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A new route for chitosan immobilization onto polyethylene surface

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ABSTRACT

Low-density polyethylene (LDPE) belongs to commodity polymer materials applied in biomedical applications due to its favorable mechanical and chemical properties. The main disadvantage of LDPE in biomedical applications is low resistance to bacterial infections. An antibacterial modification of LDPE appears to be a solution to this problem. In this paper, the chitosan and chitosan/pectin multilayer was immobilized via polyacrylic acid (PAA) brushes grafted on the LDPE surface. The grafting was initiated by a low-temperature plasma treatment of the LDPE surface. Surface and adhesive properties of the samples prepared were investigated by surface analysis techniques. An antibacterial effect was confirmed by inhibition zone measurements of *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). The chitosan treatment of LDPE led to the highest and most clear inhibition zones (35 mm^2 for *E. coli* and 275 mm^2 for *S. aureus*).

1. Introduction

Several modification methods are commonly used to modify the polymer surface. One of the most frequent consists in an immersion in the strong acid solution. Nevertheless, such wet chemical methods are technologically complicated and environmentally unfriendly especially because hazardous chemical substances are often used. Recently, a plasma treatment is a preferred procedure considered as a progressive technique for polymer surface modification without the use of aggressive chemicals (Lloyd et al., 2010). Moreover, the plasma treatment enables surface modifications without changing the bulk properties of treated material (Vesel, Junkar, Cvelbar, Kovac, & Mozetic, 2008). The low-temperature plasma belongs to a clean, dry, ecologically method of the surface modification and it is often used in various applications, such as in automotive, electronic, aeronautic, textile, optical and paper industry (Pelletier et al., 2001). The main effect of the low-temperature plasma application consists in an increase of a surface free energy as a result of the incorporation of polar functional groups to the treated surface making the surface of LDPE more hydrophilic (Novák et al., 2007).

Etching (ablation), polymerization, or cross-linking processes take place during the plasma treatment of polymers. Moreover, species created in the plasma discharge, such as electrons, ions and excited atoms (Vesel, Drenik, Mozetic, & Balat-Pichelin, 2010b) are capable to initiate chemical processes on the polymer surface, leading to a formation of new reactive functional groups (functionalization) (Pappas, 2011; Vesel et al., 2010a; Yang, Chen, Guo, & Zhan, 2009).

The uniform layer and high surface power density of plasma can be generated by the diffuse coplanar surface barrier discharge (DCSBD) plasma generator. The equipment operates at atmospheric pressure and therefore it is suitable for continual industry applications (Černák, Černáková, Hudec, Kováčik, & Zahoranová, 2009). An another advantage of the abovementioned process is the indirect contact with the electrodes, what leads to the lower polymer surface contamination as well as longer electrode lifetime (Šimor, Ráhel', Vojtek, Černák, & Brablec, 2002). DCSBD plasma equipment consists of two parallel electrodes embedded in Al_2O_3. Several pairs of electrodes are supplied by a high frequency sinusoidal voltage (John, 2005). Such arrangement of electrodes leads to the almost macroscopically homogeneous plasma (Černák et al., 2004; Šíra & Trunec, 2005).

A bacterial surface growth on the polymer surface, also called a biofilm formation is a widespread problem (Hallab, Skipor, & Jacobs, 2003). Anti-infective properties of polymers can be reached by the surface treatment of medical polymer materials. This antibacterial

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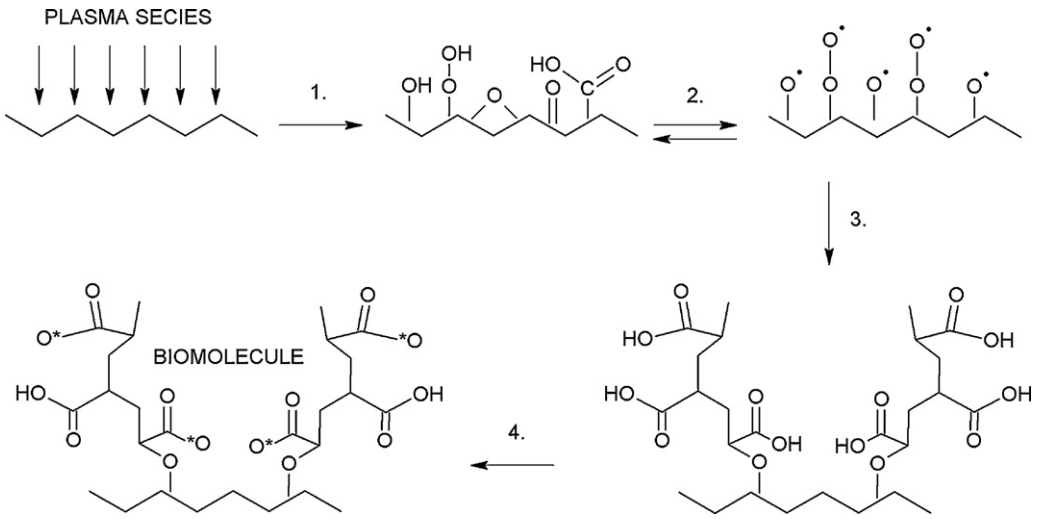

Fig. 1. Multistep approach of polysaccharides binding: (1) plasma treatment, (2) radical generation, (3) AA radical graft polymerization, and (4) polysaccharides immobilization.

surface modification is controlled by physicochemical interactions between the antibacterial and polymer surface substance (Zhang et al., 2006) by application of a multistep approach (Kenawy, Worley, & Broughton, 2007).

