and bronopol-coated LDPE samples.

3.3. Surface morphology

Changes in the surface roughness of untreated and antibacterial-treated LDPE were determined by AFM (Figure 6). Details related to the increase or decrease of the surface roughness were compared via the profile roughness parameter (R_a). The R_a value represents the average height of the irregularities in a perpendicular direction toward the sample surface, and, in the case of untreated LDPE, was being 38.1 nm in 10 μm of scan area (Figure 6a) as a consequence of the procedure used to prepare the foils. The plasma treatment led to a decrease in the roughness, as indicated by an decrease in the R_a value to 21.9 nm (Figure 6b) as a result of surface changes induced by the functionalization and ablation processes. In the case of the AA radical graft polymerization step, the R_a value increased compared to that of the plasma-treated LDPE surface because PAA brushes were created on the LDPE surface (Figure 6c). The agglomerates created from bronopol resulted in an increase of the surface roughness; therefore, the R_a values bronopol reached 37.2 nm

(Figure 6d) while benzalkonium chloride formed more uniform layer and R_a was 29.2 nm (Figure 6e).

3.4. Surface chemistry analysis

Semi-quantitative information about chemical changes in the near-surface region was obtained from FTIR-ATR measurements. The spectrum of pristine LDPE is characterized by bands (Figure 7, curve a) associated with $-\text{CH}_2-$ stretching (2848 and 2915 cm^{-1}), asymmetric C-H and $-\text{CH}_3$ bending in plane (1460 and 1470 cm^{-1} , respectively), symmetric C-H bending in plane (1377 cm^{-1}), and C-H ($-\text{CH}_2-$) $_{n \geq 6}$ rocking (doublet with maxima at 720 and 731 cm^{-1}) [27]. The plasma surface treatment of LDPE resulted in significant spectral changes (Figure 7, curve b), which provided clear evidence of the incorporation of certain oxygen-containing groups, i.e., $-\text{OH}$ (region 3700 – 3080 cm^{-1}) and/or other oxygen-containing products at the surface of the polymer (region 1845 – 1510 , 1280 , 1126 , 1150 cm^{-1} , acid, ketone, aldehyde) [28]. The modification of LDPE by PAA grafting and by subsequent treatment with benzalkonium chloride and bronopol led to additional spectral changes. Characteristic peaks of PAA grafted onto LDPE were observed, specifically, the most intense peak at 1712 cm^{-1} (carbonyl band, C=O stretching) and some unresolved peaks in the fingerprint region (1300 – 1100 cm^{-1} , C–O stretching and CH_2 bending), as shown in Figure 7, curve c. The presence of benzalkonium chloride was confirmed by the presence of the absorbance band at 702 cm^{-1} , which is attributed to C–Cl vibrations (Figure 7, curve d). The spectrum of LDPE treated with bronopol shows characteristic peaks at 3300 cm^{-1} ($-\text{OH}$), 1557 , 1340 cm^{-1} ($-\text{NO}_2$) and 852 cm^{-1} (C–Br) resulting from the incorporation of bronopol into the LDPE surface (Figure 7, curve e).

XPS was used to quantify the characteristic presence of typical chemical elements after each step of the antibacterial treatment. The surface composition of the LDPE samples, which is shown in Table 2, was measured at two different spots, allowing the average surface composition to be calculated.

The high resolution C1s spectra of all samples are shown in Figure 8. In the spectrum of untreated LDPE, only a C1s peak was present (Figure 8a), which is attributed to C–C/C–H bonds. The greatest amount of oxygen and also nitrogen functional

Figure 6. AFM images of LDPE sample surfaces and the samples' corresponding R_a values: a) untreated; b) plasma treated (A); c) A + PAA grafted (B); d) B + benzalkonium chloride coated; and e) B + bronopol coated

