



**Tomas Bata University in Zlín**  
**Faculty of Technology**  
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*Doctoral thesis*

**Preparation of Antibacterial Packaging Materials**

Příprava antibakteriálních obalových materiálů

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## ABSTRACT

Antibacterial materials can be prepared by several ways. Incorporation of antibacterial agents on the surface of modified synthetic polymer materials appears to be one of the best. Modification of polymer synthetic materials involves changing the chemical composition of top surface layer, while bulk properties, especially mechanical, remain unchanged and the product achieves adequate strength and flexibility. Such approach can be applied in food packaging field or in medical area, such as infection-prevention related to medical instruments, implants and equipment.

Prostheses, artificial replacements, heart valves, urinary catheters, vascular grafts and other devices introduced into the body are often easy targets for pathogenic organisms. Their attachment and growth arise many complications, costs are increasing, treatment is prolonged and it can lead even to mortality.

In this work, the attention is primarily focused on the treatment of commodity polymer material – low density polyethylene (LDPE) by multistep physico-chemical method. The polymer surface is activated by air plasma discharge. Then, the surface was functionalized by radical copolymer reactions with monomers forming a polymer brush on the surface, which is able to bind commercially produced antibacterial agents. The surface sample was characterized by contact angle measurement, ATR infrared spectroscopy, scanning electron microscopy and X-ray photoelectron spectroscopy. Antibacterial activity was assessed using agar diffusion test.

This work provides insight into the problems of bacterial adhesion to polymer surfaces and surface modification methods to achieve antibacterial properties. So prepared materials application can be found in food and packaging industry as well as medical tools in healthcare.

**Key words:** *polyethylene • plasma • polymer brush • antibacterial activity*

## ABSTRAKT

Antibakteriální materiály jsou připravovány několika možnými cestami. Jako nejlepší z nich se jeví nanášení antibakteriálních činidel na povrch modifikovaných syntetických polymerních materiálů. Modifikace polymerních syntetických materiálů spočívá ve změně chemického složení povrchové vrstvy, přičemž celkové vlastnosti substrátu, zejména mechanické, zůstávají nedotčeny a celkový výrobek dosahuje odpovídající pevnosti a pružnosti. Takovýto postup pak nachází využití jak v oblasti balení potravin, tak jako prevence vzniku infekce při používání lékařských nástrojů, protéz a zařízení.

Povrchy protéz, umělých náhrad, chlopní, močových a žilních katétrů a jiných zařízení zaváděných do těla jsou často snadným cílem patogenních organismů. S jejich rozšířením pak vznikají mnohé komplikace, léčba se prodlužuje, zvětšují se její náklady a může končit i smrtí.

V této práci je pozornost zaměřena na úpravu běžně používaného polymeru – nízkohustotního polyetylen (LDPE) více stupňovou fyzikálně-chemickou metodou. Povrch polymeru je aktivován plazmovým výbojem v atmosféře vzduchu. Na tomto povrchu pak proběhla radikálová kopolymerační reakce s monomery tvořícími na povrchu polymerní řetězec („polymer brush“), jež je schopný vázat průmyslově vyráběná antibakteriální činidla. Povrch vzorků byl charakterizován měřením kontaktních úhlů smáčení, infračervenou ATR spektroskopií, elektronovou rastrovací mikroskopií a rentgenovou fotoelektronovou spektroskopií. Antibakteriální aktivita byla hodnocena pomocí tzv. difúzního testu na agaru.

Tato práce přináší náhled do problematiky bakteriální adheze na povrch a způsoby úpravy polymerního substrátu tak, aby dosáhl antibakteriálních vlastností. Aplikace takto připravených materiálů může být nalezena v potravinářství a obalové technice, tak jako v medicíně coby povrch zdravotnických prostředků.

**Klíčová slova:** *polyetylen • plasma • polymer brush • antibakteriální aktivita*

*I dedicate this work to my Lord Jesus Christ.*

# LIST OF PAPERS

## Paper I

**Preparation of active antibacterial LDPE surface through multistep physicochemical approach: I. Allylamine grafting, attachment of antibacterial agent and antibacterial activity assessment**

František Bílek, Táňa Křížová, Marián Lehocký

*Colloids and Surfaces B: Biointerfaces* 88 (2011) 440.

## Paper II

**Anti-bacterial Treatment of Polyethylene by Cold Plasma for Medical Purpose**

Anton Popelka, Igor Novák, Marián Lehocký, Ivan Chodák, Ján Sedliačik, Milada Gajtanská, Mariana Sedliačiková, Alenka Vesel, Ita Junkar, Angela Kleinová, Milena Špírková and František Bílek

*Molecules*, 17 (2012) 762.

## Paper III

**A new route for Chitosan immobilization onto polyethylene surface**

Anton Popelka, Igor Novák, Marián Lehocký, Ita Junkar, Miran Mozetič, Angela Kleinová, Ivica Janigová, Miroslav Šlouf, František Bílek, Ivan Chodák

*Carbohydrate Polymers* 90 (2012) 1501.

## OTHER PAPERS

- **Preparation of active antibacterial LDPE surface through multistep physicochemical approach II: Graft type effect on antibacterial properties**  
František Bílek, Kateřina Sulovská, Marián Lehocký, Petr Sáha, Petr Humpolíček, Miran Mozetič, Ita Junkar *Colloids and Surfaces B: Biointerfaces*, IN PRESS. DOI: 10.1016/.colsurfb.2012.08.026
- **Polysaccharides Coatings on Medical-Grade PVC: A Probe into Surface Characteristics and the Extent of Bacterial Adhesion**  
Asadinezhad, A. J., Novák, I., Lehocký, M., Bílek F., Vesel, A., Junkar I., Sáha P., Popelka, A., *Molecules* 15 (2010), 1007.

# THEORETICAL BACKGROUND

## 1. Antibacterial packaging materials

The right packaging should comply with all these features:

- protect the product from spoilage, harmful mechanical, climatical, biological and social environmental influences
- to be a rational handling unit adapted for weight, shape and constructions requirements of transport, trade and consumers
- to be a visual medium of communication among the trade partners

In view of packaging as products protection is especially the critical strength of packaging, differentiated impermeability to water and water vapour, gases, grease and dust resistance, chemicals and microorganisms. Furthermore, resistance against daylight and UV irradiation has adequate damping effects, good closing and cleaning ability and is harmless.

### 1.1 Active packaging

Active packaging changes the conditions of packaged foods during storage. Thus they are able to extend their shelf life, safety, but also visual and sensory features, such as taste, smell, appearance, structure and nutritional properties. Packaging can be divided into several groups.

- *Oxygen absorption*

Oxygen absorption is the foremost type of active packaging. There were patented more than 50 types of absorbers since 1989. Their applications can reduce the residual oxygen concentration of the package to less than 0.01% (based on a simple package without changing the atmosphere). The oxygen causes discolouration, nutrient loss, increase microbial growth and also participates in the formation of harsh tastes. The oxygen uptake may be performed by oxidation of metals or their oxides, oxygen absorbents based polymer or enzymatic oxidation. These compounds are applied in the form of bags, which are inserted into the packaging, attached to the inner wall of the package incorporated into polymer material.



- ***Humidity control***

Systems affecting the humidity can be divided into systems absorbing released water (for example the pads in packages of cut meat, poultry or of frozen fish and seafood) and products or systems regulating moisture on the surface of the packaged product (packaging materials with anti-condensation coating).

- ***Absorption of undesirable flavors and smells***

This is a rarely used technique because of its high cost. Enzymes are mainly used forming or removing sensorially active substance immobilized on the packing material.

- ***Release of antimicrobial agents***

For all foods that may be subjected to microbial destruction, must cover a perfect barrier against microorganisms which can penetrate into the food from the external environment vicinity. The container must be airtight and can be an active carrier of antimicrobial substances. Bacterial growth can be counteracted by the removal of oxygen or by number of antibacterial substances which are part of the packaging material. These may be bacteriocins (nisin, pediocin), potassium sorbate or benzoic acid anhydride. The widespread applications include antimicrobial packaging systems releasing ethanol and carbon dioxide [1-4]. The stability of fresh meat can be controlled by using of antimicrobial agents as organic acids, bacteriocides and spice extracts [5, 6]. Garlic oil (sulphur compounds – allicin, diallyl disulfide and diallyl trisulfide) possesses good antibacterial activity [7]. Chitosan (ChT) is also a frequently used antimicrobial agent, since film can be prepared by evaporating from dilute acid solutions [8 - 11]. Chlorhexidine (CH) was used as vegetable preservative [12]. Silver or copper ions (Ag-zeolite is the most common agent), quaternary ammonium salts and natural compounds are generally considered as safe antimicrobial agents [13]. Triclosan-based antimicrobial agents, which are commercially developed (Microban<sup>®</sup>, Sanitized<sup>®</sup> and Ultra-Fresh<sup>®</sup>), have activity against a wide range of food pathogens [14, 15]. Other group of compounds with antimicrobial effects used in food packaging are natural plant extracts [16 - 18].

## 2. Nosocomial infection

Infection diseases are still a critically important issue in a variety of areas, such as medical devices, health care products, water purification systems, hospitals, dental office equipment, food storage and household sanitation [19]. Infections cause the highest threat to patients during the hospitalization time. These infections are called nosocomial (from Latin nosocomium “hospital”) or hospital-acquired infections (HAI). HAI are able to make patient unhealthy and often are reason of mortality. The 2002 there were approximately 1, 7 million patients infected in USA hospitals and almost 100,000 of them died, Germany, Italy and Switzerland show similar results. The situation in developing countries can embrace an own chapter, because there is very high risk of bacterial infection [20]. From 5% to 15% of hospital in-patients and about 20% of patients in an intensive care unit develop an infection during their admission just in western countries [21, 22].

Pathogens can enter hospital environment from other infected patients, hospital staff, visitors or other persons. Transfer of pathogens occurs by several path (vehiculum) shown in table 1.

*Table 1: The ways of pathogens transmission in hospital [23]*

<b>Direct non-specific vehiculum</b>	<b>Indirect specific vehiculum</b>
Air, water, food, linen items and tools, waste, insect.	Operative wounds, infusion solutions, central venous catheters, urinary catheters, artificial ventilation systems, endoscopes, installation of prostheses or other foreign objects, stomatological, haemodialigical or other medical tools.

Indeed developing of infection in hospital depends on many factors. The main factors are related to hygiene of hospital staff and patient. Besides, the current patient condition is also important (age, geographical location, sex or level of immunity, use of antibiotics). Pathogens can enter the human body through 3 large (skin, respiration system, alimentary tract) and through 2 small (eye conjunctivas, urogenital tract) epithelium surfaces. Apart from these regular ways, there is always a potential risk of infection after interruption of epithelium surface due to invasive intervention. These nosocomial infections are then called specific. Any foreign object from outside environment (anaesthesia and ventilation tubes, prosthesis, urinary and intravascular catheters or prostheses are the most common) introduced into the body significantly increase risk of infection [23].

### 3. Bacterial surface colonization

Beside infective pathogens causing usual infective illnesses like salmonella, flu, hepatitis and others, there are so called specific nosocomial illnesses, given rise by bacteria conditionally pathogenic and non-pathogenic in prevalent community.

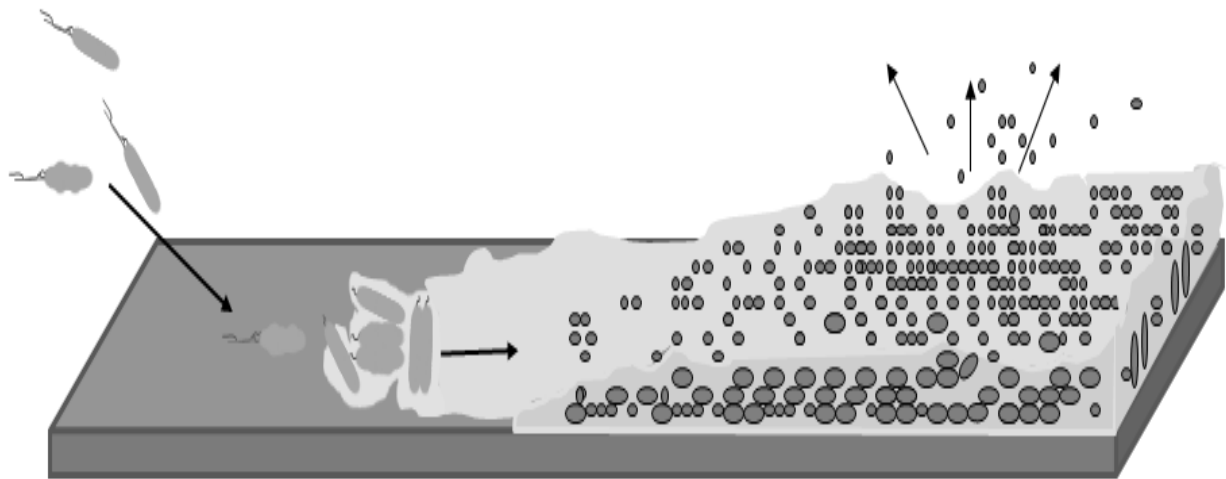
Table 2: Infection rate of common specific nosocomial pathogens [24]

Biomedical device	Infection rates (%)	Pathogens
Hip	0, 23 - 2, 23	<i>Staphylococcus aureus</i> , <i>Cryptococcus neoformans</i> , <i>etc.</i>
Knee	0, 4 - 5	<i>Staphylococcus species</i> , <i>Streptococcus pneumonia</i> , <i>etc.</i>
Shoulder	0, 04 - 4, 4	<i>Staphylococcus species</i> , <i>Mycobacterium avium-</i> <i>intracellulare</i> , <i>Propionibacterium acnes</i> , <i>etc.</i>
Spinal	2 - 2, 76	<i>Staphylococcus aureus</i> , <i>Mycobacterium tuberculosis</i> , <i>Gram-negative rods</i> , <i>etc.</i>
Fracture fixation	1 - 30	<i>S. aureus</i> , <i>coagulase-negative</i> <i>staphylococci</i> , <i>Gram-negative</i> <i>rods</i> , <i>etc.</i>
Dental implants	5 - 10	<i>Porphyromonas gingivalis</i> , <i>Enteric and Candida species</i> , <i>etc.</i>
Central venous catheters	10 - 40	<i>Staphylococci</i> , <i>Klebsiella</i> <i>species</i> , <i>Candida albicans</i> , <i>etc.</i>
Urinary catheters	10 - 30	<i>Candida</i> , <i>Klebsiella</i> <i>pneumoniae</i> , <i>Escherichia coli</i> , <i>Pseudomonas species</i> , <i>etc.</i>

Bacteria strains *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae* and fungus *Candida albicans* are the most prevalent nosocomial pathogens [25]. Pathogens related to particular biomedical device are listed in table 2. Situation in hospital environment is often complicated by bacteria with antibiotic resistance, where methicilin resistant *Staphylococcus aureus* (MRSA) is the most significant and it is increasingly reported from many countries worldwide [26].

Bacteria are spread in the environment (air, liquid) in plankton state until their reach any suitable abiotic surface or other cell for attachment and growth. According to that, success of microorganism relies on its ability of adhesion in physicochemical point of view.

The mechanism of bacterial adhesion can be divided into two steps (Fig.1). When the distance between bacteria cell and other surface is larger than 50 nm, movements of bacteria occur due to reversible physicochemical interaction, which is the first step. These include Brownian motion, van der Waals attraction, gravitation forces, surface electrostatic charges, hydrophobic interaction, chemotaxis and haptotaxis. It has been shown, that bacteria attach more on hydrophobic and non-polar surfaces. After, the distance become lower than 5 nm, the bacteria is attracted to surface and the second – molecular and cellular step of adhesion started. Bacteria cell is adhered by hydrogen bonds, ionic and dipole interaction and various membrane structures (such as capsules, fimbriae, pili or slime) engage in specific irreversible molecular reaction with substratum. Cells multiply or aggregate at the same time. Released extracellular matrix serves as a protective covering for bacteria cells and therefore viability of bacteria is much higher. Extracellular matrixes predominantly consist of polysaccharides, but also contain proteins, nucleic acid and lipids. Bacteria on the surface together with their extracellular matrix structure are called biofilm. They are structural complexes with dynamic architecture and due to this fact they can proliferate on many abiotic surfaces. Biofilm is highly hydrated structure composed of discontinuous channels, which should serve as nutrient or communication ways for bacteria microcolonies. Settled bacteria in biofilm develop own resistance and susceptibility to antimicrobial agents by activating genes changing their envelope. By reaching its critical concentration, biofilm becomes a source of new infection (daughter cells escape out from the surface to start colonization process again) and increase invasions factors including toxins, haemolysins, proteases and other tissue degrading enzymes. When bacteria overcome the local host defence system, chronic infection occurs [27 – 32].



*Figure 1: Biofilm formation*

#### **4. Protection of material against microbial colonization**

Factor influencing bacterial adhesion to the surface can be divided into three main areas:

- **Nature of the environment** (pH, ionic strength, protein presence, antibiotics or calcium cations, magnesium, iron presence, zinc cations, cobalt, silver, nickel, blood flow, salts, time)
- **Type of microorganism** (concentration, membrane hydrophobicity, zeta-potential, membrane features - such as pili or flagella, proteins, and lipopolysaccharides, susceptibility to antibiotics, extracellular substances)
- **Substratum properties** (surface functional groups, roughness, porosity, hydrophobicity).

As mentioned above bacterial attachment on solid substrate surface is highly dependent on material properties, so that their target changing leads to decrease of infection risk. Polymeric materials are frequently used in medical care due to their great flexibility of design, as it is seen in table 3. However, they need to be surface treated to resist bacterial colonization. Only few polymers are resistant against bacterial by themselves (e.g. ChT), but unfortunately they do not provide appropriate mechanical properties.

Table 3: Application of polymer in medicine [33, 34]

<b>Synthetic polymer</b>	<b>Application</b>
Polyethylene	<i>tubing drains, catheters, artificial hip or prosthetic joints</i>
Polyvinyl chloride	<i>blood containers and solution storage bags, surgical packaging, tubes of central venous catheter, ventilation tubes, cannulae, cardiovascular implants.</i>
Polypropylene	<i>catheters, orthopaedic implants (where more rigidity is required)</i>
Polyethylene terephthalate	<i>artificial vascular implants, heart valves</i>
Polyamides	<i>catheters, packaging films, artificial tendons, sutures</i>
Polyurethanes	<i>artificial skin, mammary prosthesis, vascular catheters, vascular graft, artificial heart diaphragms and valves, tubing</i>
Polyvinyl alcohol	<i>surgical threads</i>
Polystyrene	<i>filter wares</i>
Polymethyl methacrylate	<i>blood pump and reservoirs, implantable ocular lens, artificial vascular grafts</i>
Polytetrafluoroethylene	<i>catheters and artificial vascular grafts</i>

Materials evince antibacterial properties can be prepared by various ways. One method is to incorporate an organic or inorganic biocide to the polymer bulk during the material processing or we can modify monomer to be antibacterial and then to polymerize it. Another method is to change polymer properties to antibacterial after processing. Polymer can be coated by antibacterial layer, grafted by antimicrobial low molecular agents which are slowly realising or covalently bonded [35 - 39]. Other novel way is to incorporate bacteriophage onto polymer surface [40, 41] or change surface morphology towards avoiding prospective attachment ability of the bacteria [42, 43].

## 5. Treating of polymer surface by grafting

Numerous studies have been devoted to the problem of polymer surface modification. Surface is an interface between material bulk and the environment and therefore its characteristics are responsible for interaction behaviour. Conventional polymeric materials have hydrophobic, chemically inert surface due to this fact, problems with adhesion, coating, painting, colouring, lamination, packaging, colloid stabilization, undesirable protein absorption or poor biocompatibility related to certain area of their application are arising. Physical or chemical processes can be used for their modification. Physical processes include surface segregation, electromagnetic waves irradiation of and oxidation with gases; while chemical use wet-treatment, blending, coating and metallization.

Grafting is a physicochemical process. This method is the most favourable one, because of the monomer introduction control, space density or exact location of chains. The chains covalently bonded to the surface avoid its delaminating. By this technique, surface can be tailored by introducing a specific functional group: one end of grafted polymer is attached to surface while the other part of the polymer with appropriate functional groups is capable of consequent surface interaction [44].

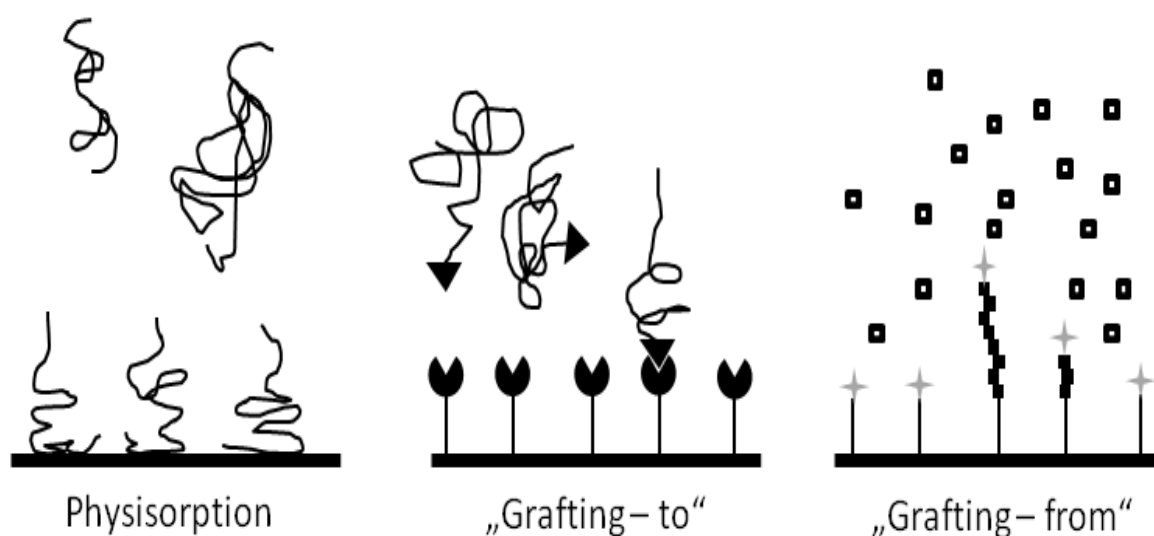


Figure 2: Polymer brush formation techniques

### 5.1 Synthesis of grafted surfaces

Polymer chain interaction with solid state surface could be both reversible and irreversible process. Grafted surface can be obtained generally by physisorption or covalent attachment (Fig. 2). *Physisorption* method is a reversible process. One polymer block interacts strongly with the substrate, while opposite part of the chain interacts weakly forming the graft. Covalently bonded polymers make the polymer



more robust and resistant to common chemicals. When pre-synthesized polymer interacts to polymer substrata, it is called "*grafting to*" approach. However, the "*grafting from*" approach occurs, when a polymer chain "grows" (polymerizing) on the backbone surface. Nevertheless, it can be synthesized polymer higher density structure by "*grafting from*" approach because monomers can easily reach reactive sites, but the polymer backbone needs to have a radical initiator which can supply the radical which is necessary for radical polymerization [45].

Polymer surface initiators are essential for polymerization initiation. They can be surface immobilized by several synthetic methods, such as cationic and anionic polymerization, living free radical polymerization and free radical polymerization [46]. The active surface sites can be generated by UV irradiation in presence of photoinitiators or photosensitizer, as well as by ozone oxidation or by various plasma surface treatments.

The graft chains conformation strongly depends on graft density. Firstly, at low densities, a "mushroom" structure is formed with a random-coil dimension [47]. With graft density increase, chains try to stretch away from surface forming polymer brush. Numerous AFM studies or contact angle measurements showed proper information related to design and architecture of so-called polymer brushes. It has been shown, that varying chain length can be done by optimizing the monomer concentration or irradiation time in case of photopolymerization. Grafted chains parameters respond to reaction conditions (mainly pH and ionic strength) and their intermolecular interaction by moderating of their shape and dynamic surface characteristics. Polymer brush properties offer a wide range of applications. Adequately formed brush can eliminate surface electrostatic charge generation, increase of interactive forces at the interface and significantly lower friction coefficient value. Grafted chains are capable of absorbing substances like both small molecules and large biomacromolecules, with application as slowly releasing antibacterial agents [48, 44].