For our work, polyacrylic acid (PAA) was chosen for antibacterial an immobilization (Fig. 1). PAA can be easily grafted on the plasma treated LDPE surface, creating effective interfacial favorable for the effective antibacterial agent bonding (Noto, Matsumoto, Takahashi, Hirata, & Yamada, 2009; Zhao & Brittain, 2000). To increase the effect of the biocide molecule anchoring, carboxyl groups of grafted PAA should be activated using N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDAC) (Asadinezhad et al., 2010b; Bazaka, Jacob, Crawford, & Ivanova, 2011).

Many polysaccharides have an appropriate structure for the immobilization. These usually contain characteristic moieties, by which they can be firmly anchored at the created brushes. The polysaccharide based on chitosan is an important compound with a chemical stability and non-volatility and therefore it can be immobilized on the pre-treated polymer surface (Kenawy et al., 2007). Chitosan is a linear cationic polysaccharide derived from deacetylation of chitin (Muzzarelli, 2010; Salmah & Azieyanti, 2011). The significant features of chitosan, such as biocompatibility, nontoxicity, and antibacterial characteristics led to the development of a number of eco-friendly products (Prasanna & Sailaja, 2012; Zhang, He, Liu, & Qia, 2009). It is often used in pharmaceutical, cosmetic (Renault, Sancey, Badot, & Crini, 2009), and food industry applications (Park, Marsh, & Dawson, 2010). Chitosan is composed from randomly distributed β-(1–4)-linked D-glucosamine and *N*-acetyl-D-glucosamine; these in contact with a bacterial cell lead to its denaturation. Chitosan is sometimes used together with pectin (Marudova, Lang, Brownsey, & Ring, 2005). By such a way, more uniform layers are obtained as demonstrated in (Elsabee, Abdou, Nagy, & Eweis, 2008). Pectin is safe for a human and it has been successfully tested as an effective gelling and thickening agent, as well as food additives (Muzzarelli et al., 2012). Pectin, a structural heteropolysaccharide contained in primary cell walls of terrestrial plants, is one of the most widely investigated polysaccharides in a field of colon-specific drug delivery. The characteristic structure of pectin is the backbone consisting of a linear chain of α-(1–4)-linked D-galacturonic acid (Asadinezhad et al., 2010a).

The chitosan and pectin multilayer using a layer-by-layer assembly reflects in their better wettability and surface uniformity. It has been noted that chitosan gives the stable alternating multilayer with pectin over the solid surface. Antibacterial agents themselves and also the multilayer confirmed an excellent antibacterial performance against two representative bacteria, namely *Staphylococcus aureus* (*S. aureus*) which is the reason for wound and urinary tract infections and *Escherichia coli* (*E. coli*) which is causing a number of diseases such as intestinal disease, peritonitis, mastitis, pneumonia, and septicemia (Elsabee et al., 2008).

This paper is aimed to the description of a new route for polysaccharide immobilization to the LDPE surface by applying the plasma treatment using the atmospheric coplanar discharge plasma and consequently grafted by a high density polymer brush on it based on the acrylic acid monomer for the chitosan and chitosan/pectin multilayer immobilization with a prospective application in medical devices. This antibacterial multistep approach was first used for the LDPE surface in this work. In addition, the peel strength of the adhesive joint was thoroughly studied for these samples.

2. Experimental

2.1. Materials

LDPE (BRALEN FB 2-17) foils 20 μm thick made by Slovnaft MOL (Slovakia) containing no additives were used for our experiment. This LDPE grade complies with Food Contact Regulations and it is suitable for a food packaging as well as for a manufacturing of pharmaceutical products. Pectin obtained from apple (with 70–75% esterification) was supplied by BioChemika (USA). Acrylic acid (99.0%, anhydrous), and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDAC, 98.0%) were obtained from Fluka (USA). Chitosan (from crab shells with medium molecular weight and a 75–85%° of deacetylation), sodium metabisulfite (99.0%, Reagentplus), glutaraldehyde (as 25.0 wt.% aqueous solution), ethylene glycol (99.8%, anhydrous), diiodomethane (99.0%, reagentplus), formamide (99.5%, molecular biology grade), and glycerol (99%, for molecular biology) were supplied by Sigma–Aldrich (USA).