Figure 7. FTIR-ATR spectra of LDPE samples: a) untreated; b) plasma treated (A); c) A + PAA grafted (B); d) B + benzalkonium chloride coated; and e) B + bronopol coated

groups (19.8 and 4.0 at%, respectively) were observed in the plasma-treated LDPE sample. In this case, the carbon C1s peak represents C–C/C–H, O=C–O, C=O, and C–O groups (Figure 8b) and the nitrogen N1s peak is composed from different chemical bonds of nitrogen atoms such as C–N, C–NH₃⁺, and –ONO₂. The spectrum of the LDPE sample grafted with PAA showed mainly the presence of an O1s peak as a consequence of the incorporation of carboxyl groups (Figure 8c); however, this peak may also indicate the presence of other oxygen groups created during plasma treatment. These samples also contained some iron traces and sulfur

Table 2. Surface composition of LDPE samples, as obtained from XPS measurements

LDPE samples	C1s	N1s	O1s	Cl2p	Si2p	S2p	Br3d
	[at%]						
Untreated	100.0	0	0				
Plasma treated (A)	76.3	4.0	19.8				
A + PAA grafted (B)	84.1	0	15.6			0.4	
B + benzalkonium chloride coated	87.8	3.0	4.7	4.5			
B + bronopol coated	91.5	0	7.1		1.3		0.1

Figure 8. High resolution C1s spectra of LDPE samples: a) untreated; b) plasma treated (A); c) A + PAA grafted (B); d) B + benzalkonium chloride coated; and e) B + bronopol coated

impurities (approximately 0.4 at%) which probably originated from dust or chemicals. XPS spectra of the benzalkonium chloride-coated LDPE sample revealed the presence of N and Cl (Figure 8d), which originate from the antibacterial agent. The presence of a small amount of Br (0.1 at%) was detected in LDPE samples coated with bronopol, which is likely associated with the creation of the very thin bronopol layer.

3.5. Antibacterial effect

The antibacterial effect was determined by measurement of the inhibition zones. The results were averaged to obtain the one representative value when all three repeated measurements of the LDPE samples treated in the same manner exhibited some antibacterial activity. The measurements clearly indicated an antibacterial effect against *S. aureus*. In Figure 9, the inhibition zone values for each step

of the antibacterial treatment are presented. Samples coated with bronopol or benzalkonium chloride were active against *S. aureus*, which confirmed presence of the antibacterial agent. The best results were obtained for LDPE samples coated with benzalkonium chloride. Moreover, the use of PAA as an active platform for the immobilization of antibacterial agents had a more pronounced effect on the antibacterial activity against *S. aureus* in comparison to, e.g., LDPE samples grafted by allylamine, reported elsewhere [29]. Antibacterial activity against *E. coli* was not sufficiently demonstrated.

4. Conclusions

This work was focused on the anchoring of selected antibacterial agents to the surface of LDPE through the use of low-temperature plasma and PAA grafting on pre-treated samples. Such treated LDPE samples exhibited changes in their surface and chemical composition. The immobilization of the antibacterial agents led to changes in the surface roughness of LDPE which was confirmed by the R_a value increase from 21.9 nm for the plasma treated LDPE to 29.2 and 37.2 nm for PE surfaces grafted with benzalkonium chloride and bronopol, respectively. Moreover, this antibacterial treatment significantly affected the wettability and adhesion of LDPE as a consequence of the increase in roughness and hydrophilicity, as previously mentioned, whereas the γ^{tot} of LDPE increased from 31.7 to 43.1 mJ/m² and 45.6 mJ/m² for LDPE surfaces covered with benzalkonium chloride and bronopol, respectively. The increase in hydrophilicity was caused by the introduction of polar functional groups present in the applied antibacterial agents, as was proven by FTIR-ATR and XPS measurements. The antibacterial effect of such

treated LDPE films was confirmed by measurements of the inhibition zone of *S. aureus*; the most pronounced antibacterial activity was exhibited by LDPE samples treated with plasma, grafted with PAA, and subsequently coated with benzalkonium chloride. The antibacterial effect against *E. coli* was not sufficiently proven.

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Figure 9. The inhibition zone area of LDPE samples after each step of the antibacterial treatment

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