## 6. Plasma treatment of polymer materials

Plasma is a set of charged and neutral particles in different quantum states (electrons, ions, excited states, neutral atoms, molecules), which forms the approximately equal to zero space charge (the so-called quasineutrality). Plasma is also called as the fourth state of matter. Its outward behaviour most resembles the gas from which it differs mainly by the presence of free charge carriers. It is capable of electric and magnetic fields generation and response to such field presence. Plasma is not a unique phenomenon, it is estimated that up to 99% of the total space is found right in this state. It is all about stars, but also a variety of nebulae and galaxies. Plasma can be found less often in earth conditions, because its existence has a large energy demands (high temperature, pressure, radiation, etc.), it will not last long as for example flashes and other discharges.

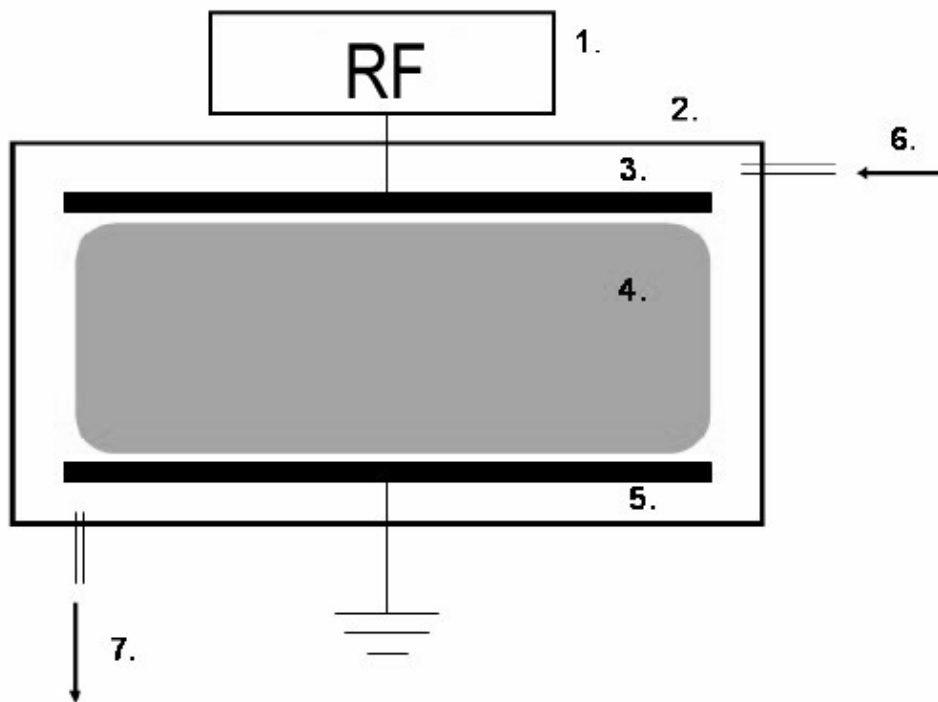


Figure 3: Example of plasma reactor: radio frequency plasma discharge chamber: 1 – radio frequency source; 2 – reactor vacuum chamber; 3 - radio frequency electrode; 4 – plasma discharge; 5 – grounded electrode; 6 – plasma gas input; 7 – gas output [51]

It is essential to release electrons from atoms or molecules in the gaseous phase or to be subjected of ionization for plasma creation. The ionization occurs when the atom or molecule gains sufficient energy - it can be accepted from secondary sources, but also from mutual collisions with neighboring particles. The generation and maintenance of plasma is source of energy causing ionization. Generally, the gas

plasma triggers when electrodes are connected to the appropriate voltage (Fig. 4). Breakthrough energy depends on the gas pressure and discharge gap width. The lowest breakthrough energy comes at low pressures. At atmospheric pressure occurs an arc discharge. Breakthrough energy can be reduced more by using radiofrequency source, laser, microwave, magnetic field, etc. [49, 50]

Plasma is a very chemical reactive environment in which unusual reactions take place. The high density of ionized and excited particles inside the plasma can change the properties of otherwise inert material. Plasma also modifies the surface energy of material, which has a consequent effect on the adhesion strength characteristics of surface coatings and biocompatibility. Ingestion plasma editing biomaterials offers the following *benefits*:

- reliable treatment which does not affect bulk properties; modifications depth reaches only a few angstroms.
- can be applied on any material substrate: metals, polymers, ceramics, composites, ...
- may influence variety of surface characteristics: chemical, tribology (friction), electrical, optical, biological, ...
- sterilizes treated surfaces
- treatment is environmentally friendly
- selection of the processing carrier gas influences the type of chemical modification

Of course plasma treatments comprise some *disadvantages*:

- treatment must take place mostly in a vacuum chamber, which increases the total number of discontinual operations and finally leads to the total cost increase
- the processes in plasma are very complex. It is difficult to understand well all the prospective interactions between the plasma and the surface and for example accurately control the functional groups quantity
- launching of industrial production from laboratory experiments is complicated

The species responsible for plasma – surface reaction are electrons, ions and radicals. Which reaction is initiated by these species depends mainly on used reaction gas, its feed rate, as well as the energy level of the plasma and type of treated material. The noble gas plasma (helium, neon, argon) used only its

mechanical energy of particles and caused mainly physical changes of surface or forming free radical by splitting off surface hydrogen. This type of radical formation is happening also simultaneously with chemical reactions when no-inert gases are used. Activation of polymer surface by plasma by free radical introduction onto the surface is well described technique, where radicals serve as initiators for further copolymer reaction (“*grafting from*”) and forming of polymer brush [52]. Processes using oxygen plasma are most common in the modification of polymer surfaces. It is known that oxygen in the plasma reacts with a variety of polymers to form oxygen-functional groups C-O, C = O, O-C = O, etc. Besides pure oxygen is widely used, its compounds, such as CO, CO<sub>2</sub>, SO<sub>2</sub> or H<sub>2</sub>O incorporate hydroxyl groups into the surface. Gases as N<sub>2</sub>, NH<sub>3</sub>, F<sub>2</sub>, HF and F<sub>4</sub>S are used for surface wettability alterations. Extraordinary physical properties such as micro-hardness, optical reflection angle and tightness can be reached by reaction of polymer in hydrocarbons (methane, ethane, ethylene, acetylene, benzene). Possible surface application of plasma is shown in table 4 [53].

## 6.1 Dielectric barrier discharge

Contemporary trend in plasma technology development is oriented to treatment at atmospheric conditions without expensive vacuum technology. The barrier discharge is generated between electrodes, at least one of which is covered with dielectric layer at atmospheric pressure. Glass, ceramics, corundum or polytetrafluorethylene are mostly used as the dielectric. Dielectric barrier has two basic roles: discharge at high pressure is split into large numbers of micro-discharges homogenously distributed in time and space throughout the discharge area. Second role is the inhibition of transition to arc discharge by limitation of charge transferred by each micro – discharge. Micro – discharges, occurred at atmospheric pressure, last typically nanoseconds. Dielectric application also leads to altering or pulsed voltage supplying. Dielectric barrier discharge can be divided into three basic groups according to configurations: *volume*, *surface* and *coplanar* barrier discharge (Fig. 4).

*Volume barrier discharge* can be generated, when a dielectric barrier is inserted in the area between electrodes. The most often is one of electrodes covered by dielectric. Volume plasma discharge consists of many micro-discharges perpendicular to electrodes, which enlarges its volume in contact with dielectric and gradually covered whole dielectric surface by rising voltage.

*Surface barrier discharge* arises between electrodes firmly attached to dielectric barrier. Typically, the first electrode is square shaped and second electrode is ordered in comb structure. Plasma appears in gaps of combed electrode. The most active plasmachemical place is founded on the top of electrodes and on the peak of

discharge. The combed electrode lifetime is restricted because of its direct contact with plasma and subsequent spraying.

*Coplanar barrier discharge* is combination of previous two. Couples of parallel electrodes excited by opposite polarity are placed inside dielectric close to the surface (under one millimeter depth). Distance between electrodes can be 100  $\mu\text{m}$  and less. Plasma is generated on the dielectric surface and consists of narrow microfilaments, which enlarge along the electrodes and forms diffuse area. Researches from Masaryk University in Brno optimized geometry of electrodes producing macroscopically homogenous plasma. The discharge is called diffuse coplanar surface barrier discharge (DCSBD) and it is characterized by long electrodes lifetime and short treatment time due to dense diffuse plasma (approximately  $100 \text{ W/cm}^3$ ) [54, 55].

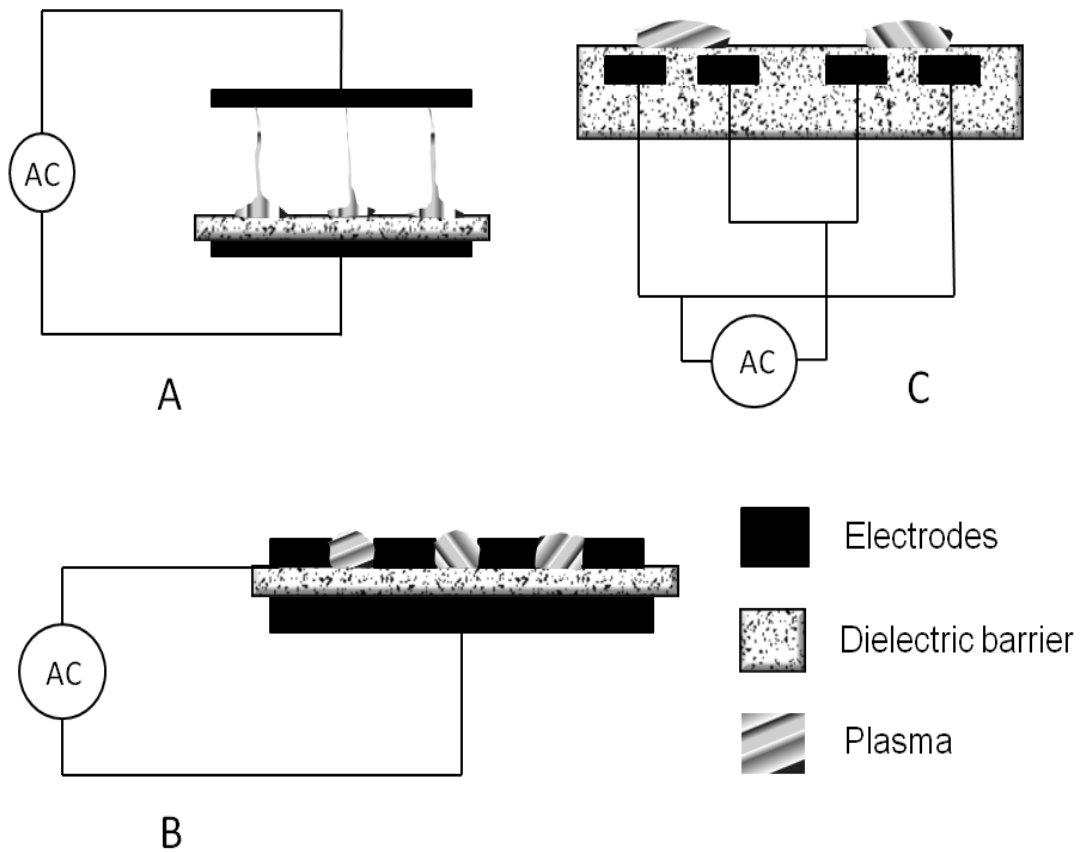


Figure 4: Barrier discharges: A - volume barrier discharge; B - surface barrier discharge; C-coplanar barrier discharge

Table 4: Possible surface modifications by plasma [56]

<b>Application</b>	<b>Description</b>
Sputter-deposition	<i>Ions (and atoms) from the plasma impact the target, and release atoms (or molecules) of the target material. The sputtered atoms diffuse through the plasma and reach at the substrate, where they can be deposited.</i>
Plasma enhanced chemical vapour deposition	<i>By chemical reactions in the plasma gas (mainly electron impact ionization and dissociation), different kinds of ions and radicals are formed which diffuse towards the substrate and are deposited by chemical surface reactions.</i>
Etching	<i>Removing material from a surface (by sputtering, chemically).</i>
Plasma-immersion ion implantation	<i>Injection of an energetic ion beam into a material, changing herewith the atomic composition and structure.</i>
Plasma polymerization	<i>Plasma dissociation and excitation of an organic monomer gas and subsequent deposition and polymerization of the excited species on the surface of a substrate.</i>
Cleaning	<i>Atomic level removal of all possible undesired residues, such as oxides, metallic and organic contaminants.</i>
Surface activation and functionalization	<i>The bombardment by energetic particles breaks the covalent bonds at the surface, leading to the formation of surface radicals. The latter can react with the active plasma species to form different chemically active surface functional groups.</i>

## 7. Antibacterial agents

### Chlorhexidine:

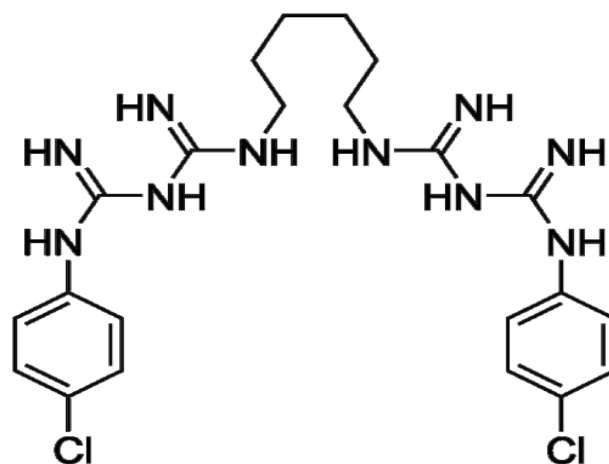


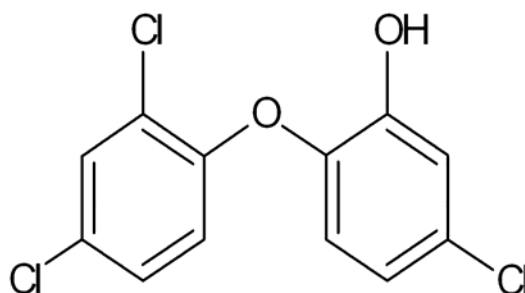
Figure 5: Structure of chlorhexidine molecule

CH molecule contains two 4-chlorophenyl rings and two biguanide groups connected with a central hexamethylene chain (fig. 5). It evinces germicidal activity against bacteria, yeasts, moulds dermatophytes and some lipophilic viruses. Its effect depends on concentration. Agent is bacteriostatic at low concentrations, whereas an increase of concentration changes the effect to bactericidal [57]. CH mode of action is the following. Cationic CH molecule is attached to negative charge bacteria surface. The adsorption is specific and it binds to phosphate containing compounds, which modifies bacteria cell membrane structure. CH increases permeability of inner membrane by reaction with phospholipids, which causes outflow of low molecular weight components (e.g. potassium ions).

The connection of high antibacterial activity, low mammalian toxicity and strong affinity to skin binding can serve as a useful anti-infective agent. Dentists found CH for many years as an excellent agent for plaque control and active compound in mouth rinses and toothpastes [58]. Surgical scrubs, health care personnel soaps, preoperative skin preparations, skin antiseptics, skin cleaners, wound protectants, burn ointments, acne creams, cosmetic preservatives, or ophthalmic solution are next most common CH applications.

Even CH is known by its low cytotoxicity levels, some studies showed that CH is able to alter the macrophages functions [59]. Some studies described accumulation of CH in natural river biofilm [60]. The p-chloroaniline, a CH degradation product can cause health problems [61].

## Triclosan

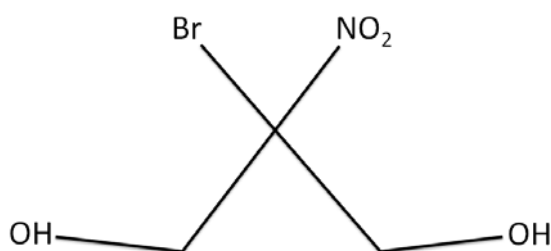


*Figure 6: Structure of triclosan molecule*

Triclosan (5-chloro-2-(2,4 dichlorophenoxy)phenol: (TC) is a non-ionic, odourless, and tasteless powder with excellent chemical stability (Fig. 6). Brand names of TC include Irgasan DP300, Aquasept, Sapoderm or Ster Zac. The application has dramatically increased over last 10 years, mainly due to its good thermostability. It is commonly used in toothpastes, acne creams, deodorants, hand soaps and it started to be incorporated more frequently into kitchen tiles, children's toys, cutting boards, toothbrush handles or athletic clothing. Production of TC exceeded 1500 tons per year [62].

TC is effective against many bacteria types as well as fungi. It acts on multiple cytoplasmatic and membrane sites through cell wall. As a result, RNA and fatty acids synthesis are disturbed. TC mass production leads to its higher amounts indicated in the wastewaters. Water treatment technologies for usually cannot remove this agent and it remains in the environment [63].

## Bronopol



*Figure 7: Structure of bronopol molecule*

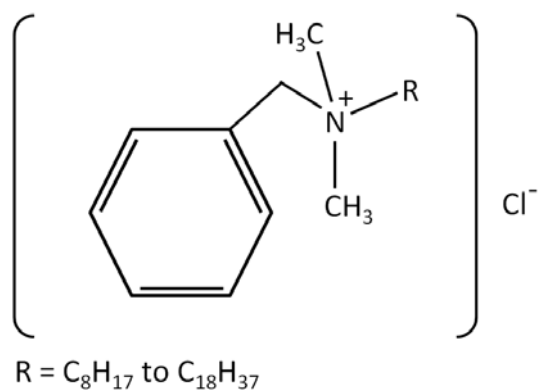
2-bromo-2-nitropropionamide, Bronopol (BR) is one of the widely used antibacterial preservatives, which belongs to the group known as formaldehyde – releasing compounds (fig. 7) [64]. It can be found as a preservative agent in many



areas, mainly in preservation of milk analysis samples and in cosmetics and hygiene products or leather conservation [65 - 67]

BR mechanism of action is supposed to be based on catalytic oxidation of thiol within the cell wall and free radicals generation [68]. Formaldehyde is released during BR decomposition which can cause allergy and irritation [69].

### Benzalkonium chloride



*Figure 8: Structure of benzalkonium chloride molecule*

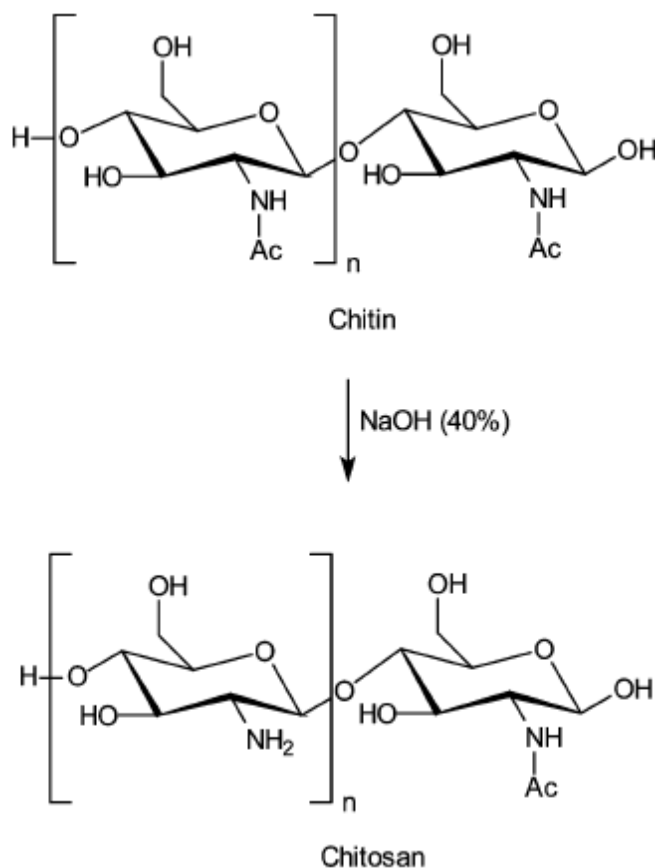
Benzalkonium chloride (BCH) is a cationic surfactant and an antimicrobial agent commonly used in nasal sprays, ophthalmic preparations, mouth rinses, cosmetic products or infant care products. Chemically, it is a quaternary ammonium compound; a mixture of alkylbenzyltrimethylammonium chloride with various even-numbered, straight alkyl chains with 8 to 18 carbons (fig. 8) [70]. It behaves highly lipophilic and it charges positively in an emulsion of host lipids [71].

Mechanism of antibacterial activity depends on N-alkyl chain length. Optimal activity against gram positive bacteria and yeast is achieved with chain lengths of 12–14 alkyls, while the lengths effective against gram negative is 14-16 alkyls and compounds containing less than 4 or more than 18 alkyls in a structure are almost inactive.

BCH influence the cell membranes integrity. Positively charged nitrogen of BCH molecule is attracted to acidic phospholipids of bacterial membranes. Then, hydrophobic BCH chain incorporates into the bacterial hydrophobic membrane core [72].

However, number of studies shown appreciable toxic effects on corneal and conjunctive epithelium including irritation, tear film instability or allergic reactions [73].

## Chitosan



*Figure 9: Preparation of ChT from chitin [19]*

Chitosan (ChT) belongs to group of antibacterial agents that are classified as biocide biopolymers. It promises not harmful effect on environment by any residual toxicity or permeating through skin [19]. ChT is a linear copolymer of  $\beta$ -(1-4) linked 2-acetamido-2-deoxy-  $\beta$  -D-glucopyranose and 2-amino-2-deoxy-  $\beta$  -D-glucopyranose. It is obtained by deacetylation of its parent polymer chitin, a polysaccharide occurring in natural sources, such as exoskeleton of insect, crustaceans or fungi (fig. 9). Its molecule contains important functional groups: amino and primary and secondary hydroxyls, which allows variety of possible chemical modifications [74]. Besides other applications, ChT molecule can form hydrogels. Polymer structure exhibit a large amount of useful biological properties such as anticholesteremic action. The greatest ChT interest is devoted to its bacteria and fungi inhibition growth.

Although action mechanism is still not completely understood, there are six theoretical mechanisms, which are based on: leakage of cell membrane due to interaction of ChT amine group and cell membrane, blocking of cell's active centres, inhibition of toxin production cell growth and by selective chelating of metals, blocking the entry of nutrients into the cell by fully covering of the cell, penetration of low-molecular weight species into the cell and interference with the synthesis of mRNA and proteins or disturbing the physiological activities in the cell [75].

This compound is relatively non-toxic, biocompatible material [76].

## **AIM OF THE WORK**

The primary aim of the presented thesis is the preparation and characterization of an active antibacterial polymer surface by using multistep physicochemical approach including plasma activation and polymer brush formation. It could be used for chemical commodities and polymers for potential application in medicine and food industry.

The main attention is paid to the investigation of antibacterial activity of prepared samples against *S. aureus* and *E. coli* strains. The optimization of preparation approach towards achieving the best antibacterial properties is next goal of thesis.

## SUMMARIES OF PAPERS

The presented thesis examines novel physicochemical approach used for the treatment of common commodity polymer surface to gain antibacterial activity.