2.2. Plasma treatment

The surface of LDPE foils was activated under dynamic conditions at atmospheric pressure using DCSBD plasma equipment produced by Comenius University (Department of Experimental Physics, Faculty of Mathematics, Physics and Informatics) in Bratislava. The design of this equipment is shown in Fig. 2. The foils

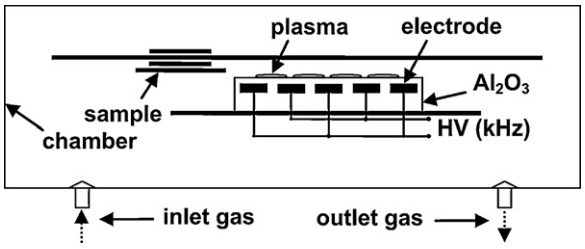

Fig. 2. Scheme of DCSBD plasma equipment.

were treated at power density of 1 W/cm^2; the plasma treatment was performed for 15 s in air as a carrier gas. Both foil sides were treated. Two parallel banded systems of electrodes (1 mm wide, 50 μm thick, with 0.5 mm spacing between the strips, made of Ag-paste) generate plasma by an effective way. Strips are embedded in 96% Al_2O_3. A high frequency sinusoidal voltage (~15 kHz at U_m ~10 kV) was used. Plasma generated by this equipment is macroscopically homogenous leading to the uniform surface treatment.

2.3. PAA Grafting

Immediately after the plasma treatment, the samples were immersed into 10 vol.% aqueous solution of AA for 24 h at 30 °C in order to achieve a radical graft polymerization of AA. The solution contained 0.1 wt.% of sodium metabisulfite as a relevant reductant agent to inhibit an AA homopolymerization. The AA polymerization led to the creation of PAA brushes that are suitable for the immobilization of antibacterial agents. After the grafting the samples were washed in deionized water for 5 min at 30 °C in order to remove weakly bounded or unreacted AA.

2.4. Chitosan and chitosan/pectin immobilization

PAA grafted LDPE foils were immersed into 0.1% (w/v) aqueous solution of EDAC at 4 °C for 6 h, for activation of carboxyl groups. The activation reaction of carboxyl groups by EDAC led to the formation of O-acylisourea with ability to react with some reducing agents (Nakajima & Ikada, 1995). Then the pre-treated samples with activated carboxyl groups were immersed into 1% (w/v) chitosan in 2% (v/v) acetic acid aqueous solution for 24 h at 30 °C. In another case, the samples were dipped into chitosan and consequently pectin solution (2% (v/v) acetic acid aqueous solution/prepared by the same way as described above for chitosan); the dipping was repeated nine times with 20 min duration in each solution. Finally, the samples prepared by any of the procedures described above were immersed in 1% (w/v) glutaraldehyde aqueous solution overnight at 4 °C to achieve the immobilization of two polysaccharides via crosslinking processes. The crosslinking reaction occurred with imine formation resulted in the reaction of primary amine with aldehyde (Carey & Sundberg, 2007). The prepared samples were then thoroughly washed and dried for 24 h at room temperature.

2.5. Surface wettability evaluation

Wettability changes of the LDPE surface after the polysaccharides immobilization by the multistep process were obtained from the contact angle measurements. The surface energy evaluation system (SEE system with CCD camera, Advex Instruments, Czech Republic) was used for experiments and a sessile drop technique was performed. A volume of 3 μl for each drop of testing liquid placed on a sample was used for investigation of a static contact angle. Ten separate readings were averaged to obtain one representative contact angle value for each liquid. The contact angle is referred as an angle between the solid/liquid and liquid/vapour interface. Deionized water, ethylene glycol, glycerol, formamide, and diiodomethane were used as testing liquids. Contact angle of each drop was measured after approximately 3 s which is sufficient for an achievement of a thermodynamic equilibrium between solid, liquid, and gas phases was reached. The testing liquids were used for a calculation of total (γ^{tot}), polar (γ^{p}) and dispersive (γ^{d}) components of the surface free energy. Owens–Wendt–Rable–Kaeble regression model using the method of least squares was used for the evaluation of γ^{tot}, γ^{p}, and γ^{d} (Salimi, Mirabedini, Atai, Mohseni, & Naimi-Jamal, 2011). The graft yield (GY) was calculated according to the equation GY (%) = $((W_2 - W_1)/W_1)\cdot 100\%$, where W_1 and W_2 represent weights of the samples before and after the surface treatment, respectively (Işiklan, Kurşun, & İnal, 2010).

2.6. Adhesive properties assessment

An adhesion between two materials was characterized by the peel strength (force per unit width). The peel test was used for peel strength measurements of the adhesive joint formed of LPDE foils and poly(2-ethylhexyl acrylate) as an adhesive agent deposited onto polypropylene foil of 15 mm wide. Measurements were performed as 90° peel test at a rate of peel 10 mm per minute using 100 N universal INSTRON 4301 dynamometer (UK). The both ends of the LDPE sample and PP with adhesive were firmly fixed into dynamometer jaws to achieve an even tension distribution across the entire width.

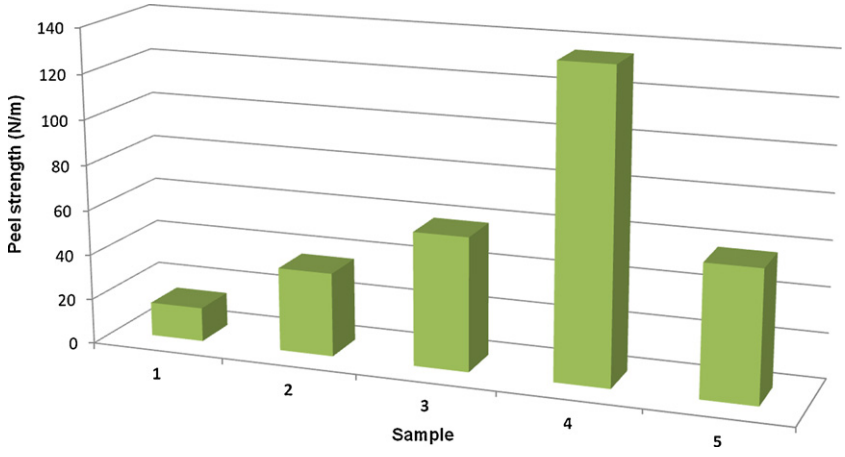

Fig. 3. Peel strength vs. surface treatment of LDPE sample: 1 – untreated, 2 – plasma treated, 3 – PAA grafted, 4 – chitosan coated, and 5 – chitosan/pectin coated.

Table 1
Surface properties of LDPE treated by multistep process (θ – contact angle; γ^{tot}, γ^{d}, γ^{p} – total surface free energy, its dispersive and polar component, respectively; GY – graft yield).

LDPE sample	θ_w (°)	θ_e (°)	θ_g (°)	θ_d (°)	θ_f (°)	γ^p (mN/m)	γ^d (mN/m)	γ^{tot} (mN/m)	GY (%)
Untreated	99.2 (±0.6)	70.9 (±1.2)	85.3 (±0.9)	48.4 (±1.2)	80.7 (±0.9)	0.2	31.5	31.7	–
Plasma treated (A)	77.5 (±1.1)	51.0 (±2.8)	67.1 (±2.8)	36.0 (±1.2)	52.8 (±1.5)	1.1	41.4	42.6	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)	4.5	43.7	48.1	0.5
B + chitosan coated	69.2 (±0.8)	36.0 (±2.1)	68.3 (±1.2)	35.9 (±1.9)	33.1 (±2.2)	6.1	38.8	44.9	3.1
B + chitosan/pectin coated	59.1 (±1.1)	30.0 (±2.8)	53.40 (±1.3)	37.8 (±2.6)	33.8 (±2.7)	11.9	36.1	48.0	8.2

w: deionized water; e: ethylene glycol; g: glycerol; d: diiodomethane; f: formamide.