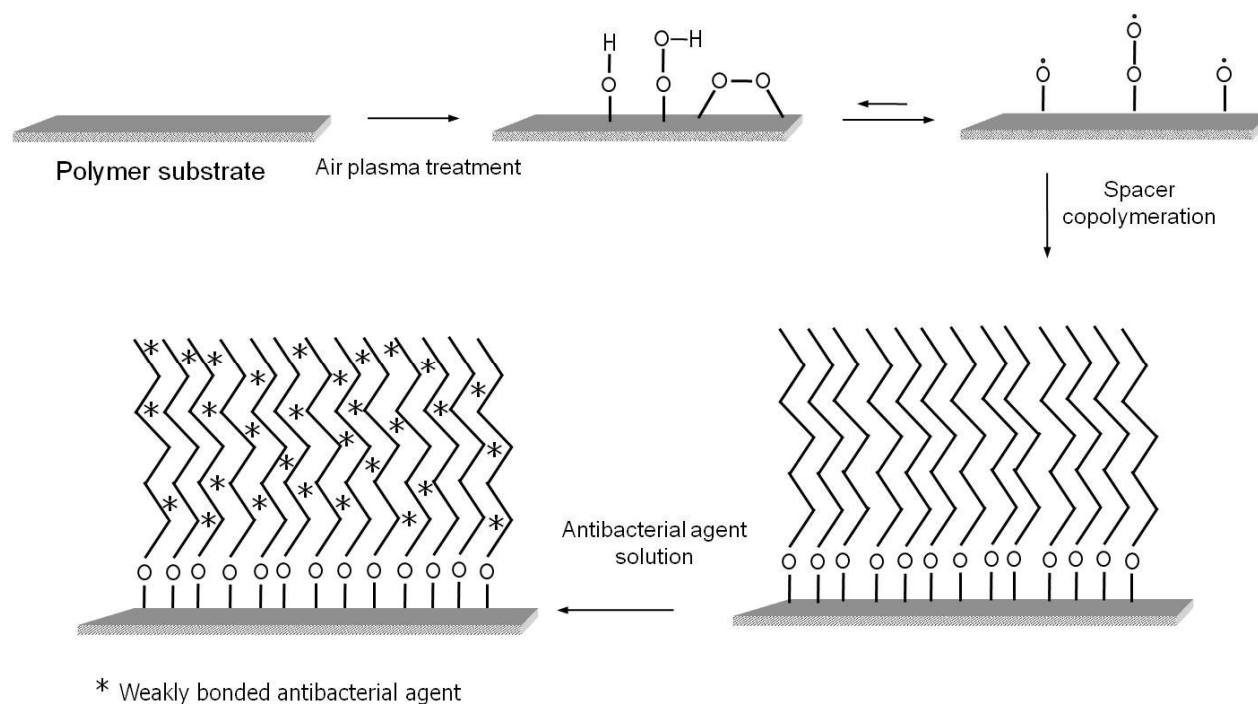


Figure 10: Multistep approach for preparation antibacterial surface

Firstly, the air plasma glow-discharge was used for reactive species generation on clean LDPE surface (fig. 10). The discharge used in **Paper I** was operated at the frequency of 13.56 MHz in the capacitive coupled radio frequency reactor. The DCSBD generator in air at atmospheric pressure serves as plasma source in **Paper II** and **Paper III**. Parallel banded electrode system and supply voltage ( $\sim 10$  kV, at frequency  $\sim 15$  kHz) leads to macroscopically homogeneous diffusion plasma generation. Due to the plasma treatment surface the free energy increased as a result of polar functional groups introduction on the treated surface, thus making the surface of LDPE more hydrophilic and reactive. Then, the plasma treated surface is exposed to saturated vapours of AA (**Paper I**) or grafting monomer solution of AAc (**Paper II** and **Paper III**) which chemically react with the activated surface and functionalize the polymer surface. Results from XPS and FTIR analyses or SEM images confirmed the presence of these functionalities and so called polymer brush structure formation on the surface. Finally, samples were immersed into the antibacterial agents' solutions. BCH, BR, CH and TC were applied in **Paper I**, TC and CH in **Paper II**. Surface analysis and mainly inhibition zone, measurement of antibacterial activity, against *S. aureus* and *E. coli* confirmed antibacterial agent immobilization on the AA grafted surface contrary to samples without grafting. The

highest increase of antibacterial activity was observed by the sample containing TC and grafted by AA. On the other hand, samples covered by BR did not show any antibacterial activity. The multilayer coating was created by several alternating dipping into ChT and pectin solutions and final crosslinking by glutaraldehyde in **Paper III**. However, samples grafted by AAc observed more antibacterial inhibition zone efficiency than samples coated by multilayer chitosan/pectin system.

Surface wettability was investigated by static contact angle measurement using sessile drop technique. Acid – Bases theory were chose for surface energy evaluation. Generally, significant increase of total surface energy (mainly basic part) was observed after the plasma treatment because characteristics reactive polar functional groups were introduced onto the LDPE surface. The monomer grafting led to the further increase of basic part of surface energy, whereas AA and AAc contain polar amine and carboxylic groups. Surface energies of antibacterial coated samples indicate their presence on the surface and alter according to polarity and amount of attached molecules.

The ATR-FTIR and X-ray photoelectron spectroscopy (XPS) described chemical changes during samples preparation. The results showed that the LDPE samples treatment by the low temperature plasma in air caused oxygen-containing groups' introduction to the surface. XPS analysis together with FTIR spectroscopy confirmed the grafted layer and antibacterial agent presence on the surface, because of detection their characteristic groups.

The amount of attached antibacterial agent, estimated from characteristic groups' intensity was generally higher for all samples covered by grafted layer. XPS analysis revealed to be more suitable analysis then FTIR. Because compared to the FTIR technique, XPS depth of analyzed layer is substantially smaller and thus the description of surface characteristics is not distorted by signal of non-modified subsurface layer and surface changes are easily detectable. Surface morphology changes were observed by scanning electron microscopy (SEM) (**Paper I and III**) and atomic force microscopy (AFM) (**Paper II**). The plasma effect led to the slightly increase of LDPE surface roughness as a result of functionalization and mechanical ablation processes. Further grafting by AA or AAc created a brush-like pattern appropriate for subsequent modification, which is more noticeable by AFM analysis. Antibacterial agent deposition caused smoothening of SEM images.

## CONCLUSION

Health damages caused by various infections as a consequence of bacterial growth on polymeric products or deterioration of packed food by bacteria are two main reasons of active antibacterial material improvement. The material protection key point lies in the adhesion controlling as its first step of bacterial surface colonization.

The thesis deals with effective polymer surface treatment, while bulk properties remain unchanged. This advantage enable to use mentioned approach after product processing, so that there are reduced problems related to mixing antibacterial agent into bulk polymer during processing, such as blend homogeneity, antibacterial agent degradation or antibacterial agents impact on bulk properties. Moreover, antibacterial agents' consumption is significantly reduced, considering their impact on environment.

Our results showed, that it can be prepared antibacterial active polymer surface that resist most effectively *E. coli* and *S. aureus* strains. Most effective configuration reveals to be TC attachment to AA or AAc as grafting monomer and activation of surface by diffuse coplanar surface barrier air discharge at atmospheric pressure. This approach can be advantageously integrated for example to packaging foil production line. However, there are still a lot of opened questions before final industrial application. Antibacterial effect's durability, peel resistance, potential cytotoxicity and *in vivo* assay need to be tested.

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## LIST OF ABBREVIATIONS

AA	polyallylamine
AAc	polyacrylic acid
AFM	atomic force microscopy
ATR – FTIR	attenuated total reflectance - Fourier transform infrared (spectroscopy)
BCH	benzalkonium chloride
BR	bronopol
DCSBD	diffuse coplanar surface barrier discharge
CH	chlorhexidine
ChT	chitosan
HAI	hospital-acquired infections
LDPE	low-density polyethylene
mRNA	messenger ribonucleic acid
MRSA	methicilin resistant <i>Staphylococcus aureus</i>
SEM	scanning electron microscopy
TC	triclosan
XPS	X-ray photoelectron spectroscopy



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# **PAPER I**



# Preparation of active antibacterial LDPE surface through multistep physicochemical approach: I. Allylamine grafting, attachment of antibacterial agent and antibacterial activity assessment

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## ABSTRACT

Low-density polyethylene (LDPE) samples were treated in air plasma discharge, coated by polyallylamine brush through copolymeric grafting surface-from reaction and deposited four common antibacterial agents (benzalkonium chloride, bronopol, chlorhexidine and triclosan) to gain material with active antibacterial properties. Surface characteristics were evaluated by static contact angle measurement with surface energy evaluation ATR-FTIR, X-ray Photoelectron Spectroscopy (XPS) and SEM analysis. Inhibition zone on agar was used as *in vitro* test of antibacterial properties on two representative gram positive *Staphylococcus aureus* (*S. aureus*) and gram negative *Escherichia coli* (*E. coli*) strains. It was confirmed, that after grafting of polyallylamine, more antibacterial agent is immobilized on the surface. The highest increase of antibacterial activity was observed by the sample containing triclosan. Samples covered by bronopol did not show significant antibacterial activity.

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## 1. Introduction

Despite a great progress in the area of medical science, solving of problems referred to nosocomial infections are maintained at approximately the same level [1,2]. Nosocomial infections are diseases emerging during hospitalization and at once they are not related to the problem that the patient is hospitalized with. Such disorder brings worsening of health condition and patient comfort, extending the time of healing, additional antibiotic therapy, and therefore its price, the patient also becomes a source of infection for his environment and these complications frequently cause patient death [3]. Nosocomial infections may be endogenous or exogenous origin. Infections of endogenous (internal) origin are caused by microorganism normally occurred in the human body, that is weakened during immunodepression. For exogenous (external) source of nosocomial infections lies a factor in the surroundings: other patients, hospital staff, insects, air, food, but highly potential source of infection are foreign objects introduced into the patient body as medical devices. These are for example: urinary catheters, central vascular cannulae, tubes of respirators or artificial prostheses [4]. The surface of such devices can be easily colonized by bacteria, when hardly removable biofilm is created as a source and

centre of further infection [5]. This infection may be partly reduced by compliance with hygienic-epidemiological regime of nursing staff. Equally important way how to reduce foreign object infection risk on in human body on minimum is to treat its surface to gain active antibacterial properties [6].

Materials with antibacterial properties can be prepared by several approaches [7–9]. One of them is immobilization of antibacterial agent on the surface of polymer material [10]. This seems to be more effective than incorporation of the active species into the polymer bulk with respect to the relatively short application period of these devices which is mostly not extending 2 weeks. Surface modification of synthetic polymer materials changes the chemical composition of the upper layer [11–13]. The overall substrate properties, especially mechanical, remain unchanged and the substrate achieves adequate strength and flexibility [6,14].

Surface modification can be achieved by several methods, i.e.: mechanical treatment, flame treatment, wet chemical etching by strong oxidizing acids, corona or plasma treatment [15–17]. The last listed method seems to be the most effective as well as environmental friendly. Plasma treatment of polymer materials in air as a carrier gas is an effective tool to modify the surface via incorporation of oxide containing groups onto the surface structure, i.e.: hydroxyl, carbonyl, carboxyl, ether, hydroperoxide, etc. Some of the groups are unstable and system leads to the reaction forming active radicals [18–20]. These metastables are capable to react with suitable monomer creating polymer “brush-like” structure

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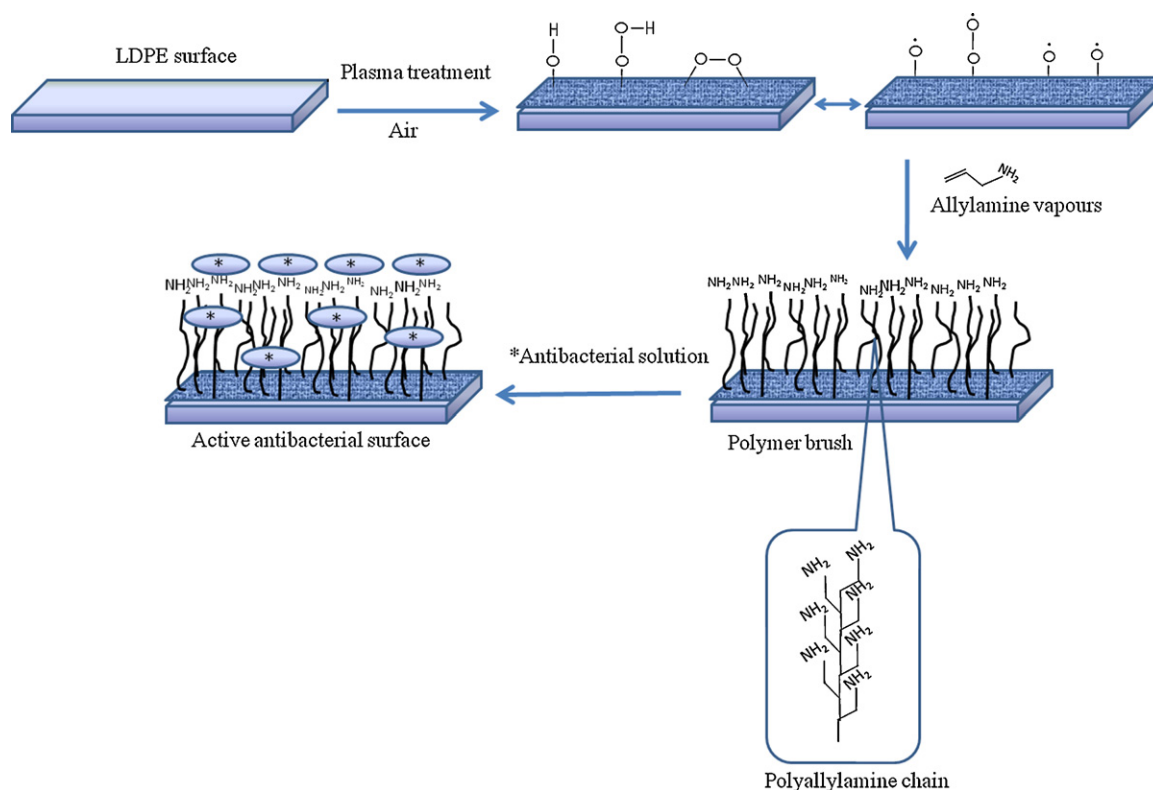


Fig. 1. Modification of LDPE surface.

[21–23]. This kind of structure is suitable for reaction with antibacterial agent molecule functionalities by creation of intra-molecular forces. It is worth noting that chemical bond due to its relative high strength would not lead to final application because of the relatively low antibacterial agent diffusion from the surface structure and thus low active antibacterial activity [24–26].

In this work, the radiofrequency (RF) plasma discharge was selected for surface activation of low-density polyethylene (LDPE) substrates as a first operation in the multistep approach. After surface modification the allylamine (AAm) monomer was grafted onto the surface to create amine containing polymer brush structure via radical “surface from” reaction in gaseous phase. The last step is connected with the anchoring of the active antibacterial species into the surface structure.

Benzalkonium chloride, bronopol, chlorhexidine and triclosan were chosen as common antibacterial substrates in our study with respect to the final application which does not allow utilization of potentially hazardous antibacterial chemical reagents which cannot be used in direct contact with the body [6,27–29].

In this paper, the active antibacterial material multistep preparation approach, surface characterization by contact angle measurements, ATR-TIR spectroscopy, Scanning electron microscopy, X-ray Photoelectron Spectroscopy (XPS) and antibacterial assay is described in order to prepare a novel active functional material which can bring a new potential in healthcare reducing the amount of nosocomial infections and therefore to improve the comfort of the patients amongst others. These results can be useful not only in the development of packaging or medical material, but can contribute to the further examination of phenomena happening between polymer surface and bacteria cell.

## 2. Methods

The LDPE film samples of the 0.1 mm thickness with square size of 5 cm × 5 cm were washed in solution of cationactive ten-

side, cleaned in water and consequently in deionized water. After cleaning, they were dried in laboratory conditions for 2 h. Such prepared samples were subjected to RF plasma treatment from both sides by using FEMTO Diener (Germany) plasma reactor. The discharge was operated at the frequency of 13.56 MHz at the capacitively coupled radiofrequency reactor. The discharge matching power was 50 W for all experiments. For all samples, 5 min was the duration of plasma process. Flow rate of air as a carrier gas was 50 sccm. After that, the samples were placed in allylamine (Fluka) vapours for 15 s in order to create the amine polymer brush. Then, the samples were separately rinsed into the reaction vials containing antibacterial agent solutions and kept standing for 24 h at laboratory conditions. Concentration of antibacterial solution were following: 2% (w/v) Triclosan (TC) in absolute ethanol, 2% (w/v) bronopol (BrP) in absolute ethanol, 2% (w/v) benzalkonium chloride (BCh) aq. solution, 2% (w/v) chlorhexidine (ChlH) in isopropylalcohol 70% (v/v) aq. solution. Finally, the samples were taken out of the solution and cleaned by stream of water followed by ultrasonication for 5 min in deionized water to eliminate the unanchored antibacterial agent from sample surface. The last cleaning was done by deionized water. Cleaned samples were dried in ambient conditions for 2 h and then separately placed in desiccator.

### 2.1. Reagents

LDPE polymer was obtained as packing foil (The Dow Chemical Company), Triclosan ([5-chloro-2-(2,4-dichlorophenoxy) phenol]) 97.0%, bronopol (2-bromo-2-nitropropane-1,3-diol), chlorhexidine (1,1-hexamethylene bis[5-(4-chlorophenyl)biguanide]), benzalkonium chloride, absolute ethanol and allylamine were supplied by Sigma Aldrich (USA). Isopropylalcohol 99.7% was gained from Lach-Ner (Czech Republic). All reagents were used as received without further purification.

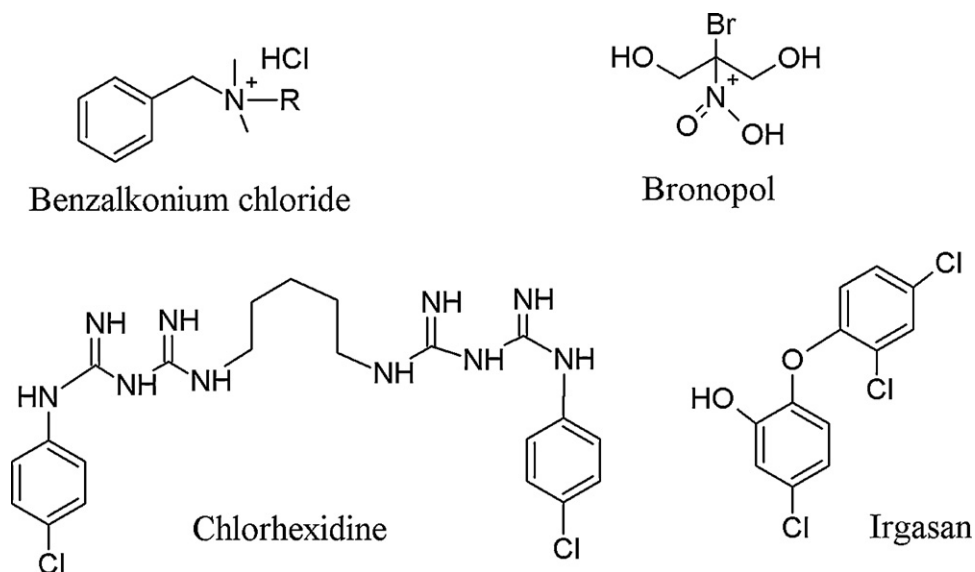


Fig. 2. Antibacterial agents.

## 2.2. Surface characterization

For surface energy evaluation and contact angle measurement, the “See system” (surface energy evaluation system) (Advex Instruments, Czech Republic) was used in this study. Deionized water, ethylene glycol (Sigma–Aldrich) and diiodomethane (Sigma–Aldrich) were used as the testing liquids; the droplets volume of testing liquids was set to 2  $\mu\text{l}$  for all experiments. Ten separate readings were averaged to obtain one representative contact angle value. Substrate surface free energy was evaluated using these data by “acid–base”.

ATR-FTIR spectroscopy (Avatar 320 Nicolet, USA) was used for surface chemical characterization. Spectra were collected at spectral resolution of 2  $\text{cm}^{-1}$ , ZnSe crystal was placed to incident angle 45°.

X-ray photoelectron spectroscopy analysis was performed on polymer films using Escalab 200A (VG Scientific, UK) with PISCES software for data acquisition and analysis. For analysis, a monochromatic Al K $\alpha$  X-ray source operating at 15 kV (300 W) was used, and the spectrometer, calibrated with reference to Ag 3d<sub>5/2</sub> (368.27 eV), was operated in CAE mode with 20 eV pass energy. Data acquisition was performed with a pressure lower than 10<sup>-6</sup> Pa. Spectral analysis was performed using peak fitting with Gaussian–Lorentzian peak shape and Shirley type background subtraction.

Table 1  
Surface energy values.

Sample	Surface energy components (mN/m)				
	$\gamma^{\text{total}}$	$\gamma^{\text{LW}}$	$\gamma^{\text{AB}}$	$\gamma^+$	$\gamma^-$
1. Untreated	33.9	33.7	0.2	0.12	0.09
2. Plasma treated	45.0	39.7	5.3	0.35	19.8
3. Plasma + AAm grafted	42.4	39.8	2.6	0.05	34.7
4. Plasma + BCh	40.0	37.4	2.2	0.17	7.1
5. Plasma + AAm + BCh	44.4	38.2	6.2	0.38	25.0
6. Plasma + ChIH	41.5	41.2	0.3	0.00	10.8
7. Plasma + AAm + ChIH	41.2	40.5	0.7	0.01	12.4
8. Plasma + TC	44.3	41.1	3.2	0.21	12.3
9. Plasma + AAm + TC	43.0	41.5	1.5	0.03	16.2
10. Plasma + BrP	42.0	40.0	2.0	0.08	13.1
11. Plasma + AAm + BrP	41.2	38.9	2.2	0.12	10.9

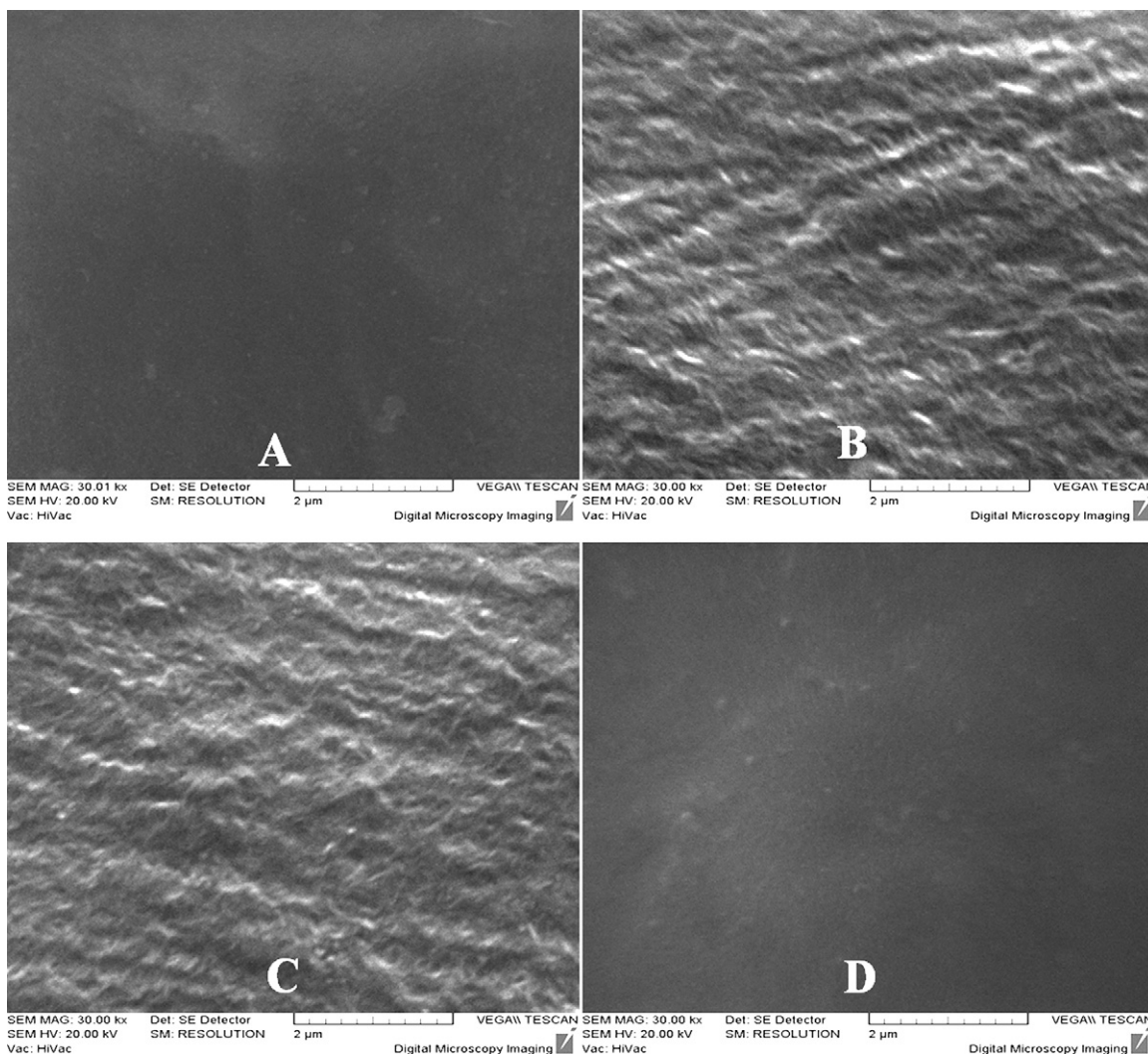
Scanning electron microscopy (SEM) was used for surface morphology evaluation of samples. The images were obtained by VEGA II LMU (Tescan, Czech Republic) microscope. A 30,000 $\times$  magnification was used and samples were 45° tilted for better observation.