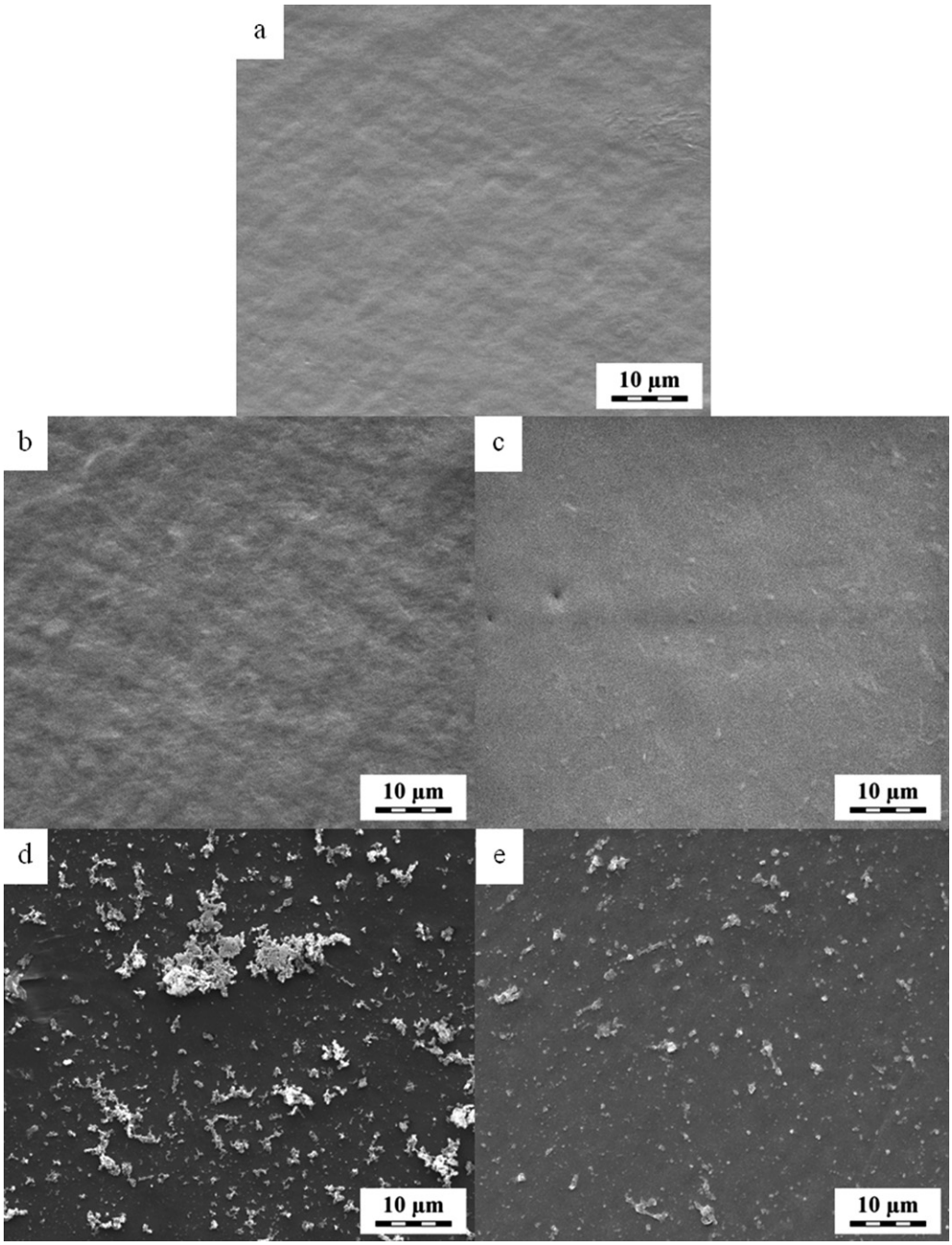

Fig. 4. SEM micrographs showing surface morphology for LDPE samples: a – untreated, b – plasma treated, c – PAA grafted, d – chitosan coated, and e – chitosan/pectin coated.

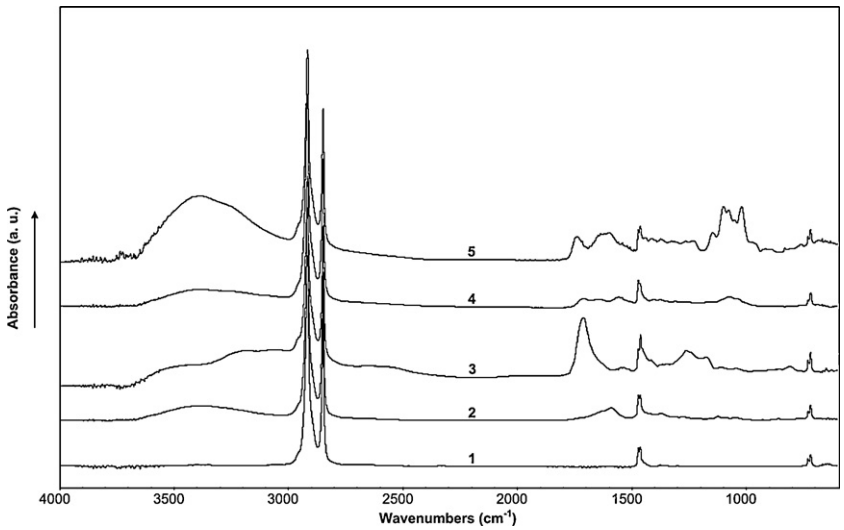

Fig. 5. FTIR-ATR spectra of LDPE samples: 1 – untreated, 2 – plasma treated, 3 – PAA grafted, 4 – chitosan coated, and 5 – chitosan/pectin coated.

2.7. Surface morphology analysis

Scanning electron microscope (SEM) was used for a characterization of the surface morphology and local surface heterogeneities of LDPE samples. The surfaces of both untreated and antibacterial-treated LDPE films were observed by the SEM microscope (Quanta 200 FEG; FEI, Czech Republic) using secondary electrons detector and accelerating voltage 30 kV. Before measurement, the samples were sputter-coated by a thin layer of Pt (~4 nm). All samples were analyzed at several locations (≥3) in order to find characteristic and significant surface features.

2.8. Surface chemistry investigation

2.8.1. X-ray photoelectron spectroscopy

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

2.8.2. Infrared spectroscopy

Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR–ATR) was used for an investigation of the surface chemical composition. The spectra were recorded by the FTIR NICOLET 8700 spectrometer (Thermo Scientific USA) through the single bounce ATR with Ge crystal at 45° incident angle. The spectral resolution and the number of scans were 2 cm^{-1} and 64, respectively for each measurement. The pressure clamp was used to obtain the highest quality of the spectra. The acquired spectra were analyzed using OMNIC™, v. 8.1 software. Each measurement was triplicate to obtain the average spectra for different spots.

2.8.3. Antibacterial activity assessment

The antibacterial activity of prepared samples was tested against two bacterial strains *Staphylococcus aureus* (CCM 4516) and *Escherichia coli* (CCM 4517) by the inhibition zone method (diffusion test) on agar. Nutrient agar No. 2 M1269 – 500 g from HiMedia Laboratories PII. Ltc. was used for our experiments. Tested samples were cut in a circular shape (d = 8 mm), washed in ethanol, dried and placed on an agar plate inoculated by the bacterial suspension (volume = 100 μl, concentration = 10^7 units/ml). The samples were incubated for 24 h at 37 °C and diameters of the inhibition zone were measured in 5 directions to obtain average values for inhibition zone calculations. The test with each sample was triplicate.