Antibacterial activity of samples was tested against two bacteria strains: *Staphylococcus aureus* (CCM 4516) and *Escherichia coli* (CCM 4517) by measuring inhibition test (diffusion) zone on agar (Nutrient Agar No.2 M1269 – 500 g from HiMedia Laboratories Pvt. Ltd.). The circular shape specimens ( $d=8$  mm) were cut both from modified and unmodified samples, washed in ethanol, dried and placed on agar plate inoculated by bacterial suspension (volume: 100  $\mu\text{l}$ , concentration: 10<sup>7</sup> units/ml, incubation time: 24 h at 37 °C). Then, inhibition zone diameter was measured in 5 directions, and the average value was calculated. Each test was triplicate.

## 2.3. Substrate modification

Air plasma glow-discharge was used to introduce initiators onto samples surfaces (Fig. 1). The applied plasma process generated wide range of reactive species, which undergo consecutive chemical reactions, creating thus several oxygen based functionalities at the interface such as carbonyl, carboxyl, ether, peroxides and specially radicals which serve as a initiators of copolymerization reaction of other polymers [14]. Then, this pretreated surface was exposed in AAm vapour saturated atmosphere for 15 s. Monomer of AAm reacts with free radicals formed and immobilized on the surface and the propagation of copolymerization is started. Side chain of poly (AAm) is growing while this reaction is competing with homopolymerization of AAm in monomer vapours. Monomer units react and polymerize with each other and do not attach to surface. Copolymeric reaction is inhibited by oxygen molecules from air as well due to the bonding to the vinyl group.

Using plasma for initiating the surface together with “grafting from” approach is very advantageous for formation of copolymerized chains – high density polymer brush. Attached polyallylamine brush carries number of amine groups capable of bonding to four antibacterial agents mentioned above. Antibacterial agent’s molecules bond the poly (AAm) brush after 24 h of immersing in their solution by physico-chemical forces. The intermolecular origin



**Fig. 3.** SEM micrographs (A–D): A. untreated (sample 1), B. Plasma treated (sample 2), C. Allylamine grafted (sample 3), D. Plasma + AAm + Chlorhexidine (sample 7).

bonds formed between amine group and antibacterial agent allows antibacterial agent to diffuse into surroundings and provide LDPE material with active antibacterial surface.

### 3. Results and discussion

#### 3.1. Surface energy measurement

Table 1 shows results of surface energy analysis of tested samples. The relative standard deviation values for mentioned results were mostly 3% or less. Untreated PE (sample 1) reveals the lowest value of the total surface energy ( $\gamma^{\text{total}}$ ), due to its natural hydrophobic character. Dispersion component of surface energy ( $\gamma^{\text{LW}}$ ) is approximately the same value as a component of the total surface free energy. For these reasons, the polar component ( $\gamma^{\text{AB}}$ ) achieves low values, which corresponds to the theory and previous measurements [30].

The sample 2 processed in the low-temperature plasma has a significant increase in total surface energy. Moreover, there is an increase of polar component, which is due to incorporation of polar oxygen functional groups on the surface of PE during the plasma treatment.

In the case of the sample 3 (grafted by AAm), it can be noticed large increase of  $\gamma^-$  basic component which indicate presence of basic groups, amine group in this case, together with slight decrease

in total surface energy, mainly because of the AAm hydrocarbon chain presence. This could prove that AAm is successfully bonded to the surface.

BCh added to plasma treated surface (sample 4) decreased its total surface energy (especially polar part) compared to plasma treated (sample 2); while BCh added to AAm grafted (sample 5) surface increased its total, respectively polar surface energy component, compared to AAm grafted sample almost three times (sample 3). This could be explained by different type of reaction that occurs between activated or grafted surface and BCh. Significant rise of  $\gamma^-$  at sample 5 compared to sample 4 may confirm more basic group of BCh, what means greater amount of antibacterial agent were attached to the surface, because BCh molecule contains numerous basic groups. Thus, the antibacterial agent is anchored in higher amount in case of the previous reaction with AAm. The same consequence can be found for other substrates: sample 7 polar surface energy is higher than sample 6, especially increase of basic groups is significant. These groups can be chloride atoms of attached chlorhexidine molecules, which carries partially negative charge. TC bonded to samples 8 and 9 caused in both case reduction of mainly  $\gamma^-$  component. The decrease is higher at sample 9, where TC is added on polyAAm layer then on sample 8 – plasma treated only. Samples 10 and 11 covered by BrP showed similar behaviour. That decrease is here even higher than at TC samples Fig. 2.



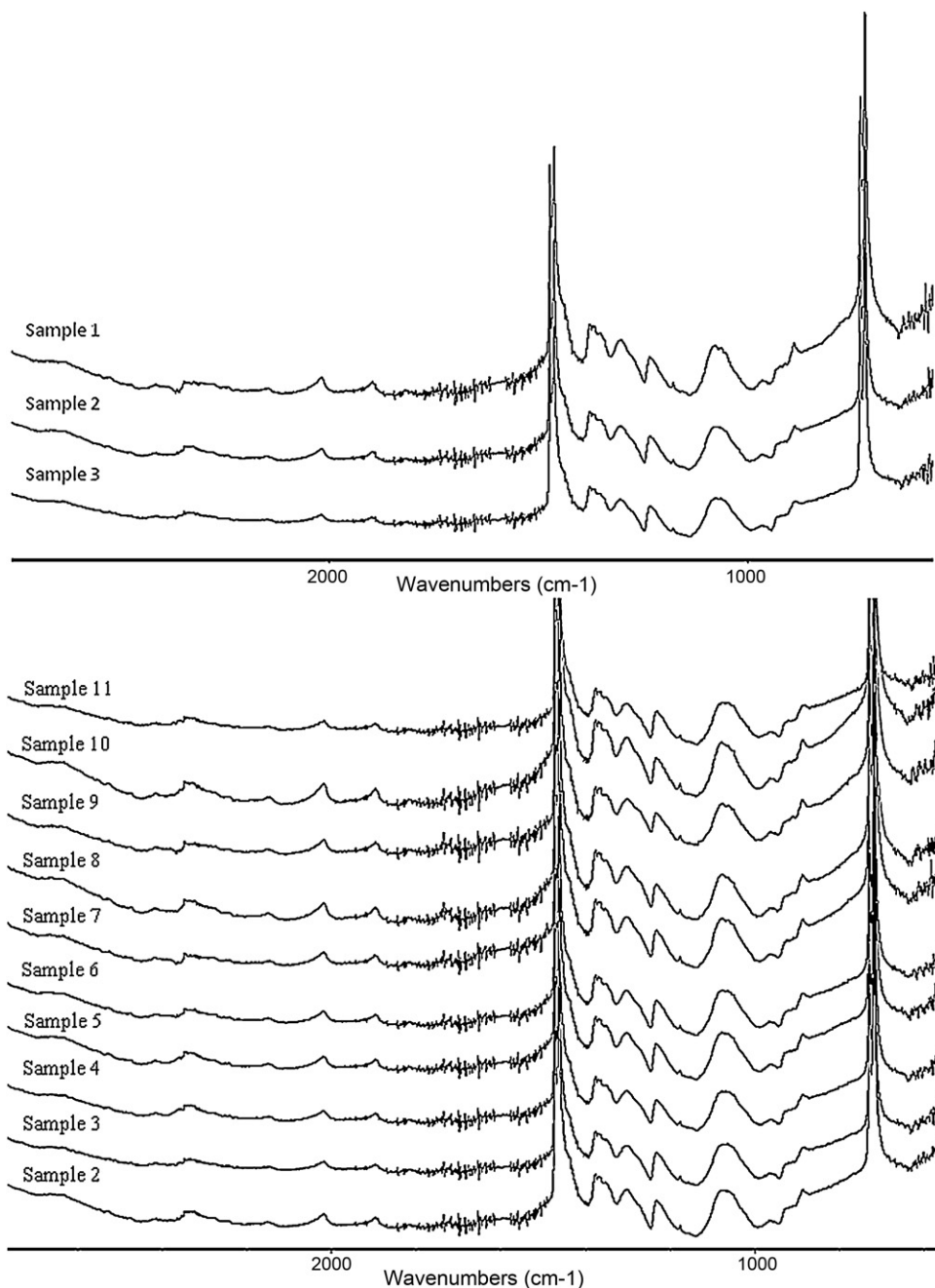


Fig. 4. FTIR results.

### 3.2. Surface morphology (SEM)

From the LDPE surface image (sample 1) is clear that the surface exhibits a low degree of surface density morphology (relatively smooth surface, even at high magnification).

An increase of roughness occurs in the case of the sample 2, which was modified by low temperature plasma. This is caused by the various particles bombardment, resulting in the temperature effect on the surface layer, which passes into the liquid phase and subsequent surface re-crystallization.

Sample 3 morphology seems to be similar to sample 2. Thus, AAm grafting on plasma-activated surface does not have significant effect on surface morphology.

An image corresponding to the samples on which the layer of antibacterial agent was applied shows that this layer covers the sur-

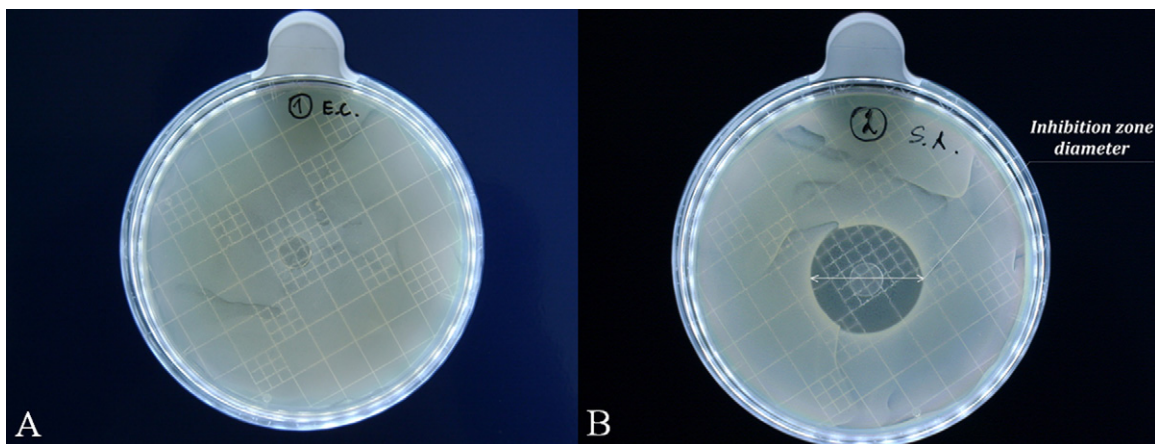
face topography. Images are shown in Fig. 3. These experiments and obtained results are in agreement with other polymeric substrates [31].

#### 3.2.1. FTIR spectroscopy

Very weak broad signal can be found in the area  $3000\text{--}3100\text{cm}^{-1}$  in case of plasma treated substrate (sample 2), which corresponds to  $\text{--OH}$  vibration (Fig. 4). Another minor change can be found around wave number  $1720\text{cm}^{-1}$ , where occurs the signal of  $\text{C=O}$  group. It can be also observable very weak peak in the area from  $1580$  to  $1600\text{cm}^{-1}$  after deeper analysis and subsequent enlargement, which corresponds to the carboxylate vibration. These oxygen-containing peaks in the spectrum disappear after reaction with AAm (sample 3). AAm molecules create bonds with surface through oxygen groups,

**Table 2**  
Surface elemental compositions and ratios of samples 1–6 obtained from XPS analysis.

	C 1s %	O 1s %	N 1s %	C 12p %	Br 3d %	O/C	N/C	Cl/C	Br/C
1. Untreated	98.6	1.4	–	–	–	0.0142	–	–	–
2. Plasma treated	87	11.8	1.2	–	–	0.1356	0.0138	–	–
3. Plasma + AAm Grafted	87.9	8.1	3.8	–	–	0.0922	0.0432	–	–
4. Plasma + BCh	86.6	11.8	1.3	0.3	–	0.1363	0.015	0.0035	–
5. Plasma + AAm + BCh	84.6	9.4	3.6	2.4	–	0.1111	0.0426	0.0284	–
6. Plasma + ChIH	86.8	11.9	1.1	0.2	–	0.1371	0.0127	0.0023	–
7. Plasma + AAm + ChIH	84.4	7.2	6.3	2.1	–	0.0853	0.0746	0.0249	–
8. Plasma + TC	86.8	11.3	1.4	0.5	–	0.1302	0.0161	0.0058	–
9. Plasma + AAm + TC	84.5	12.1	0.8	2.6	–	0.1432	0.0095	0.0308	–
10. Plasma + BrP	87.1	11.6	1.1	–	0.2	0.1332	0.0126	–	0.0023
11. Plasma + AAm + BrP	86.9	9.7	2.8	–	0.6	0.1116	0.0322	–	0.0069

**Fig. 5.** Images of Antibacterial inhibition zone test: A – sample 1 (untreated) *E. coli* test, without inhibition zone. B – sample 9 (plasma treated + AAm + TC) *S. aureus* test, with 27 mm diameter of inhibition zone.

which are not detectable. However, these groups also appear very weakly in the spectrum due to their presence of the antibacterial substrates, particularly in the  $-\text{OH}$  group BrP and TC (samples 8–11). Antibacterial agents are responsible for presence weak signals of aromatic benzene ring at wave numbers around  $1630\text{ cm}^{-1}$ . In the spectrum of the sample containing BrP was found very weak signal around  $1520\text{ cm}^{-1}$ , which is associated with the vibrations of  $\text{NO}_2$ , however presence of  $\text{C}-\text{Br}$  vibration in our sample with BrP (samples 10, 11) failed because of the very low spectral intensity in the subject area. Also presence of chlorine is not clearly visible. It may not correspond to the signals in the range of wave numbers  $600\text{--}700\text{ cm}^{-1}$  in samples where chlorine is present, especially in TC and ChIH (samples 6–9). The presence of nitrogen, which is structured in AAm molecules and antibacterial agents, mainly in ChIH (sample 6,7), was found as a broad peak at wave numbers  $3330\text{ cm}^{-1}$ . However, the spectrum contains even  $\text{C}=\text{N}$  vibration at wave number  $1640\text{ cm}^{-1}$ . Sample covered by BrP (sample 11) showed small peak in the area of  $1520\text{ cm}^{-1}$ , which corresponds to  $\text{NO}_2$  vibration. On the other hand, presence of  $\text{C}-\text{Br}$  vibration cannot be proven in our case, because of very weak signal intensity in supposed wave number area Fig. 5.

### 3.2.2. X-ray photoelectron spectroscopy (XPS)

XPS is employed to gain a quantitative insight into the elemental composition of the surface. Compared to the FTIR technique, depth of analyzed layer is substantially smaller and thus the description of surface characteristics is not distorted by signal of non-modified subsurface layer. The results are the analysis of the spectrum and its calculated surface atomic compositions and ratios clearly entered into Table 2.

The resulting values indicate that the untreated sample (sample 1) has a very significant proportion of carbon in the surface struc-

ture, as expected. There was even found small amount of oxygen, which could mean presence of impurities. Plasma treatment (sample 2) considerably increases the concentration of oxygen in the surface structure, up to a value of almost 12%. Respectively, ratio of oxygen and carbon atoms increased almost 10 times. This is because of incorporation of oxygen-containing groups during the modification by low-temperature radio frequency air plasma treatment. The same spectrum exhibits 1, 2% of nitrogen containing functional groups, 0.0138 in the ratio with carbon. It was determined after the peak deconvolution; that the nitrogen is present in the amine form due to the appropriate binding energy value. Concentrations of carbon on surface decreased proportionally. Upon AAm grafting (sample 3), the oxygen quantity compared to the plasma treated samples decreases on 8.1% with the slight 0.9% increase of carbon content and there is a significant increase in the value of nitrogen in the surface structure. Ratio of N/C elements increased 3 times, compared to sample 2, that means 3.8% of nitrogen on the surface. This is a direct consequence of bonding the AAm chains with the oxygen groups, which are covered over and their signal is decreased. XPS analysis evidently confirmed presence of antibacterial agent on samples 4–11. Chlorine groups are in 0.3% presence on the surface of sample 4, plasma treated and covered by BCh. Samples 5 analysis showed eight times increase of chlorine amount, on the value of 0.284 Cl/C ratio. This result confirms the importance of AAm brush structure preparation, which immobilizes more molecules of antibacterial agents by the creation of intra-molecular forces then simple plasma treated surface. Similar results are given for samples 6 and 7. Also here the chlorine presence was analyzed and its amount was even 10, 5 times higher for sample 7 coated by AAm followed by ChIH immobilization then for plasma treated (sample 6). C 12p elements represented 2, 1% of analyzed surface (0.0249 of Cl/C ratio). This increase was the greatest from others samples and

**Table 3**  
Inhibition zone area measurement.

	Inhibition zone (mm <sup>2</sup> )			Average value (mm <sup>2</sup> )
	1.	2.	3.	
<i>Escherichia coli</i>				
1. Untreated	0	0	0	0
2. Plasma treated	0	0	0	0
3. Plasma + AAm grafted	0	0	0	0
4. Plasma + BCh	0	0	0	0
5. Plasma + AAm + BCh	0	0	0	0
6. Plasma + ChIH	0	0	0	0
7. Plasma + AAm + ChIH	44.7	62.8	44.7	50.8
8. Plasma + TC	44.7	62.8	62.8	56.8
9. Plasma + AAm + TC	103.6	150.7	126.4	126.9
10. Plasma + BrP	0	0	0	0
11. Plasma + AAm + BrP	0	0	0	0
<i>Staphylococcus aureus</i>				
1. Untreated	0	0	0	0
2. Plasma treated	0	0	0	0
3. Plasma + AAm Grafted	0	0	0	0
4. Plasma + BCh	82.4	44.7	44.7	57.3
5. Plasma + AAm + BCh	150.7	126.4	150.7	142.6
6. Plasma + ChIH	176.6	103.6	150.7	143.7
7. Plasma + AAm + ChIH	329.7	365.0	401.9	365.5
8. Plasma + TC	62.8	82.4	82.4	75.9
9. Plasma + AAm + TC	522.0	480.4	480.4	494.3
10. Plasma + BrP	0	0	0	0
11. Plasma + AAm + BrP	0	0	0	0

it shows that ChIH bonds to AAm treated surface in larger amount than other 3 tested antibacterial agents. Samples 8 and 9 covered by TC gave similar result as samples 6 and 7. There was also evident increase (more than 5 times) by the sample covered by AAm (sample 9). Sample 9 ratio of Cl/C atoms were higher (0.0308) than sample 7, because TC molecules contains more Cl atoms. Analysis of samples 10 and 11 revealed presence of Br element on surface. In case of sample 10 it was 0.2% (0.0023 Br/C ratio) and 0.6% (0.0069) in case of sample 11. This indicates again the effectiveness of AAm coating due to the amount of Br groups. It suggests that also number of Br immobilized molecules increased 3 times.

### 3.2.3. Antibacterial analysis

Table 2 shows inhibition zone area, which was calculated as sample surface area deducted from the total area of inhibition zone. The results show that untreated (sample 1), as well as plasma treated (sample 2), even after an AAm grafted one (sample 3) and samples coated by BrP (samples 10 and 11) does not report any provided antibacterial capabilities against both bacterial strains. Antibacterial effect cannot be observed in the case of bacteria *E. coli* for samples coated by BCh (samples 4, 5). Samples covered by ChIH presented antibacterial activity only when AAm was previously grafted (sample 7). This could be direct evidence that an AAm grafting is of a paramount importance for effective antibacterial layer preparation. BCh is not bonded to the surface by molecular forces and therefore it is unable to diffuse freely into the surroundings when AAm is not present, and therefore the sample does not meet expected antibacterial requirements. This statement can be supported by antibacterial activity of samples coated by TC (sample 8, 9). Sample treated by AAm (sample 9) revealed more than 2 times larger area of inhibition zone than sample without polyAAm brush. Bacterial strain of *S. aureus* reveals weaker resistance against antibacterial agents and therefore their inhibition zones held larger area. This difference can be explained by the various physicochemical characteristics of the bacteria strains and materials. Accordingly, we get similar but stronger results than in case of *E. coli*. Contrary to bacterial tests on *E. coli*, BCh samples (samples 4, 5) reported significant inhibition zone against strain *S. aureus*. The area was 2.5 times larger for sample 5, pre-treated by AAm. ChIH immobi-

lized to the samples (sample 6, 7) revealed, antibacterial activity, which increased 2.5 times when the surface was grafted by poly-AAm (sample 7). The highest increase of antibacterial activity was observed for the sample containing TC and grafted by polyAAm (sample 9). The increase was 6.5 times higher compared to sample covered by TC without AAm chains (sample 8).

With respect to above mentioned, the surface treatment approach including grafting of AAm increase antibacterial activity of PE samples, while the most effective was sample covered TC (sample 9), where inhibition zone area for *S. aureus* increased 6.5 times, 2 times respectively for *E. coli*. Satisfactory results were also obtained for sample 7, covered by ChIH, where activity increased 2.5 times for *E. coli* compared to sample 6. On the other hand, BrP samples 10 and 11 did not show any significant antibacterial activity Table 3.

## 4. Conclusion

The results show that the treatment of LDPE samples by the low temperature plasma caused an increase in surface roughness, surface free energy and introduced oxygen containing groups to the surface. A solid brush-like layer of AAm was grafted on that activated surface, while the presence was confirmed by the decrease of surface roughness and the increase of nitrogen concentration. This fact was also proven by the XPS measurement. XPS analysis together with FTIR spectroscopy confirmed the presence of antibacterial agents anchored to the surface layer. Their amount was generally higher for all samples covered by AAm polymer layer than for the comparative samples treated only by plasma discharge. XPS analysis show, that the highest increase of antibacterial amount was 10, 5 times at sample 7 – treated by plasma, grafted by AAm and coated by ChIH. Antibacterial activity and release of antibiotics into the surrounding area was measured by means of inhibition zones. Antibacterial activity appeared generally more effective against gram positive *S. aureus* than for gram negative *E. coli* strain. We noticed increase of inhibition zone area at samples, where the surface was grafted by AAm in comparison to samples, without AAm brush. These tests confirmed that after AAm grafting more antibacterial agent is immobilized on the surface. The highest increase (6.5 times) of antibacterial activity was observed by the sample containing TC and grafted by polyAAm (sample 9). Samples covered by BrP did not show any antibacterial activity.

## Acknowledgements

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## **PAPER II**

Article

## Anti-bacterial Treatment of Polyethylene by Cold Plasma for Medical Purposes

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**Abstract:** Polyethylene (PE) is one of the most widely used polymers in many industrial applications. Biomedical uses seem to be attractive, with increasing interest. However, PE is prone to infections and its additional surface treatment is indispensable. An increase in resistance to infections can be achieved by treating PE surfaces with substances containing antibacterial groups such as triclosan (5-Chloro-2-(2,4-dichlorophenoxy)phenol) and chlorhexidine (1,1'-Hexamethylenebis[5-(4-chlorophenyl)biguanide]). This work has examined the impact of selected antibacterial substances immobilized on low-density polyethylene (LDPE) via polyacrylic acid (PAA) grafted on LDPE by low-temperature barrier discharge plasma. This LDPE surface treatment led to inhibition of *Escherichia coli* and *Staphylococcus aureus* adhesion; the first causes intestinal disease, peritonitis, mastitis, pneumonia, septicemia, the latter is the reason for wound and urinary tract infections.

**Keywords:** polyethylene; grafting; plasma treatment; immobilization; triclosan; chlorhexidine; acrylic acid

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## 1. Introduction

PE is one of the most common biomedical polymers due to its excellent mechanical properties, but it suffers from insufficient biocompatibility and bioactivity [1]. PE is widely used in many biomedical applications including the production of catheters for percutaneous transluminal coronary angioplasty in medical and pharmaceutical industries [2], but infections resulting from application of this medical polymer represent the main clinical complication [3]. These infections may cause implant failure, complex revision processes and implant removal, and all can lead to patient suffering, prolonged hospitalization and even death in some cases [4]. Biocompatibility depends on many surface characteristics such as wettability [5], roughness, chemistry, surface charge, density of functional groups. The presence of hydrophobic and hydrophilic domains [6], charge [7], the functional group densities, and their conformation [8,9] play ascendant roles in affecting cell behavior [10]. Although PE has superiority concerning volume properties, its surface free energy has a low value that reflects its low wettability. This property is related to its hydrophobic and chemically inert surface without polar functional groups [11]. The solution of the problem consists in PE surface modification. Low-temperature plasma can be suggested as the appropriate procedure for the hydrophilization of the surface. Due to the plasma treatment surface the free energy is increased as a result of introduction of polar functional groups on the treated surface, thus making the surface of PE more hydrophilic [12,13].

Developing plasma techniques belong to an important class of polymer surface modification techniques where a very thin layer of the polymer surface is treated without any changes in bulk. Moreover, plasma technology is based on ecological, clean and dry processes suitable for industrial applications without the use of chemicals. Low-temperature plasma is often used in many applications, for example in the electronic, aeronautic, automotive, medical [14], biomedical, textile, optical and paper industries [15]. In this process a polymer is exposed to a plasma reactive species such as ions, electrons, excited atoms and molecules, which cleave existing chemical bonds and form new reactive functional groups, which may initiate or participate in grafting, polymerization, or cross-linking reactions on the surface. Plasma processing can significantly contribute to adhesion improvement by removing surface contamination and to surface morphology changes through increased roughness due to etching [16,17].

The Diffuse Coplanar Surface Barrier Discharge (DCSBD) plasma generator [18] appears to be an effective tool for creating macroscopically homogeneous plasmas, which has many advantages compared with conventional devices. The most important advantage considering the application of DCSBD includes performance at atmospheric pressure, which is significant in terms of continuous industrial technologies. Another advantage is that the plasma does not directly contact the electrodes, which protects these from wear [19]. DCSBD equipment consists of two parallel banded system of electrodes (usually 1-mm wide, 50 micron thick, with 0.5 mm spacing between the strips, made of

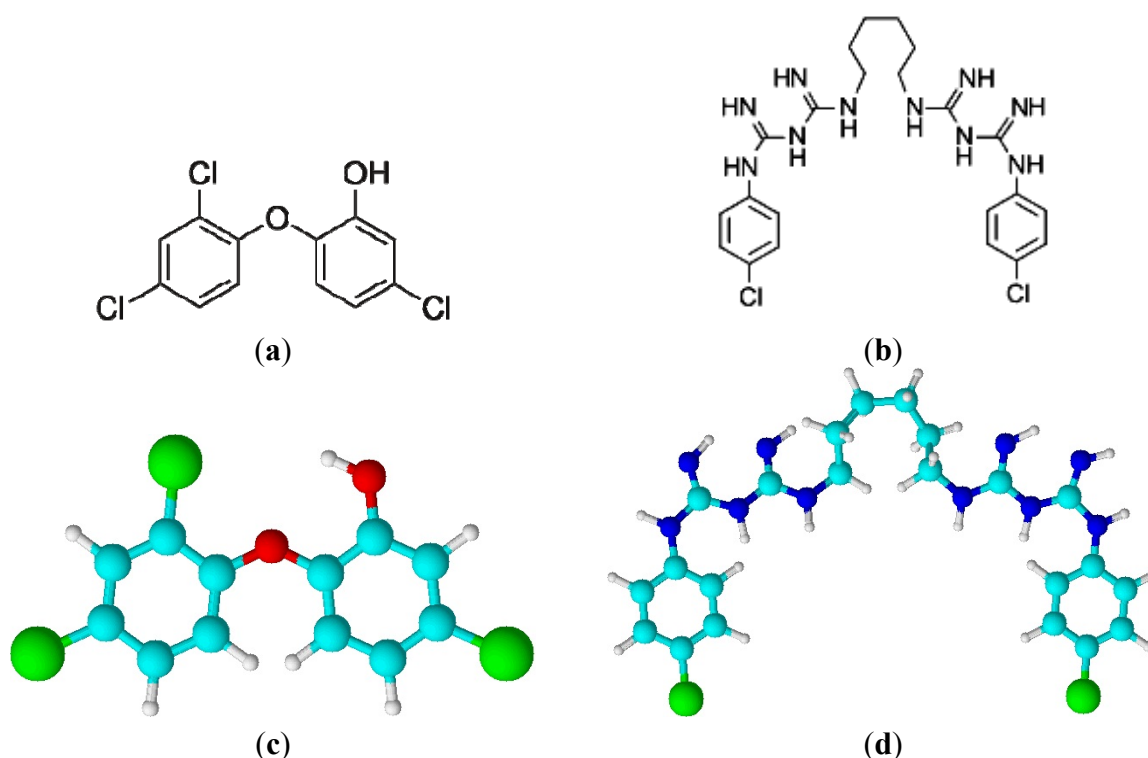


Ag-paste) embedded in 96% Al<sub>2</sub>O<sub>3</sub>-promotion of natural purity. Such an arrangement of electrodes leads to a visually almost completely macroscopically homogeneous diffusion plasma [20–22].

Adhesion and surface growth of bacteria, also called biofilm formation, is a widespread problem [23]. To prevent its formation, anti-infection modification of polymers for medical applications may be applied. Anti-infective properties of polymers can be achieved by following: (a) anti-infection agents mixed in the polymer; (b) copolymerization anti-infection agents with monomer; (c) appropriate surface treatment of medical polymers.

Antibacterial surface modification is controlled by the physical-chemical interactions between bacteria and polymer surface. This treatment has several advantages, because it does not influence the bulk properties of the polymer, antibacterial agents are not released from the polymer volume, and the technique is relative simple and effective. Triclosan [24] and chlorhexidine [25] (Figure 1) shows straight, steady, broad-spectral antibacterial efficiency and very low clinical toxicity in clinical tests. This treatment in combination with plasma can affect significantly biochemical and physical properties of LDPE [3] by following a multistep physicochemical approach [26]. In the first step, formation of functional groups on the polymer surface is necessary via the plasma species created by a DCSBD generator [27–29].

**Figure 1.** (a) triclosan; (b) chlorhexidine; (c) 3D structure of triclosan; (d) 3D structure of chlorhexidine.



In the second step, an end-functionalized polymer brush is formed on polymer surface via radical graft polymerization of acrylic acid (AA), which is anchored on the plasma treated surface [30]. The PAA grafted on the LDPE surface represents a new approach for subsequent antibacterial treatment. Finally, biomolecules are immobilized on this pre-treated surface using EDAC coupling, whereby carboxyl groups of AA are then activated and ready to provide the immobilization sites [31].

## 2. Results and Discussion

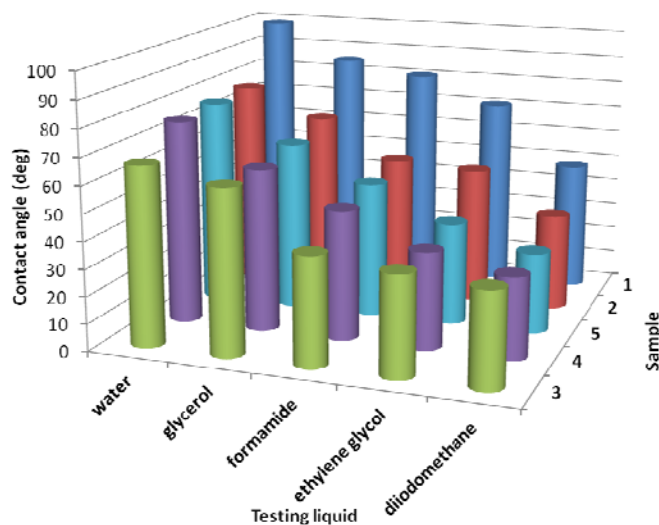
### 2.1. Surface Wettability

Wetting (wettability) can be defined as the degree to which a solid is wetted. When a drop is totally spread on solid surface and the contact angle approaches 0 deg, then the complete wettability of the surface is achieved. However, in many cases it is only a partial wettability occurs (or non-wettability). Contact angle measurements are usually used for estimating the extent to which a solid surface will be wetted. Wettability can be expressed as relative strength of cohesion (liquid/liquid) and adhesion (solid/liquid) forces. Weak cohesion with strong adhesion due to the very low contact angle is close to full wettability. If solid/liquid interactions decrease and liquid/liquid interactions increase, wettability decreases. If the contact angle of water is 90 deg or more the polymeric surface is hydrophobic. This relates with poor wettability, low surface energy and weak adhesion of the polymer. On the other side drop with a small contact angle relates to more hydrophilic surface, that causes better wettability, adhesion and higher surface energy of investigated material. The contact angles changes of testing liquid set, graft yield (GY), and surface free energy ( $\gamma^{\text{tot}}$ ) and its components of antibacterial treated LDPE are shown in Table 1. The graft yield (GY) was calculated by the following equation:  $GY[\%] = [(W_2 - W_1) / W_1] \times 100$ , where  $W_1$  and  $W_2$  represent the weight of the samples before and after surface treatment [32]. The graphic changes of contact angles of testing liquids caused by antibacterial treatment are shown in Figure 2. The water contact angle ( $\theta_w$ ) of untreated LDPE (Sample 1) achieves the highest values from the all samples because it is polymer with hydrophobic and chemical inert surface.  $\theta_w$  significantly decreased after plasma effect of the Sample 2 when different functional groups were introduced on to the surface formed from plasma species and therefore the treated surface acquired more polar or hydrophilic character. The highest decrease of the contact angle was observed in case of surface covered by polyacrylic acid (PAA, Sample 3) which corresponds to its hydrophilic character. Also triclosan (Sample 4) and chlorhexidine (Sample 5) immobilization led to  $\theta_w$  decrease. For investigation of other physicochemical parameters of the treated surface Lifshitz-Van der Waals/acid-base (LW/AB) theory was used, which allows to obtain  $\gamma^{\text{tot}}$  and its components such as non-polar LW ( $\gamma^{\text{LW}}$ ) and polar AB ( $\gamma^{\text{AB}}$ ) components. LW indicates the total dispersive Lifshitz-Van der Waals interaction and AB refers to the acid-base or electron-acceptor/electron donor interaction according to Lewis [33]. LDPE belongs to group of low-energy polymeric materials and therefore  $\gamma^{\text{tot}}$  of Sample 1 achieves very low values which correspond with difficulties during processing, such as dyeing, printing and bonding (low adhesion). This can be removed by plasma treatment of LDPE when  $\gamma^{\text{tot}}$  can significantly increases as in the case of Sample 2. The largest increase of  $\gamma^{\text{tot}}$  and  $\gamma^{\text{AB}}$  was observed for Sample 3 due to highest polarity in comparison with other samples as a result of polar oxygen group's presence. Sample 4 and 5 showed similar increases of surface free energy values, thereby confirming the increase in wettability.

**Table 1.** Surface properties of LDPE treated by multistep process.

Sample	$\theta_w$ (°)	$\theta_e$ (°)	$\theta_g$ (°)	$\theta_d$ (°)	$\theta_f$ (°)	$\gamma^-$ (mN/m)	$\gamma^+$ (mN/m)	$\gamma^{AB}$ (mN/m)	$\gamma^{LW}$ (mN/m)	$\gamma^{tot}$ (mN/m)	GY (%)
1	99.2 (±0.6)	70.9 (±1.2)	85.3 (±0.9)	48.4 (±1.2)	80.7 (±0.9)	1.0	0.1	0.7	34.5	35.2	-
2	77.5 (±1.1)	51.0 (±2.8)	67.1 (±2.8)	36.0 (±1.2)	52.8 (±1.5)	6.6	0.1	1.1	41.4	42.6	0.0
3	66.9 (±0.7)	32.1 (±2.4)	57.2 (±2.7)	32.5 (±1.6)	37.0 (±2.0)	10.4	0.5	4.5	43.7	48.1	0.5
4	75.8 (±1.6)	36.1 (±0.7)	60.4 (±1.0)	30.5 (±1.5)	48.3 (±1.2)	5.0	0.4	2.8	44.0	46.8	1.8
5	76.7 (±0.5)	38.1 (±2.5)	63.2 (±2.72)	30.0 (±1.6)	50.4 (±1.5)	5.2	0.2	2.0	44.4	46.4	2.0

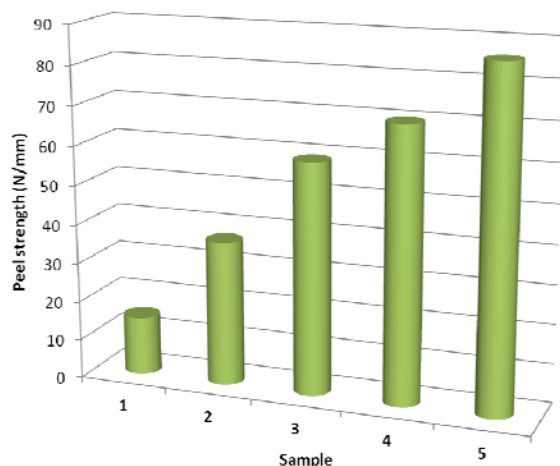
w = deionized water, e = ethylene glycol, g = glycerol, d = diiodomethane, f = formamide;  
 \* Sample 1: untreated LDPE; Sample 2: plasma-treated; Sample 3: AA grafted; Sample 4: triclosan coated; Sample 5: chlorhexidine coated.

**Figure 2.** Contact angle vs. surface treatment and vs. testing liquid; 1 - untreated LDPE; 2 - plasma-treated; 3 - AA grafted; 4 - triclosan coated; 5 - chlorhexidine coated.

## 2.2. Adhesive Properties

The results of peel strength measurements of adhesive joint to poly(acrylate) are shown in Figure 3. Surface free energy changes are closely related to adhesion between two materials in contact. Therefore, the increased wettability resulted in an increase of adhesion strength of adhesive joint to more polar poly(acrylate). However, adhesion depends not only chemical composition and the chemical nature of the surface, but also on surface morphology (roughness). The rougher is the surface the higher is the adhesion and *vice versa*. Thus, adhesion is a complex parameter consisting of several related chemical and physicochemical properties. Therefore, in the case of Sample 3 even though the surface energy reaches its highest value the peel strength is less than for Sample 4 and 5. Cross-linking occurred in Sample 5 (via glutaraldehyde) is another factor that contributes to the increase in the adhesion strength [34].

**Figure 3.** Peel strength vs. surface treatment; 1 - untreated LDPE; 2 - plasma-treated; 3 - AA grafted; 4 - triclosan coated; 5 - chlorhexidine coated.



### 2.3. Surface Morphology

Surface morphology changes (according to AFM measurements) of antibacterial treated LDPE by multistep process via DCSBD are shown in Figure 4. The relief of Sample 1 is only slightly wavy, caused by inequalities in the production of LDPE foils. The plasma effect led to the slightly increase of LDPE surface roughness as a result of surface changes by re-organization of the surface microstructure by chemical (functionalization) and mechanical (ablation) processes. The plasma grafting of LDPE by acrylic acid results in the creation of a brush-like pattern appropriate for subsequent modification. Triclosan coating alteration of the surface topography led to characteristic textures. The cross-linking agent (glutaraldehyde) was used to improved chlorhexidine binding to LDPE resulting in the formation of cross-linked structure and therefore the significant changes in the surface morphology and roughness were observed.

**Figure 4.** AFM surface changes for Sample 1–5: 1 - untreated LDPE; 2 - plasma-treated; 3 - AA grafted; 4 - triclosan coated; 5 - chlorhexidine coated.

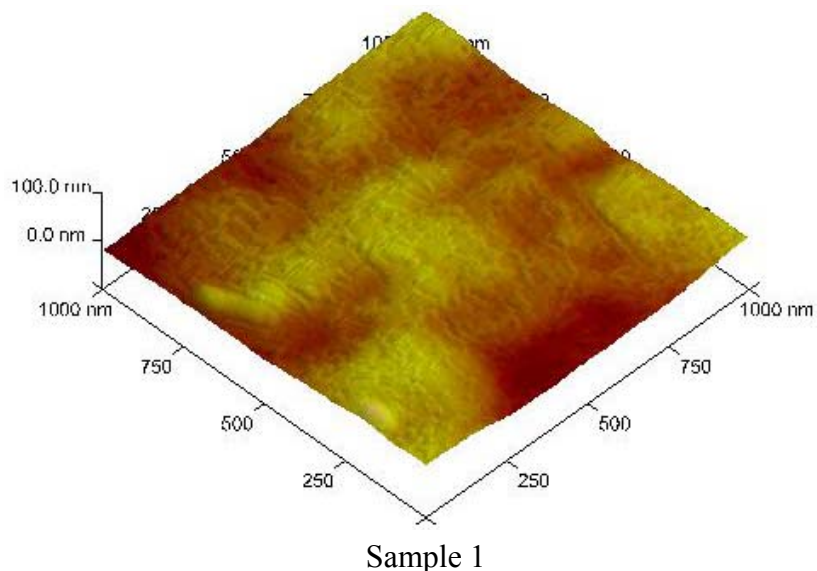
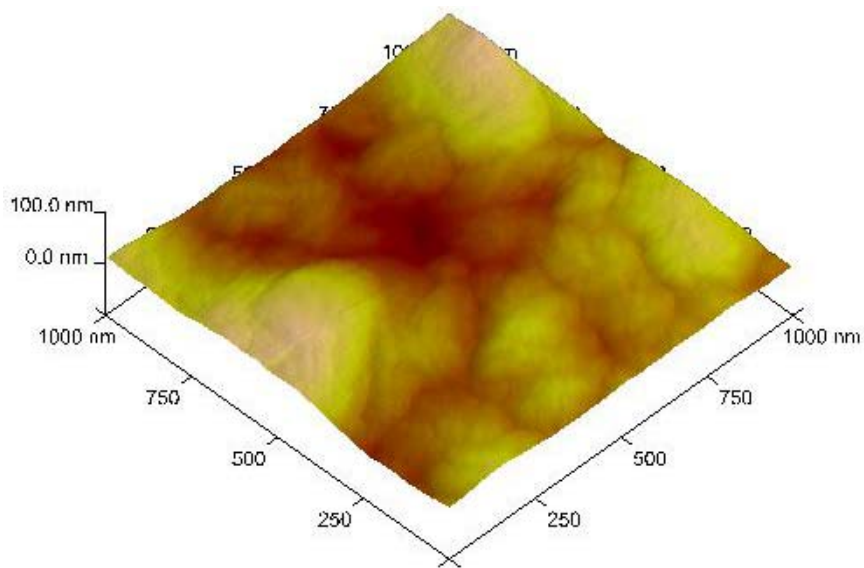
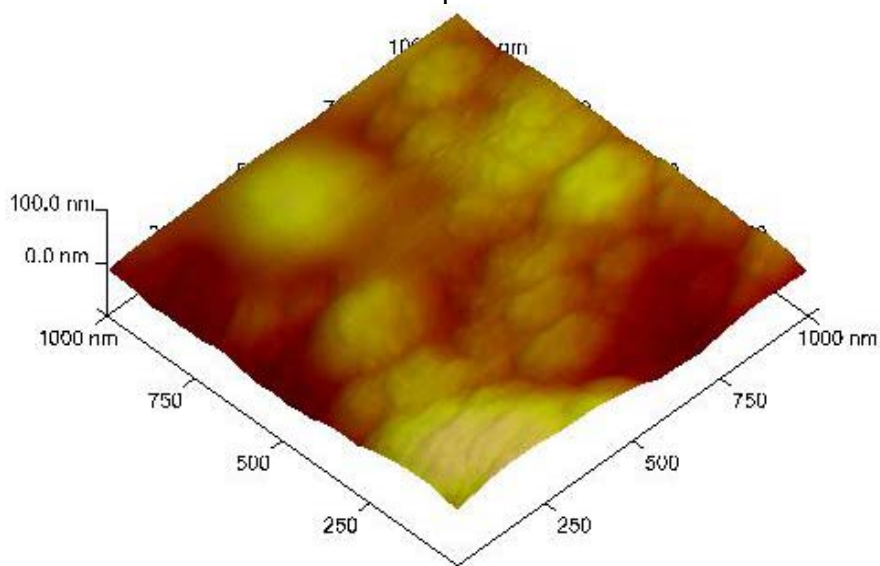


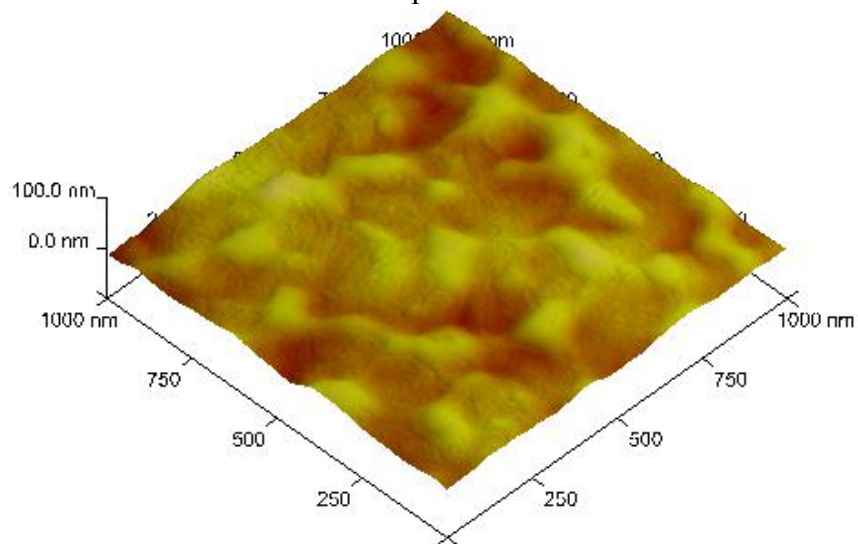
Figure 4. Cont.