3. Results and discussion

3.1. Surface wettability

Surface parameters of untreated and treated LDPE calculated from contact angle data for various testing liquid are shown in Table 1. The hydrophobic and chemical inert surface nature of untreated LDPE is the reason for high values of contact angle (θ) due to low surface wettability. The significant decrease of θ was observed after the plasma treatment because characteristics reactive polar functional groups were introduced onto the LDPE surface. The PAA grafting led to the further decrease of θ, whereas PAA contains polar carboxylic groups. In addition, the chitosan and chitosan/pectin multilayer led to the significant decrease of θ due to the presence of characteristic polar functional groups. Accordingly to the measured contact angle values, low values were calculated also for γ^{tot} of untreated LPDE associated with its hydrophobic nature. The plasma treatment leads to the increase of LDPE γ^{tot} indicating the surface polarity increase. Even greater increase of LDPE γ^{tot} was recorded for PAA grafted LDPE and for chitosan immobilized LDPE. The highest increase of γ^{tot} and γ^{p} was observed for chitosan/pectin multilayer immobilized on the LDPE surface via PAA.

3.2. Adhesive properties

The information about adhesion changes of the adhesive joint to more polar polyacrylate were obtained from peel test measurements that are shown in Fig. 3. The adhesion can be expressed by a force per width (peel strength). The peel strength closely relates to γ^{tot}, roughness and chemical nature of investigated materials forming an adhesive joint. Therefore, the increase of wettability results in the peel strength increase of the adhesive joint to more polar polyacrylate. On the other side, rougher surface results in higher

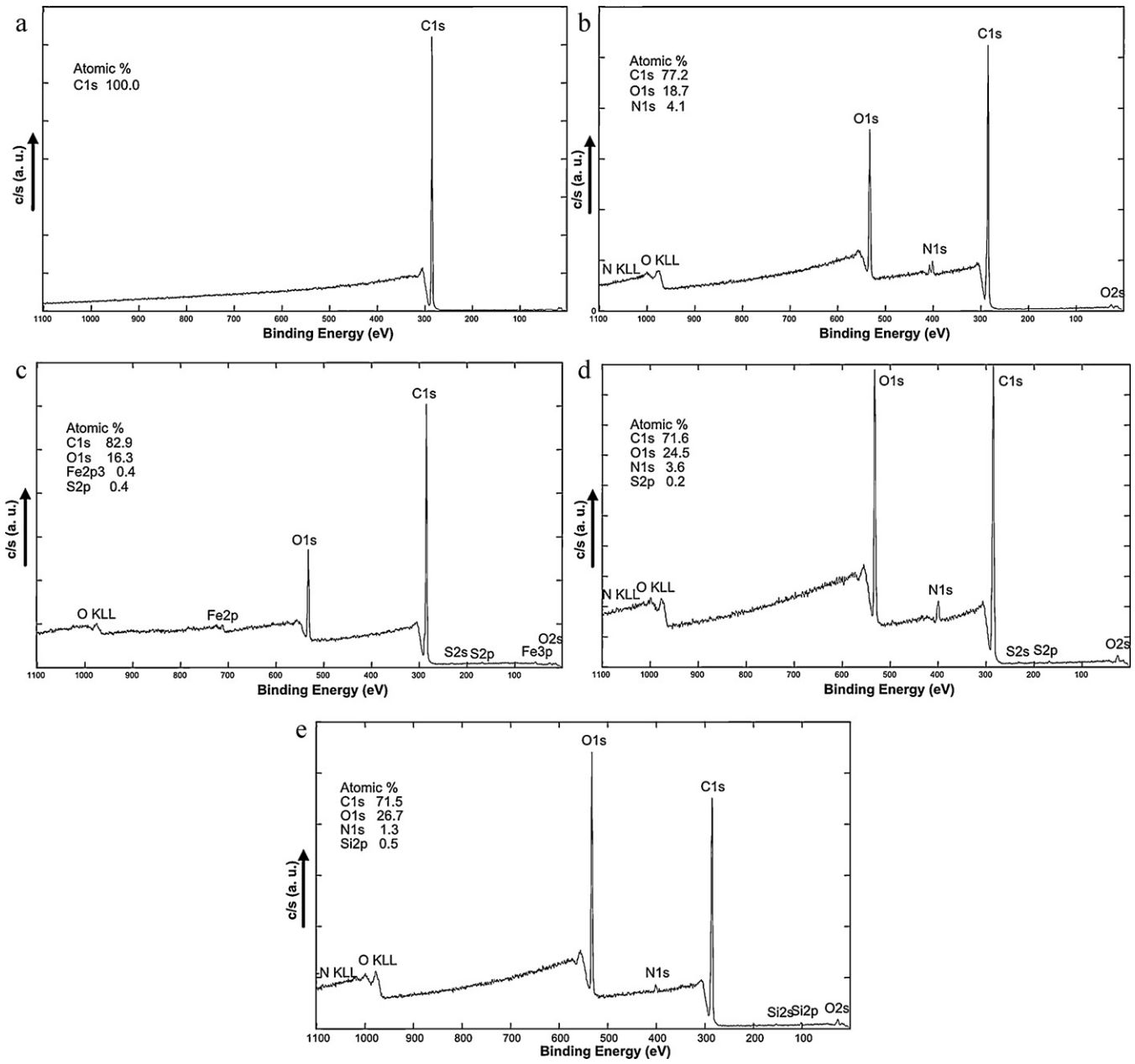

Fig. 6. XPS survey-scan spectra of LDPE samples with atomic composition: a – untreated, b – plasma treated, c – PAA grafted, d – chitosan coated, and e – chitosan/pectin coated.