Sample 2

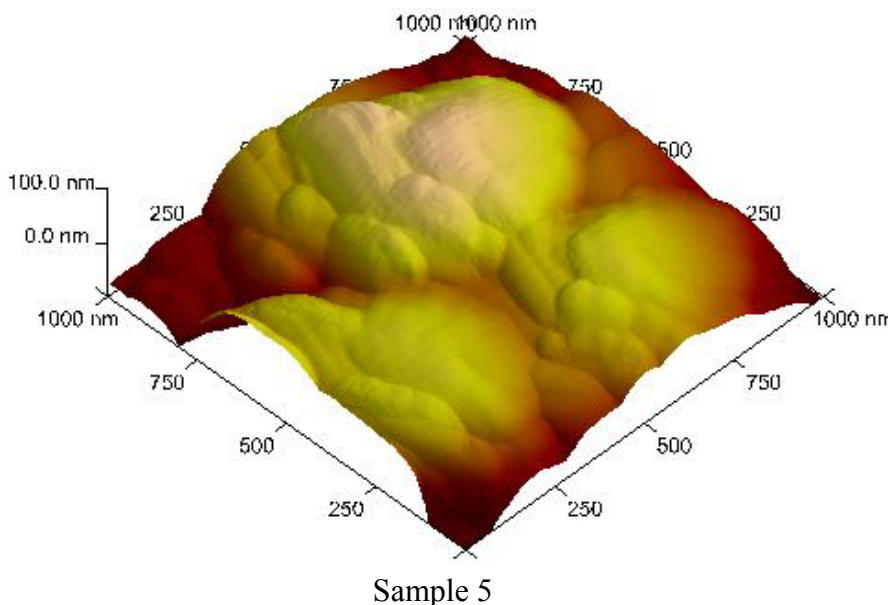


Sample 3



Sample 4

Figure 4. Cont.



## 2.4. Surface Chemistry

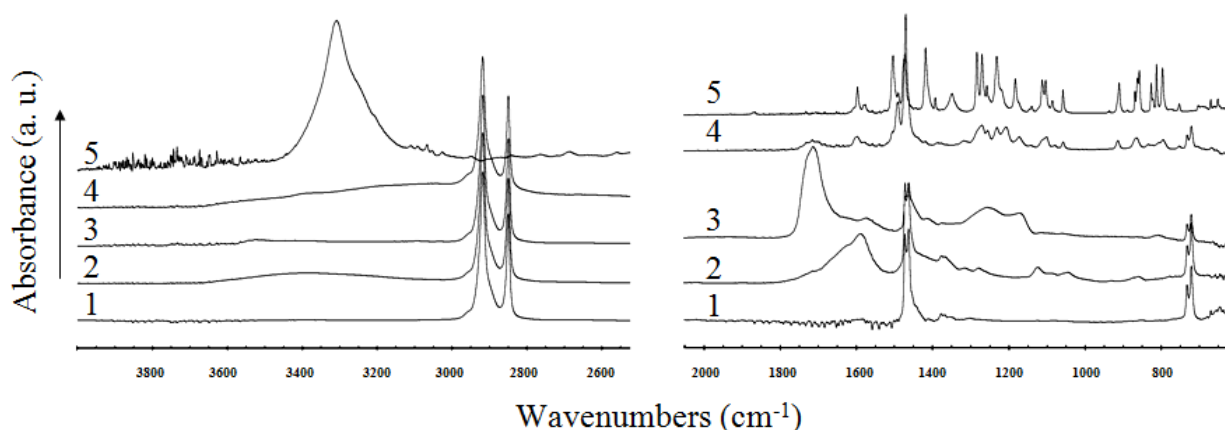
### 2.4.1. Analysis of FT-IR-ATR Spectra

The FT-IR-ATR measurements provide mostly semi-quantitative information on the chemical changes of the near-surface region, because the measured thickness of the layer is limited to 4  $\mu\text{m}$  for ZnSe crystal. The Ge crystal has by far the highest refractive index of all the ATR materials available which means that the effective depth of penetration is lower than in case of ZnSe [35]. For better visualization the infrared spectra of virgin LDPE and modified material together with the pure triclosan were split into three different wavenumber regions. The spectrum of the untreated LDPE is a typical polyethylene spectrum with a small number of characteristic peaks. After air plasma exposure of the pristine material, the characteristic oxygen functional groups were introduced and therefore significant changes in the measured spectrum have been observed. These changes seem to be caused by the incorporation of some hydroxy or peroxy groups after the plasma treatment of LDPE in air (evidence for that statement is the appearance of two broad peaks between 3,600–3,050  $\text{cm}^{-1}$  and 1,800–1,520  $\text{cm}^{-1}$ , respectively). Carbonyl stretching is one of the easiest absorptions to recognize in an infrared spectrum. It is usually the very intense band in the spectrum. In this spectrum also the appearance of two smaller peaks at 1,280  $\text{cm}^{-1}$  and 1,120  $\text{cm}^{-1}$  are seen.

Significant changes in the spectra are also observed in (both) cases of LDPE-PAA grafting and after the subsequent triclosan coating. In the spectrum of grafted material one can observe some characteristic peaks of polyacrylic acid, *i.e.*, the most intense peak at 1,712  $\text{cm}^{-1}$  (carbonyl band, C=O stretching) and also some unresolved peaks in the fingerprint region (1,300–1,100  $\text{cm}^{-1}$ , C-O stretching and CH<sub>2</sub> bending). After triclosan treatment the shape of the spectrum changes, as can be seen in Figure 5. These changes are significant almost in a whole mid-infrared region, especially in the region below 1,700  $\text{cm}^{-1}$ . Because of the simple spectrum of LDPE (small numbers of peaks) in comparison with the spectrum of triclosan, it is assumed that almost all changes in the spectrum of the triclosan coated LDPE are originated due to addition of triclosan. The presence of triclosan in the

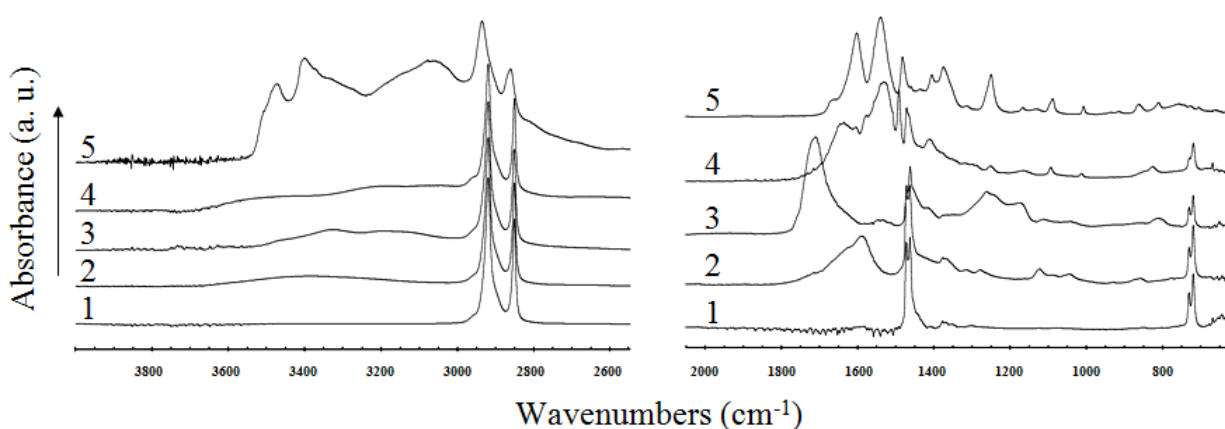
treated sample is confirmed by an appearance of a number of peaks, which are also present in the spectrum of the pure triclosan (e.g., at  $1,491\text{ cm}^{-1}$ , benzene ring vibration), and also without doubt by a peak at  $752\text{ cm}^{-1}$  (stretching mode of C-Cl in the triclosan molecule). The shifts in the maxima of individual peaks and the changes in their shapes can be probably assigned to bonding of triclosan molecules to LDPE surface and inhibition of their unrestricted motion.

**Figure 5.** FT-IR-ATR spectra of: 1 - untreated LDPE; 2 - plasma treated; 3 - AA grafted; 4 - triclosan coated; 5 - pure triclosan.



More significant changes in spectra are observed in (both) cases of LDPE-PAA grafting and also after subsequent chlorhexidine coating. After chlorhexidine treatment the shape of the spectrum changes, as seen in Figure 6. These changes are significant almost in the whole mid-infrared region, especially in the region below  $1,700\text{ cm}^{-1}$ . The presence of chlorhexidine is confirmed by the appearance of a peak at  $1,640\text{ cm}^{-1}$  (C=N vibration) and also undoubtedly by a peak at  $1,530\text{ cm}^{-1}$  (stretching mode of aromatic ring in the chlorhexidine molecule).

**Figure 6.** FT-IR-ATR spectra of: 1 - untreated LDPE; 2 - plasma treated; 3 - AA grafted; 4 - chlorhexidine coated; 5 - pure chlorhexidine.



#### 2.4.2. Analysis of XPS Spectra

LDPE samples with different coatings were analyzed by the XPS method. The purpose of these analyses was to prove the presence of the coating on LDPE samples which were previously treated in

air plasma and grafted with acrylic acid. For each sample the surface composition was measured at two different spots on the surface. This allowed calculation of the average surface composition, which is shown in Table 2.

**Table 2.** Average surface composition of the LDPE samples as revealed by XPS.

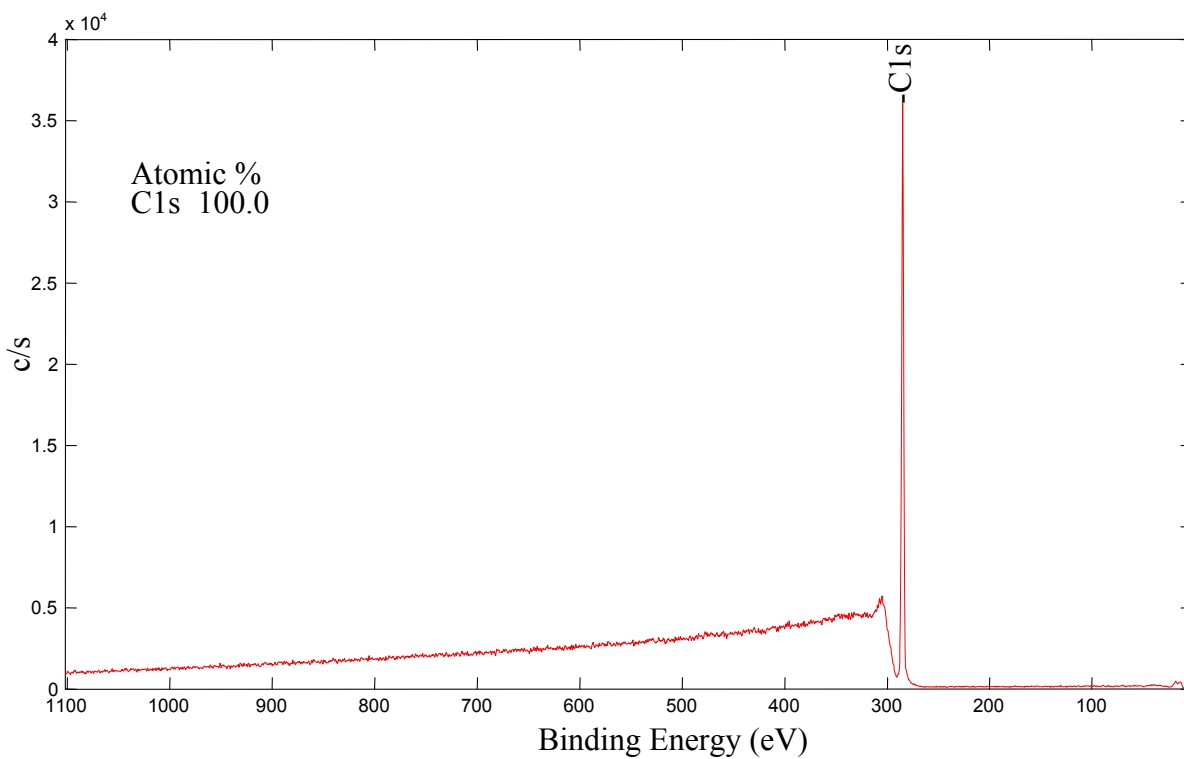
Sample	C1s	N1s	O1s	Na1s	Cl2p	S2p
1	100	0	0			
2	76.3	4.0	19.8			
3	84.1	/	15.6			0.4
4	89.1	2.0	8.4	0.4	0.2	
5	86.8	6.7	5.0		1.5	

\* Sample 1: untreated LDPE; Sample 2: plasma-treated; Sample 3: AA grafted; Sample 4: triclosan coated; Sample 5: chlorhexidine coated.

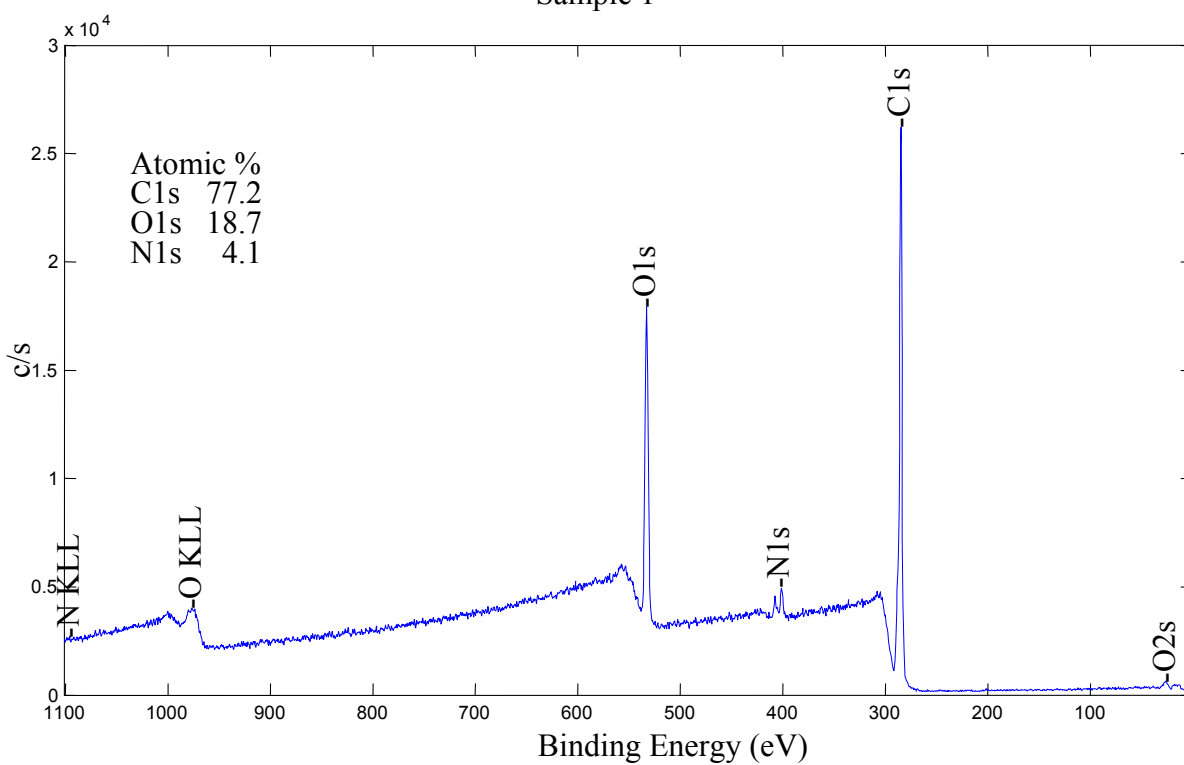
XPS survey-scan spectra of Samples 1–5 are shown in Figure 7 and the carbon C1s peaks of Samples 1–5 are shown in Figure 8. Moreover the nitrogen N1s peak for Sample 2 is shown in Figure 9. For LDPE treated in air plasma different oxygen functional groups and also some nitrogen groups were found. Sample 3 shows mostly the presence of carboxyl groups. For this sample also some traces of iron, about 0.4 at %, were detected. In case of air plasma treatment the peak could include carboxyl as well as ester groups, which could not be resolved with XPS analysis. In the case of AA grafting we believe that this peak presents only carboxylic groups which originate from AA. Furthermore air plasma treatment results also in incorporation of other oxygen functional groups, such as carbonyl and hydroxyl, which can be clearly seen from Figure 8. Comparison of carbon C1s peaks of Sample 1 and Sample 2 are shown in Figure 8. As expected, the carbon spectrum of an untreated sample has only one peak due to C-C bonds. After plasma treatment, new peaks due to different carbon-oxygen bonds appear. As seen in Table 2, some nitrogen is found as well but the C-N peak is overlapping with the C-O peak. Therefore, this peak does not appear in Figure 8 for C1s peak of Sample 2. Nitrogen N1s peak of Sample 2 is composed from different modes of chemical binding of nitrogen atoms. More details about nitrogen binding can be seen in Figure 9 for N1s of Sample 2. As immobilization of biomolecules is done by carboxylic groups, grafting of AA to plasma treated LDPE is important to obtain only carboxylic groups on the polymeric surface. Carbon C1s peak of LDPE sample grafted with acrylic acid (AA) is present in Figure 8. As expected carboxyl group due to AA is detected at the surface. For Sample 4 mostly oxygen and some nitrogen (which is not from triclosan) were found. Concentration of Cl is very low. The coating is probably very thin, since the carboxylic group, which is clearly seen in spectrum, originates from AA, which is below the triclosan. Peak due to C-O/C-OH bond is associated with the presence of triclosan. For Sample 5, nitrogen and chlorine originating from the triclosan coating were detected. See also Figure 8 showing C-N bonds from chlorhexidine coating and carboxylic part from AA.



**Figure 7.** XPS survey-scan spectra of Samples 1–5 with atomic compositions; Sample 1 - untreated LDPE; Sample 2 - plasma-treated; Sample 3 - AA grafted; Sample 4 - triclosan coated; Sample 5 - chlorhexidine coated.



Sample 1



Sample 2

Figure 7. Cont.

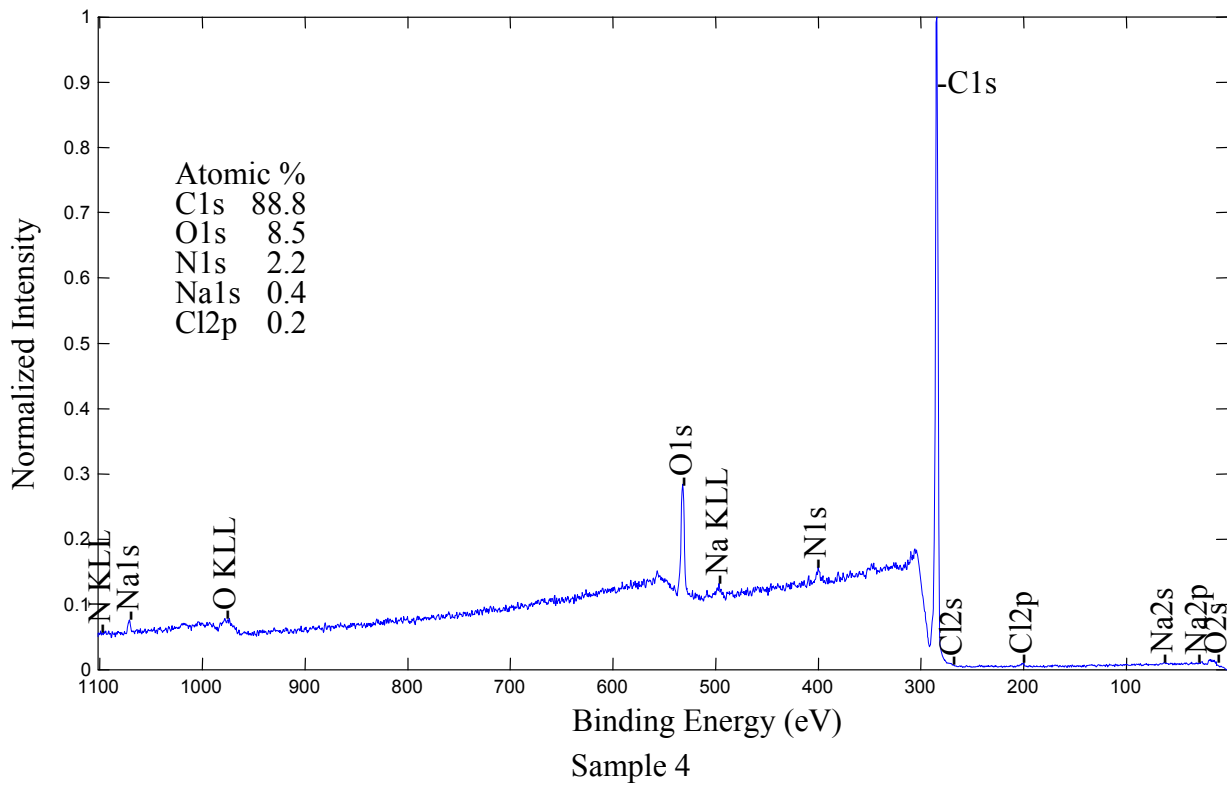
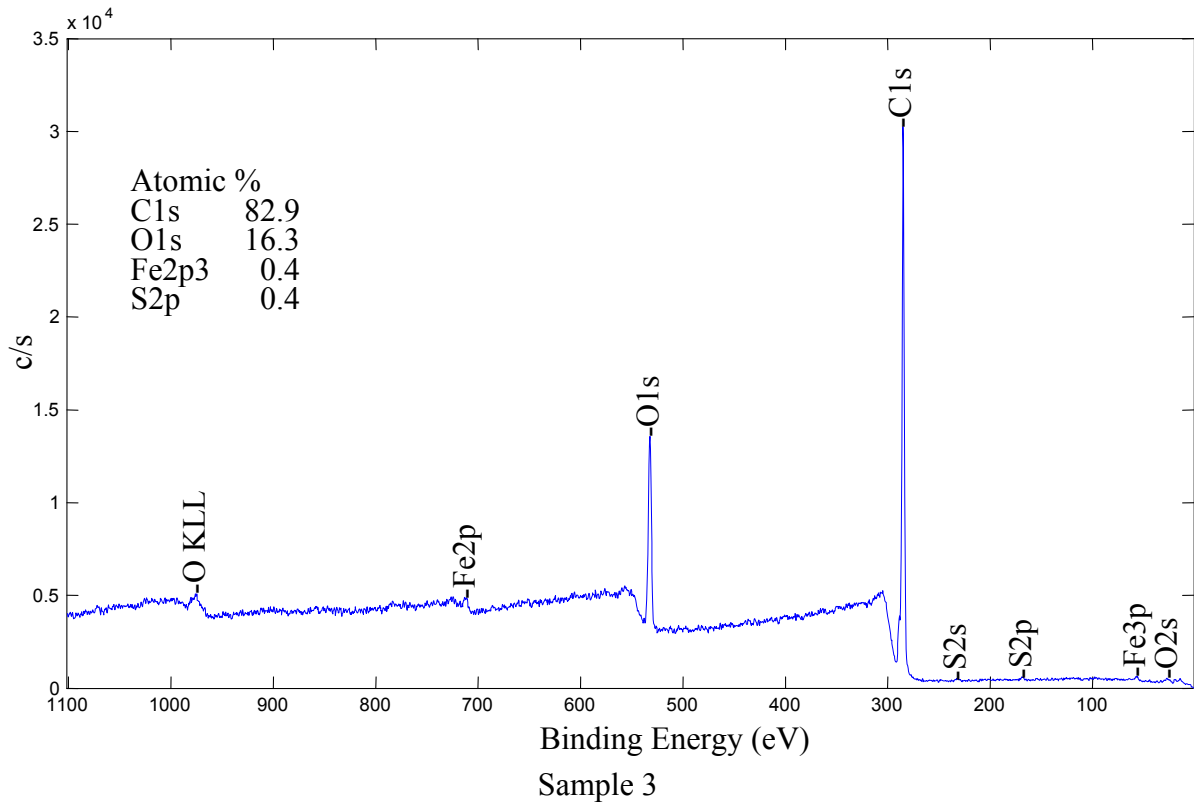




Figure 8. Cont.

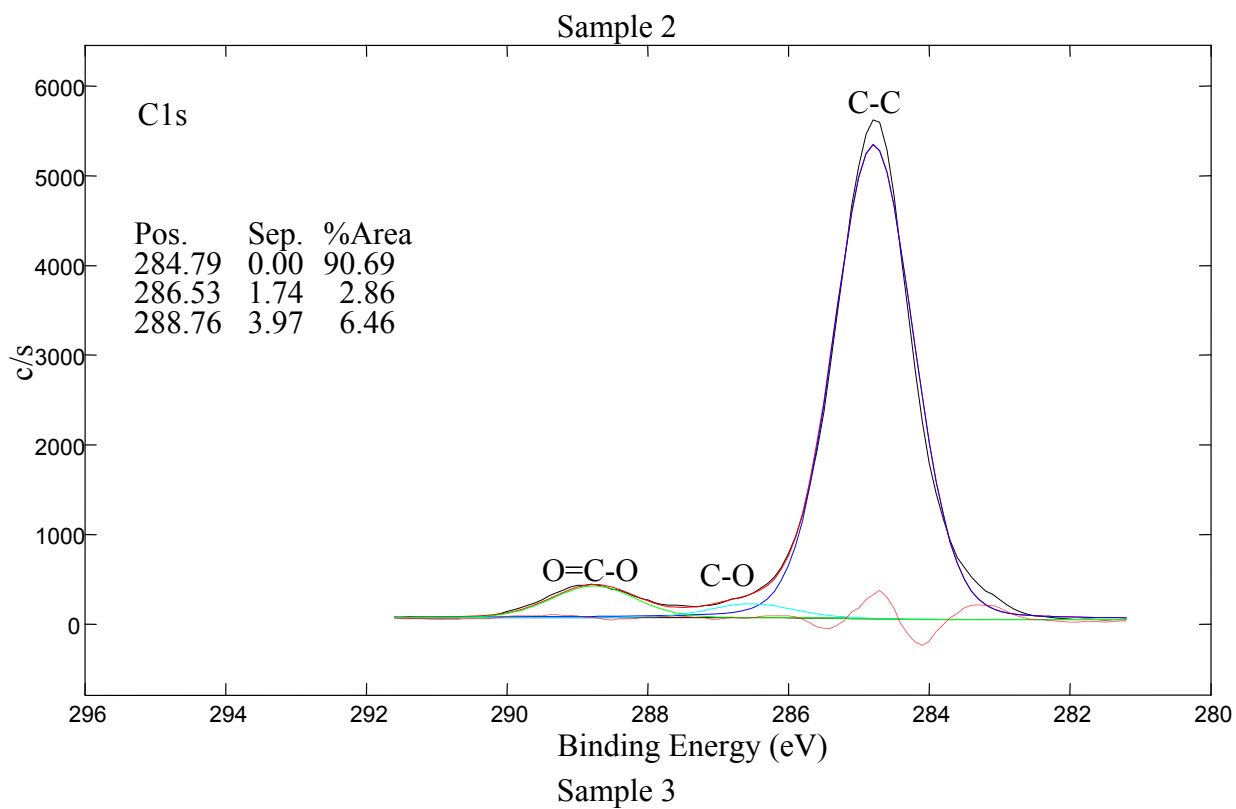
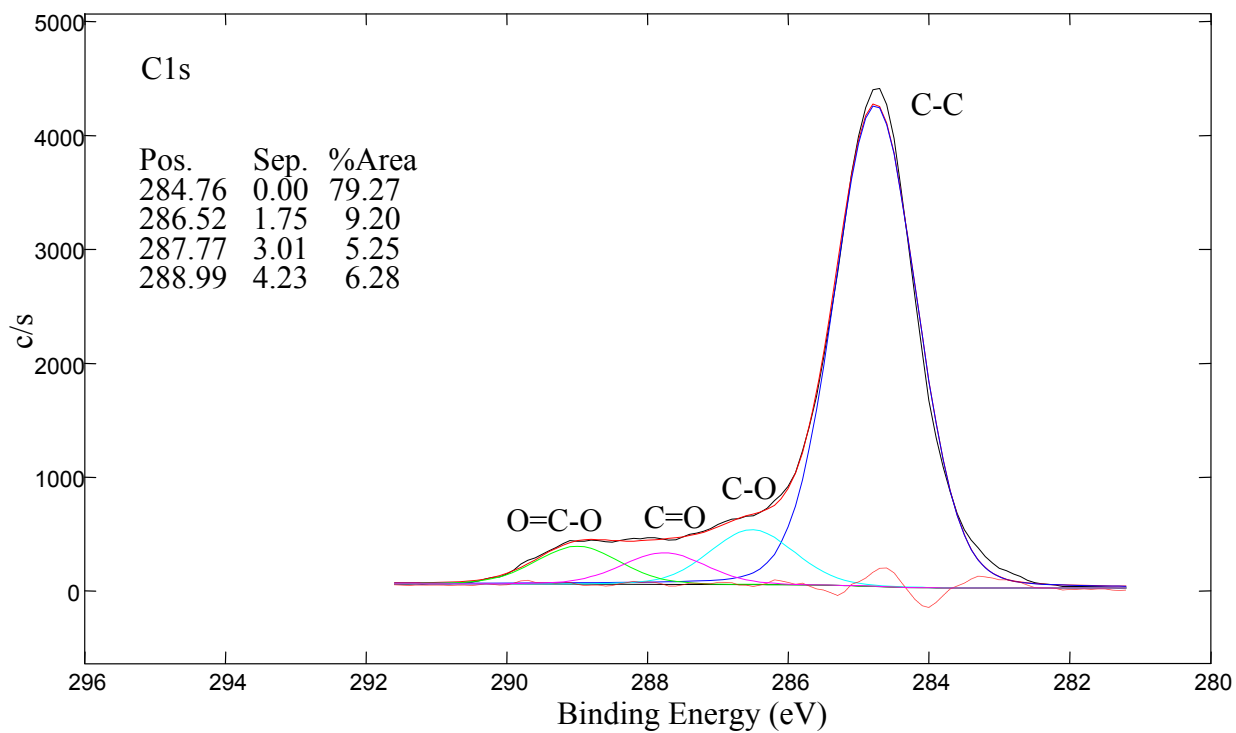
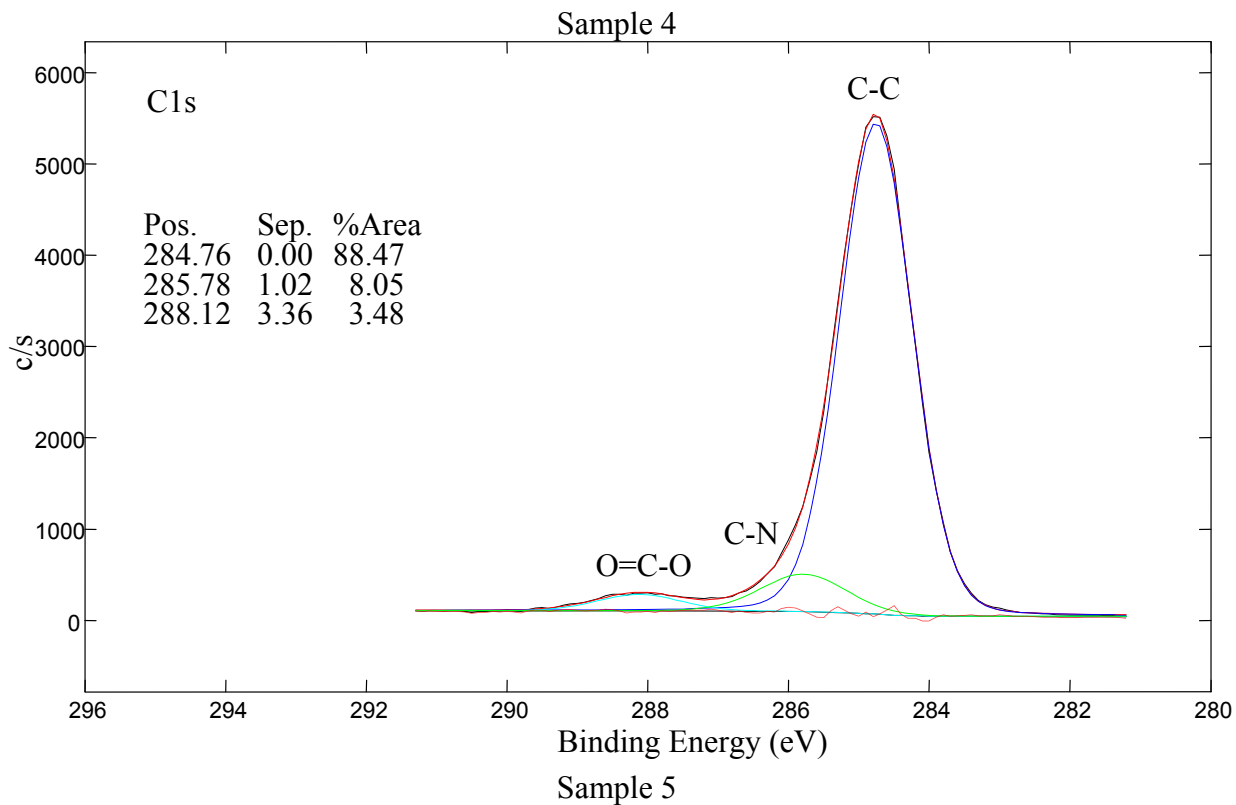
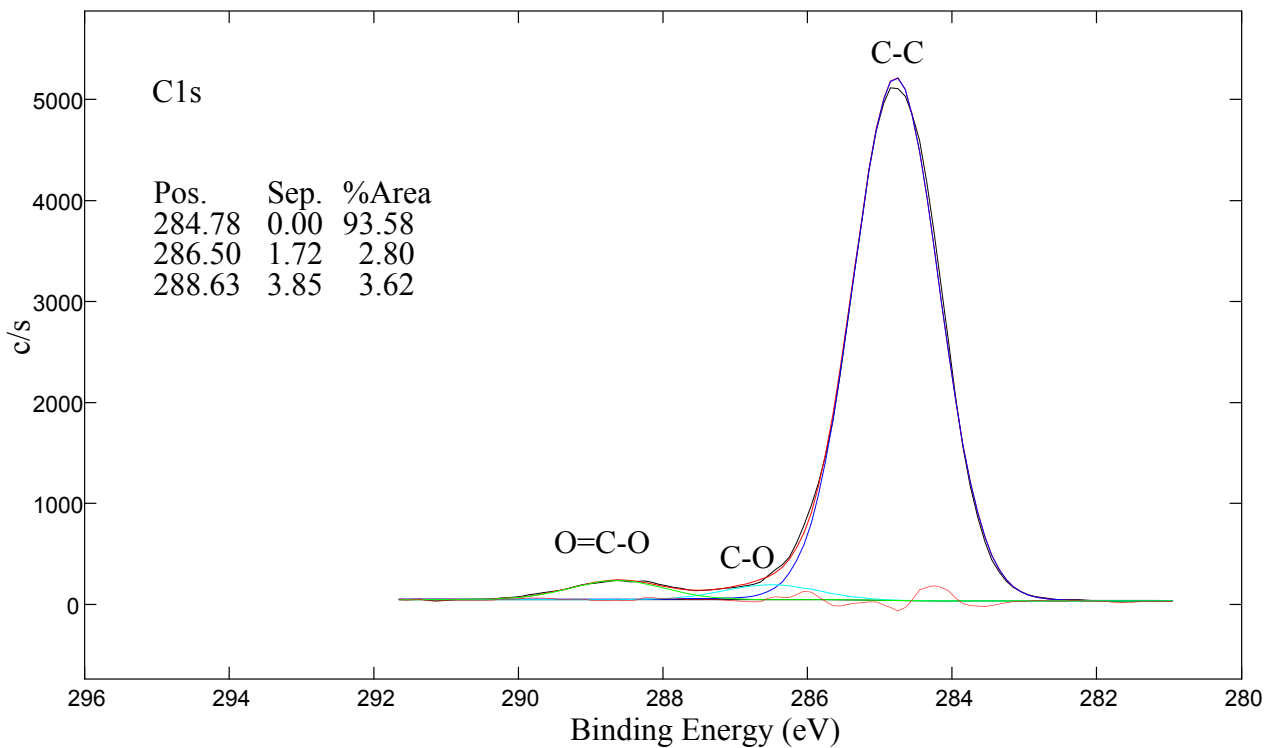
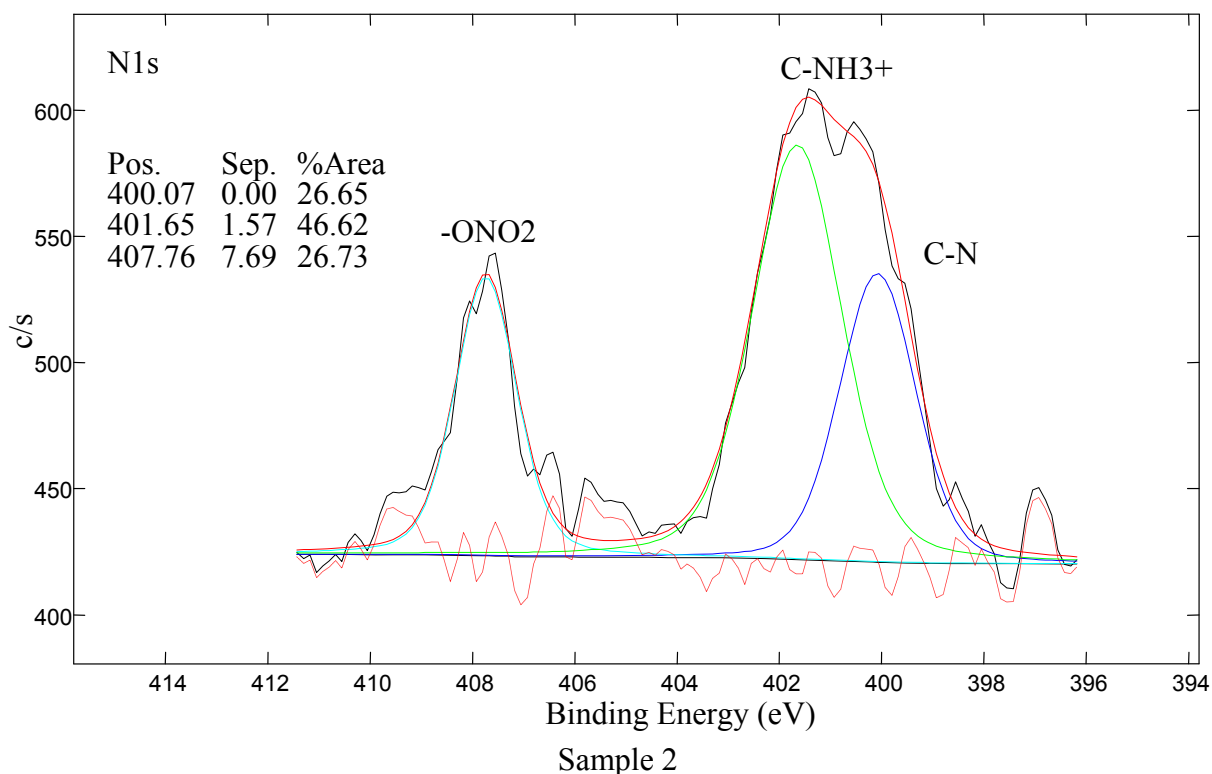


Figure 8. Cont.



**Figure 9.** Nitrogen N1s peak for Sample 2 - plasma-treated.

### 2.4.3. Antibacterial Activity Assessment

Table 3 shows inhibition zone area results. The inhibition zone area was calculated as the sample surface area deducted from the total area of the inhibition zone. The results show that untreated (Sample 1), plasma treated (Sample 2) as well as acrylic-acid grafted sample (Sample 3) do not display any antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus* strains. The sample coated with triclosan (Sample 4) does meet the expected antibacterial requirements. The average inhibition zone for the Gram-negative *Escherichia coli* strain is of 115.1 mm<sup>2</sup> and for the Gram-positive *Staphylococcus aureus* 493.1 mm<sup>2</sup>. These values prove the antibacterial activity of the prepared layers as well as confirm XPS measurements. Similar results were obtained for chlorhexidine coated samples (Sample5). The average inhibition zone value of 42.2 mm<sup>2</sup> was calculated for *Escherichia coli* and 288.1 mm<sup>2</sup> for *Staphylococcus aureus* strain. It is worth mentioning, that both antibacterial agents are more active against Gram-positive bacteria. Finally, triclosan coated samples show better results among the two antibacterial substances used.

**Table3.** Inhibition zone area measurement.

LDPE	Inhibition zone (mm <sup>2</sup> )			Average value (mm <sup>2</sup> )
	1	2	3	
<i>Escherichia coli</i>				
Sample 1	0	0	0	0
Sample 2	0	0	0	0
Sample 3	0	0	0	0
Sample 4	105.8	118.3	121.2	115.1
Sample 5	40.2	43.8	42.5	42.2

Table 3. Cont.

LDPE	Inhibition zone (mm <sup>2</sup> )			Average value (mm <sup>2</sup> )
	1	2	3	
<i>Staphylococcus aureus</i>				
Sample 1	0	0	0	0
Sample 2	0	0	0	0
Sample 3	0	0	0	0
Sample 4	475.0	496.3	507.9	493.1
Sample 5	286.4	279.3	298.5	288.1

\* Sample 1: untreated LDPE; Sample 2: plasma-treated; Sample 3: AA grafted; Sample 4: triclosan coated; Sample 5: chlorhexidine coated.

### 3. Experimental

#### 3.1. Materials

LDPE BRALEN FB 2-17 foils: Slovnaft MOL (Slovakia), containing no processing additives, the thickness of LDPE film was 20  $\mu\text{m}$ , density = 0.918  $\text{g}\cdot\text{cm}^{-3}$ , mass flow rate (MFR at 190 °C, 2.16 kg) = 2 g per 10 min, Vicat softening temperature = 96 °C. This type of LDPE is suitable for food contact. The product complies with Food Contact Regulations and the grade is suitable for manufacturing of pharmaceutical packing-products.

Triclosan (5-Chloro-2-(2,4-dichlorophenoxy)phenol): Irgasan,  $\text{C}_{12}\text{H}_7\text{Cl}_3\text{O}_2$ , Fluka Analytical (Italy), white powder, Assay  $\geq 97.0\%$  (HPLC),  $M_r = 289.54 \text{ g}\cdot\text{mol}^{-1}$ , ash  $\leq 0.1\%$ , melting point = 56–58 °C.

Chlorhexidine (1,1'-Hexamethylenebis[5-(4-chlorophenyl)biguanide]): imidodicarbonimidic diamide,  $\text{C}_{22}\text{H}_{30}\text{Cl}_2\text{N}_{10}$ , Aldrich Chemistry (Spain), white powder, Assay = 98%,  $M_r = 505.46 \text{ g}\cdot\text{mol}^{-1}$ , melting point = 134 °C.

Acrylic acid (Prop-2-enoic acid):  $\text{C}_3\text{H}_4\text{O}_2$ , colorless liquid, Acros Organics (Belgium), Assay = 99.5%, extra pure, stabilized with 180 to 220 pm monomethyl ether of hydroquinone (MEHQ),  $M_r = 72.06 \text{ g}\cdot\text{mol}^{-1}$ , flash point = 48 °C, density = 1.050  $\text{g}\cdot\text{cm}^{-3}$ , boiling point = 139 °C.

EDAC (N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride):  $\text{C}_8\text{H}_{17}\text{N}_3\cdot\text{HCl}$ , Fluka (USA), purum, Assay = 98.0%,  $M_r = 191.70 \text{ g}\cdot\text{mol}^{-1}$ , melting point = 110–115 °C.

Glutaraldehyde (Pentane-1,5-dial):  $\text{C}_5\text{H}_8\text{O}_2$ , clear liquid, was used as 25.0 wt% aq. solution,  $M_r = 100.12 \text{ g}\cdot\text{mol}^{-1}$ , density = 1.06  $\text{g}\cdot\text{cm}^{-3}$ , melting point = -14 °C, boiling point 187 °C.

Ethylene glycol (Ethane-1,2-diol):  $\text{C}_2\text{H}_6\text{O}_2$ , Sigma-Aldrich (USA), anhydrous, Assay = 99.8%,  $M_r = 62.07 \text{ g}\cdot\text{mol}^{-1}$ , flash point = 111 °C, melting point = -13 °C, boiling point = 195–197 °C.

Glycerol (Propane-1,2,3-triol):  $\text{C}_3\text{H}_8\text{O}_3$ , Sigma (Germany), for molecular biology, Assay = 99%,  $M_r = 92.09 \text{ g}\cdot\text{mol}^{-1}$ , density = 1.262  $\text{g}\cdot\text{cm}^{-3}$ , melting point = 20 °C, flash point = 160 °C, boiling point = 182 °C/20 mmHg.

Formamide (Methanamide):  $\text{CH}_3\text{NO}$ , Sigma (USA), deionized, Assay = 99.5%,  $M_r = 45.04 \text{ g}\cdot\text{mol}^{-1}$ , density = 1.132  $\text{g}\cdot\text{cm}^{-3}$ , melting point = 2 °C, flash point 150 °C, boiling point = 210 °C/760 mmHg.

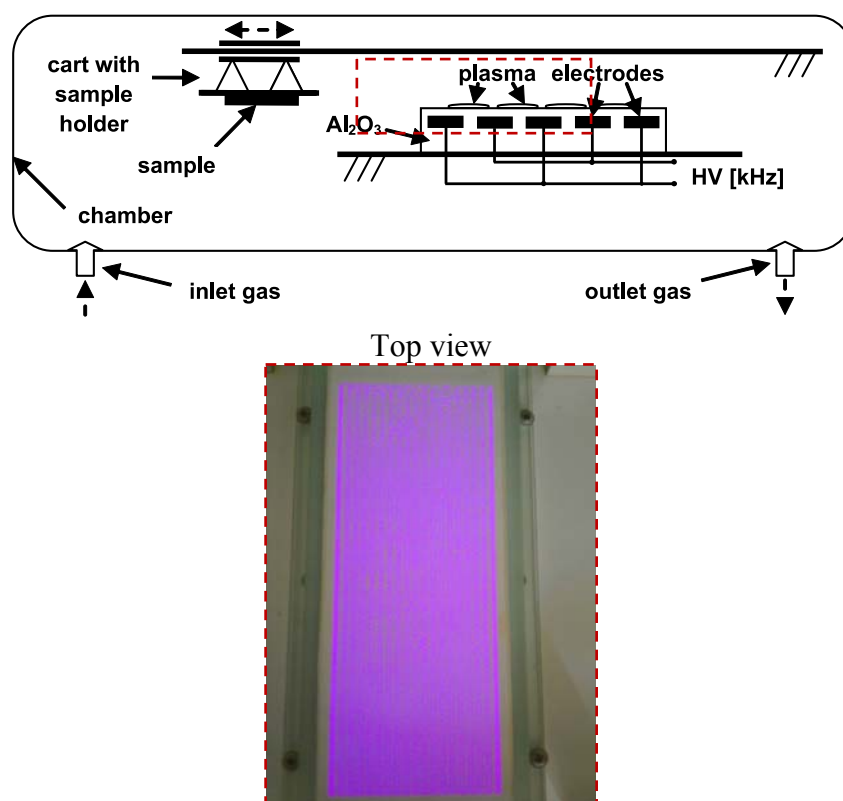
Diiodomethane:  $\text{CH}_2\text{I}_2$ , colorless liquid with chloroform-like odour, Assay = 99%, Reagent Plus, Sigma-Aldrich (Germany), containing copper as stabilizer,  $M_r = 267.84 \text{ g}\cdot\text{mol}^{-1}$ , density = 3.325  $\text{g}\cdot\text{cm}^{-3}$ , melting point = 5–8 °C, flash point = 110 °C, boiling point = 67–69 °C.

Dichloromethane:  $\text{CH}_2\text{Cl}_2$ , mikroCHEM (SVK), Assay = 99.5%,  $M_r = 84.93 \text{ g}\cdot\text{mol}^{-1}$ , density =  $1.33 \text{ g}\cdot\text{cm}^{-3}$ , melting point =  $-96.7 \text{ }^\circ\text{C}$ , boiling point =  $39.6 \text{ }^\circ\text{C}$ .