adhesion and vice versa. The adhesion is thus a complex of the several related chemical and physicochemical properties. Therefore, the peel strength of untreated LDPE achieves very low values. The plasma treatment resulted in a double increase of the peel strength caused by the changes in polarity and surface roughness. PAA grafting and chitosan/pectin multilayer coating leads to the further increase in the peel strength compared to values for plasma treated LDPE. The most pronounced increase in the peel strength of adhesive joint LDPE samples-polyacrylate was observed for the chitosan coating. The chitosan coating led to the most increase of the surface roughness.

3.3. Surface morphology

Changes in the surface morphology of untreated and antibacterial treated LDPE by the multistep process obtained from SEM measurements are shown in Fig. 4. The surface morphology of untreated LDPE (Fig. 4a) is characterized by a very low surface roughness. The plasma treatment of LDPE (Fig. 4b) results in a slight increase of the surface roughness as a result of surface changes by a combination of functionalization and ablation processes. PAA brushes formed on the LDPE surface exhibited a characteristic texture (Fig. 4c). The domain size increased as the grafting advanced. The other conclusive factor influencing the surface morphology is a grafting mechanism. A certain amount of generated radicals in the sublayer initiates the grafting reaction. The bulged top layer results from the AA monomer polymerization participating in the chain propagation process. The chitosan immobilization by glutaraldehyde as a crosslinking agent leads to the formation of chitosan agglomerates on the continuous layer of the PAA grafted surface (Fig. 4d). Pectin significantly increases the uniformity of the chitosan layer; more uniform surface morphology is obtained for the chitosan/pectin multilayer as seen in Fig. 4e.

3.4. Surface chemistry

3.4.1. Analysis of FTIR-ATR spectra

FTIR-ATR measurements provide mostly semi-quantitative information about chemical changes in a near-surface region. The infrared spectra of LDPE samples were splitted into three regions for better visualization. The spectrum of untreated LDPE is a characteristic spectrum of polyethylene with only few characteristic peaks. After plasma exposure of the untreated material, significant changes in a measured spectrum are observed. As seen in Fig. 5, the incorporation of oxygen containing groups was obvious, i.e. hydroperoxides (region 3700–3080 cm^{-1}) and/or other oxygen containing products at the surface of the material (region 1845–1510 cm^{-1}, 1280 cm^{-1}, 1126 cm^{-1}, 1150 cm^{-1}, carboxyl, carbonyl or aldehydic moieties).

Other significant changes in the spectra are observed for LDPE modified by PAA grafting, and also after the subsequent treatment by chitosan, chitosan/pectin and glutaraldehyde, respectively. The spectrum of grafted material contains several characteristic peaks of PAA, i.e. the most intense peak at 1712 cm^{-1} (carbonyl band, C═O stretching), and also some unresolved peaks in the fingerprint region (1300–1100 cm^{-1}, C—O stretching and CH_2 bending). After the chitosan and glutaraldehyde treatment, the shape of the spectrum is changing, as can be seen in Fig. 5. Because of the treatment complexity, these changes in the spectra can be interpreted with some difficulties – the spectra of chitosan, pectin and also of glutaraldehyde are very similar in the fingerprint region. Despite this statement, the spectra of samples 4 and 5 indicates also the presence of acrylic acid (carbonyl band, 1712 cm^{-1}), pectin – 1734 cm^{-1} (C═O band arising from pectin). The presence of chitosan in samples 4 and 5 is confirmed by an appearing of the band at 1653 cm^{-1} in corresponding spectra (-CNH band arising from chitosan). The presence of glutaraldehyde (suggesting as a crosslinking agent) is indicated in the spectrum at lower wavenumbers (approximately at 1100 cm^{-1} as a contribution to C—O absorbance).

The changes in the spectra are significant almost in a whole mid-infrared region, especially in the fingerprint region and they confirm the incorporation of chemicals used for the surface treatment of LDPE.

3.4.2. Analysis of XPS spectra

The LDPE samples with the different treatment were thoroughly analyzed by the XPS method. The objective was to get the evidence of the presence of the antibacterial substances coating on the LDPE surface via the plasma treatment in air and grafting with AA. The surface composition for each sample was measured at two different spots allowing the calculation of an average surface composition.

The XPS survey-scan spectra of samples with the average surface composition are shown in Fig. 6. As expected, the untreated LDPE has a characteristic spectrum composed of 100 at.% of C1s peak (Fig. 6a), belonging to C—C bonds. Different oxygen functional groups and also some nitrogen groups were found in the plasma treated sample in air (Fig. 6b). Carbon C1s peak corresponds to C—C, O═C—O, C═O, C—O groups. The nitrogen N1s peak of the LDPE sample treated in air plasma is composed of different chemical bonds of nitrogen atoms such as C—N, C—NH_3^+, —ONO_2. The sample of PAA grafted on LDPE showed mainly the presence of carboxyl groups (Fig. 6c). The oxygen groups originate mainly from PAA but also other oxygen groups are present, which were created during and immediately after the plasma treatment. In samples coated with chitosan or chitosan-pectin, large oxygen and some nitrogen content is detected. The sample coated only with chitosan has higher nitrogen content (Fig. 6d), while the sample coated with chitosan–pectin has higher oxygen content (Fig. 6e). In this sample also traces of silicon impurities were detected – about 0.4 at.%. The XPS spectrum indicates, that a number of different moieties (e.g. carbonyl, carboxyl, etc.) containing oxygen is present in treated LDPE.

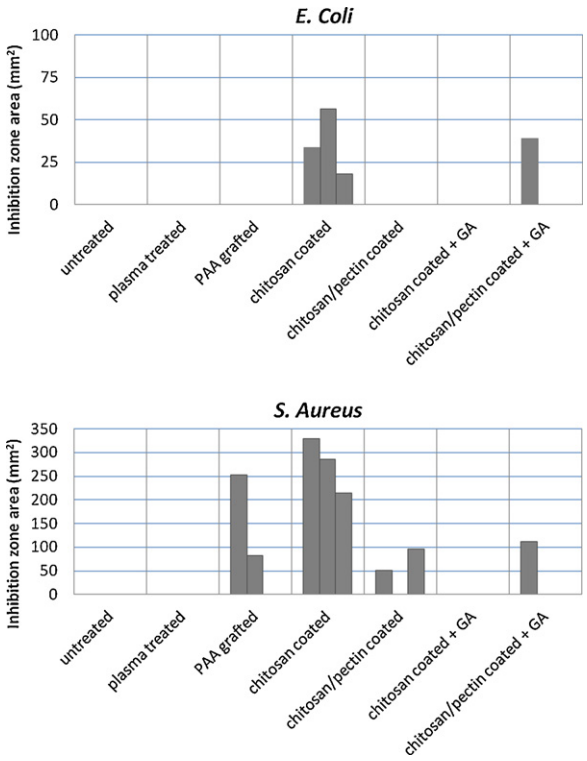

Fig. 7. Inhibition zone area of LDPE samples for *S. aureus* and *E. coli* strains. Each column represents the inhibition zone area for one experiment out of three.

3.5. Antibacterial activity

The inhibition zone area was calculated from an average diameter of the inhibition zone, whereas the area of the sample was not taken into account. Each measurement was triplication (Fig. 7). The untreated, plasma treated, and PAA grafted LDPE sample with chitosan together with glutaraldehyde did not show any antibacterial activity against *E. coli* and *S. aureus* strains. The chitosan/pectin coated sample showed minor activity only against *S. aureus*, the inhibition zone being around 70 mm^2. Similar results were obtained for the chitosan/pectin coated sample after crosslinking by glutaraldehyde. This sample showed activity also against *E. coli*. However, the antibacterial activity of these samples is not significant. The highest and most clear inhibition zones were given by samples grafted by PAA and coated by chitosan. The levels in this case were on average 35 mm^2 for *E. coli* and 275 mm^2 for *S. aureus*.

The PAA grafted sample did not show any inhibition zone for *E. coli*, nevertheless the same sample indicated the antibacterial activity for *S. aureus*. This could be explained by high sensitivity of the PAA brushes and their ability to easily absorb impurities during manipulation. As it can be seen from results, the sample grafted by PAA and coated by chitosan only demonstrated active antibacterial properties against both bacterial strains. Other samples did not prove the significant antibacterial activity. Chitosan is probably weakly bonded to the PAA surface and it can diffuse easily. On the other hand the LDPE surface treated by the multilayer of chitosan/pectin or additionally crosslinked by glutaraldehyde prevents chitosan molecule to diffuse and form the inhibition zone.

4. Conclusions

The multistep physicochemical approach was shown to be effective for binding of selected antibacterial compounds, namely the chitosan and chitosan/pectin multilayer on the LDPE surface. The DCSBD plasma treatment resulted in the increase of the surface roughness as well as the surface free energy due to introducing oxygen-based functional groups on the polymeric surface. PAA brushes synthesized via the plasma-initiated graft polymerization using AA as a monomer leads to the increase of the surface polarity representing a stable base for polysaccharides/biomolecules/antibacterial agent binding. The most effective bacterial inhibition zone was observed for the sample coated by chitosan indicating its antibacterial efficiency. The chitosan/pectin coated sample showed minor activity only against *S. aureus*, and similar results were received using the chitosan/pectin coating with glutaraldehyde having the antibacterial activity against *E. coli*. The results of this work represent the important information in a field of the biocide properties study of polysaccharides coatings on the LDPE surface using a modification process by the DCSBD plasma.

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