### 3.2. Plasma Treatment

The LDPE foils were first cleaned with dichloromethane to remove impurities. Then the LDPE foil activation was carried out under dynamic conditions at atmospheric pressure and room temperature with the DCSBD equipment developed at Comenius University (Department of Experimental Physics, Faculty of Mathematics, Physics and Informatics) in Bratislava. The schematic representation with description of this system is given in Scheme 1. The treatment was performed with the following settings: power supply = 200 W, plasma treatment time = 15 s, in air atmosphere and all samples were treated on both sides. DCSBD equipment generates macroscopically homogeneous plasma without direct contact with the electrodes, which protects the electrodes from wear. Plasma is generated by two parallel banded system of electrodes (1-mm wide, 50 micron thick, with 0.5 mm spacing between the strips, made of Ag-paste) embedded in 96%  $\text{Al}_2\text{O}_3$ -promotion of national purity, while the electrodes are supplied via high frequency sinusoidal voltage ( $\sim 15 \text{ kHz}$ ,  $U_m \sim 10 \text{ kV}$ ). Such an arrangement of electrodes and supply voltage leads to visually almost perfectly homogeneous diffusion plasma.

**Scheme 1.** DCSBD scheme and detail of burning plasma panel.



### 3.3. Grafting by PAA

Immediately after plasma treatment the LDPE foil was immersed into 10 volume % aqueous solution of AA for 24 h at  $30 \text{ }^\circ\text{C}$  in order to initiate of radical graft polymerization of AA onto activated surface of LDPE foil. This solution contained also 0.1 wt.% sodium metabisulfite as a relevant reductant to inhibit AA homopolymerization. After AA polymerization PAA brushes were

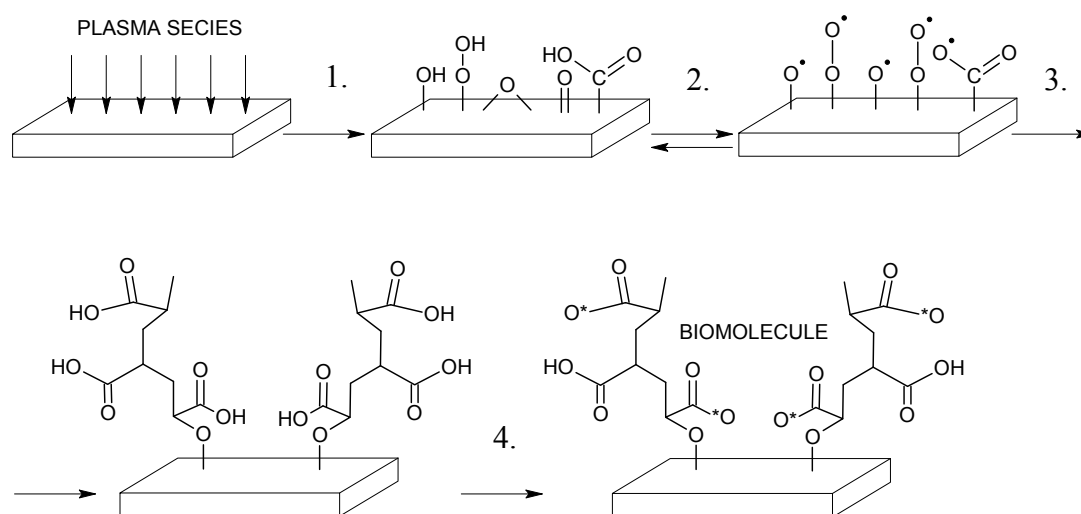


created onto LDPE surface that are suitable for binding antibacterial agents. After removing the samples from the solution the grafted foils were washed in deionized water for 5 min at 30 °C in an ultrasonic bath for removal weakly bound PAA and unreacted AA species on the surface LDPE.

### 3.4. Antibacterial Immobilization

LDPE grafted by PAA was immersed at 4 °C for 6 hours into 0.1 w/v% aqueous solution of EDAC that acts as an activator of carboxyl groups where *O*-acylisourea is produced and it has possibility to react with reducing agents. The sample pre-prepared by such way was then immersed into solution of triclosan and chlorhexidine. The first solution was prepared as 2 w/v% solution of triclosan in absolute ethanol and the latter as 2 w/v% solution of chlorhexidine in 70 v/v% isopropanol aqueous solution for 24 h at 30 °C in an oven. Moreover the coated LDPE by chlorhexidine was then yet immersed into 1 w/v% aqueous solution of glutaraldehyde overnight at 4 °C to better immobilization of chlorhexidine onto the surface via cross-linking. The antibacterial treated samples were thoroughly washed and then dried for 24 h at room temperature to constant weight. The mechanism of antibacterial treatment is described in Scheme 2.

**Scheme 2.** Multistep approach of bimolecular binding: 1. plasma treatment; 2. radical generation; 3. AA radical graft polymerization; and 4. antibacterial deposition.



### 3.5. Surface Wettability Evaluation

The wettability of LDPE treated by multistep process via PAA plasma grafted and antibacterial immobilization were carried out by the measurement of contact angle using sessile drop technique using Surface Energy Evaluation system (SEE system with CCD camera, Advex Instruments, made in Czech Republic). This system contains sensitive CCD camera with the highest resolution equal to  $1,280 \times 960$  due to high screen capture. Contact angle was measured by placing a small drop of testing liquid on a surface treated LDPE. The angle formed between the solid/liquid interface and the liquid/vapor interface is referred to as the contact angle. Deionized water, ethylene glycol, glycerol, formamide, diiodomethane were used as testing liquids, applied volume was 3  $\mu$ L (elimination of influence of gravity) and a static contact angle was measured shortly after the drop formation when a

thermodynamic equilibrium is reached between the three phases: solid, liquid, and gas. Surface energy ( $\gamma^{\text{tot}}$ ), its polar acid-base ( $\gamma^{\text{AB}}$ ), dispersive ( $\gamma^{\text{LW}}$ ), electron-acceptor ( $\gamma^-$ ) and electron-donor ( $\gamma^+$ ) components were calculated by Acid-Base regression model using method of least squares.

### 3.6. Adhesive Properties Assessment

The adhesive properties, namely peel strength (force per unit width) of the adhesive joint of antibacterial treated LDPE by triclosan and chlorhexidine via DCSBD to poly(2-ethylhexyl acrylate) deposited onto polypropylene foil (with 15 mm width), were carried out by measurements of 90° peel test at a 10 mm per minute rate of peel using a 100 N universal INSTRON 4301 dynamometer (England). Ends of the polymer film were firmly fixed in the jaws of dynamometer so that tension was evenly distributed across the entire width of the surface.

### 3.7. Surface Topography Analysis

The surface morphology and local surface heterogeneities of the modified polymer were measured by AFM. All measurements were performed under ambient conditions using a commercial atomic force microscope (NanoScope™ Dimension IIIa, MultiMode Digital Instr., USA) equipped with a PPP-NCLR tapping-mode probe (Nanosensors™, Switzerland; spring constant 39 N·m<sup>-1</sup>, resonance frequency  $\approx$  160 kHz). The surface properties of all the films were measured in x and y axis sizes between 2 to 25  $\mu\text{m}$  on different sites of the films in order to find characteristic and significant surface features. The AFM analyses were performed in tapping mode for all the images. This technique allows the obtaining either two- or three-dimensional information of both height and material heterogeneity contrast with high resolution when recording height and phase shifts simultaneously.

### 3.8. Surface Chemistry Investigation

#### 3.8.1. XPS

Samples were analyzed with a TFA XPS Physical Electronics XPS instrument. The base pressure in the chamber was about  $6 \times 10^{-8}$  Pa. The samples were excited with X-rays over a 400  $\mu\text{m}$  spot area with a monochromatic Al K $_{\alpha 1,2}$  radiation at 1,486.6 eV. The photoelectrons were detected with a hemispherical analyzer positioned at an angle of 45° with respect to the normal to the sample surface. Survey-scan spectra were made at a pass energy of 187.85 eV and 0.4 eV energy step. An electron gun was used for surface neutralization. The concentration of elements was determined by using MultiPak v7.3.1 software from Physical Electronics, which is supplied by the spectrometer producer.

#### 3.8.2. FT-IR-ATR

Attenuated total reflectance FTIR measurements were performed on a NICOLET 8700 FTIR spectrometer (Thermo Scientific) through the single bounce ATR accessory equipped with Ge crystal at an angle of incidence 45°. For each measurement the spectral resolution and the number of scans were 2 cm<sup>-1</sup> and 64, respectively. The quality of spectra depends on good contact between the crystal

and the sample. This requirement was achieved through the use of a pressure clamp. The acquired spectra were analyzed using spectroscopic software OMNIC™, v. 8.1.

### 3.9. In Vitro Antibacterial Test

Bacterial adhesion and biofilm experiments were performed using Gram-positive (*S. aureus* 3953) and Gram-negative (*E. coli* 3954) bacteria. Circular shaped specimens ( $d \approx 8$  mm) were cut from pristine and modified LDPE samples. A so called agar diffusion plate (inhibition) test was performed for antibacterial activity evaluation of tested substrates. The polymer samples were washed in ethanol and dried under laboratory conditions. The substrates prepared by such a way were placed on agar plate (Nutrient Agar No. 2 M1269, Hi Media Laboratories Pvt. Ltd.) inoculated by bacterial suspension. The bacterial suspension volume was 100  $\mu\text{L}$  for all samples. Bacteria concentration was  $10^7$  units·mL<sup>-1</sup> and incubation time was 24 h at the temperature 37 °C. After that, inhibition zone diameter was measured in 5 directions and average value was calculated. Each test was repeated in triplicate.

## 4. Conclusions

This work was aimed at examining the impact of selected antibacterial agents, namely triclosan and chlorhexidine bound to the surface of LDPE. DCSBD plasma treatment leads to increased surface free energy, roughness and surface wettability by introducing characteristic oxygen groups. A DCSBD plasma generator was used as activator of the LDPE surface for efficient binding of acrylic acid and for its transformation to polymeric form by radical polymerization. Thus the bound acrylic acid created polymer brushes on the polymer surface that provided physical forces to bind antibacterial agents in an effective manner. The presence of triclosan and chlorhexidine was confirmed by different surface analysis techniques. Moreover the antibacterial effect of such treated LDPE film was proven by *in vitro* bacterial tests against *E. coli* and *S. aureus* when adhesion of bacteria to polymer was effectively diminished.

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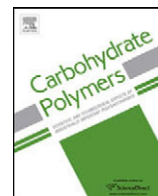
*Sample Availability:* Samples of the compounds are available from the authors.

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## **PAPER III**





## 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<sup>2</sup> for *E. coli* and 275 mm<sup>2</sup> for *S. aureus*).

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### 1. Introduction

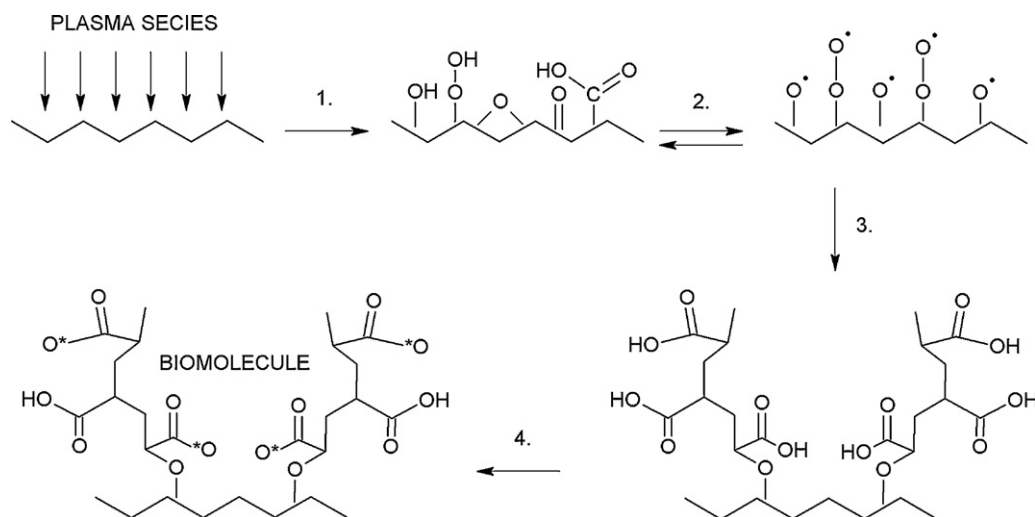
Several modification methods are commonly used to modify the polymer surface. One of the most frequent consists in an immersion in the strong acid solution. Nevertheless, such wet chemical methods are technologically complicated and environmentally unfriendly especially because hazardous chemical substances 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<sub>2</sub>O<sub>3</sub>. 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, & Qja, 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  $\beta$ -(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  $\alpha$ -(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  $\mu$ m 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

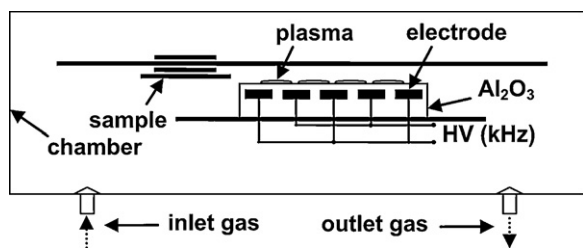


Fig. 2. Scheme of DCSBD plasma equipment.

were treated at power density of  $1 \text{ W/cm}^2$ ; 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,  $50 \mu\text{m}$  thick, with 0.5 mm spacing between the strips, made of Ag-paste) generate plasma by an effective way. Strips are embedded in 96%  $\text{Al}_2\text{O}_3$ . A high frequency sinusoidal voltage ( $\sim 15 \text{ kHz}$  at  $U_m \sim 10 \text{ kV}$ ) 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  $30^\circ\text{C}$  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  $30^\circ\text{C}$  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  $4^\circ\text{C}$  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  $30^\circ\text{C}$ . 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  $4^\circ\text{C}$  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  $3 \mu\text{l}$  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 ( $\gamma^{\text{tot}}$ ), polar ( $\gamma^{\text{p}}$ ) and dispersive ( $\gamma^{\text{d}}$ ) components of the surface free energy. Owens–Wendt–Rable–Kaeble regression model using the method of least squares was used for the evaluation of  $\gamma^{\text{tot}}$ ,  $\gamma^{\text{p}}$ , and  $\gamma^{\text{d}}$  (Salimi, Mirabedini, Atai, Mohseni, & Naimi-Jamal, 2011). The graft yield (GY) was calculated according to the equation  $\text{GY} (\%) = ((W_2 - W_1)/W_1) \cdot 100\%$ , 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  $90^\circ$  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.

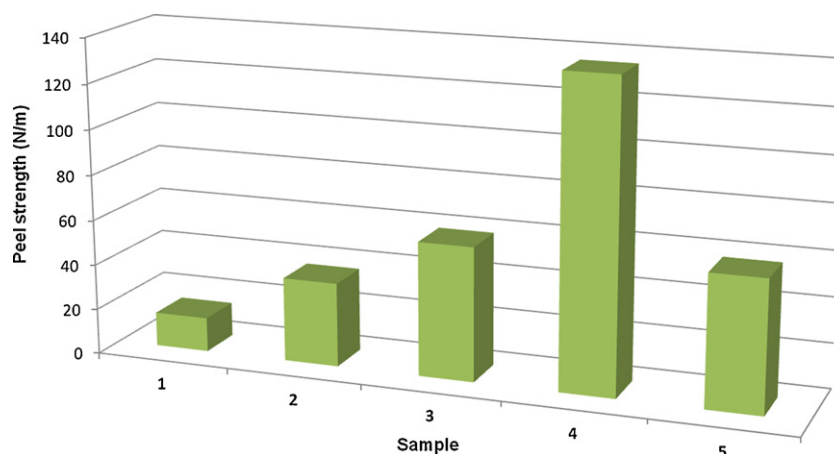
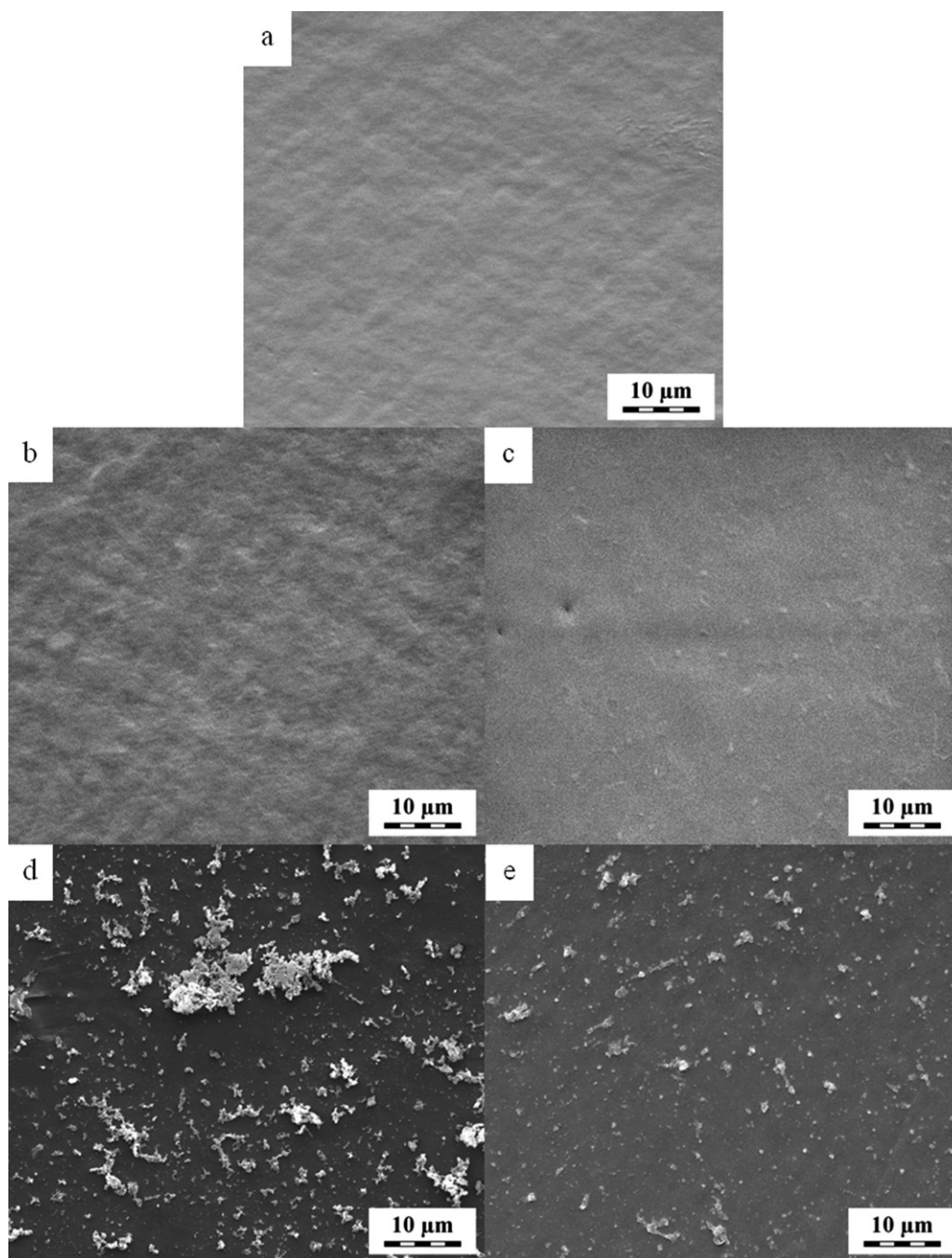


Fig. 3. Peel strength vs. surface treatment of LDPE sample: 1 – untreated, 2 – plasma treated, 3 – PAA grafted, 4 – chitosan coated, and 5 – chitosan/pectin coated.

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

LDPE sample	$\theta_{\text{w}}$ (°)	$\theta_{\text{e}}$ (°)	$\theta_{\text{g}}$ (°)	$\theta_{\text{d}}$ (°)	$\theta_{\text{f}}$ (°)	$\gamma^{\text{p}}$ (mN/m)	$\gamma^{\text{d}}$ (mN/m)	$\gamma^{\text{tot}}$ (mN/m)	GY (%)
Untreated	99.2 ( $\pm 0.6$ )	70.9 ( $\pm 1.2$ )	85.3 ( $\pm 0.9$ )	48.4 ( $\pm 1.2$ )	80.7 ( $\pm 0.9$ )	0.2	31.5	31.7	–
Plasma treated (A)	77.5 ( $\pm 1.1$ )	51.0 ( $\pm 2.8$ )	67.1 ( $\pm 2.8$ )	36.0 ( $\pm 1.2$ )	52.8 ( $\pm 1.5$ )	1.1	41.4	42.6	0.0
A + PAA grafted (B)	66.9 ( $\pm 0.7$ )	32.1 ( $\pm 2.4$ )	57.2 ( $\pm 2.7$ )	32.5 ( $\pm 1.6$ )	37.0 ( $\pm 2.0$ )	4.5	43.7	48.1	0.5
B + chitosan coated	69.2 ( $\pm 0.8$ )	36.0 ( $\pm 2.1$ )	68.3 ( $\pm 1.2$ )	35.9 ( $\pm 1.9$ )	33.1 ( $\pm 2.2$ )	6.1	38.8	44.9	3.1
B + chitosan/pectin coated	59.1 ( $\pm 1.1$ )	30.0 ( $\pm 2.8$ )	53.40 ( $\pm 1.3$ )	37.8 ( $\pm 2.6$ )	33.8 ( $\pm 2.7$ )	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.

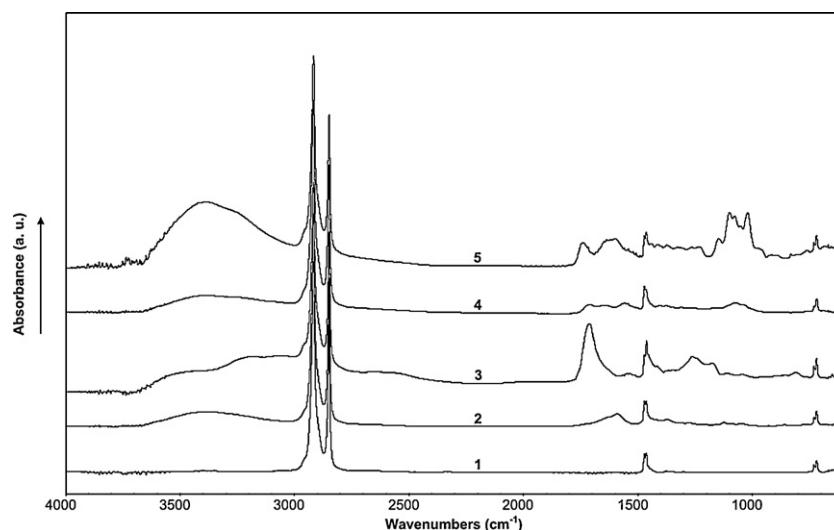


Fig. 5. FTIR-ATR spectra of LDPE samples: 1 – untreated, 2 – plasma treated, 3 – PAA grafted, 4 – chitosan coated, and 5 – chitosan/pectin coated.

### 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 ( $\geq 3$ ) 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  $6 \times 10^{-8}$  Pa. The samples were irradiated with X-rays over a  $400 \mu\text{m}$  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  $45^\circ$  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 NICO-LET 8700 spectrometer (Thermo Scientific USA) through the single bounce ATR with Ge crystal at  $45^\circ$  incident angle. The spectral resolution and the number of scans were  $2 \text{ cm}^{-1}$  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 Pll. Ltc. was used for our experiments. Tested samples were cut in a circular shape ( $d = 8 \text{ mm}$ ), washed in ethanol, dried and placed on an agar plate inoculated by the bacterial suspension (volume =  $100 \mu\text{l}$ , concentration =  $10^7$  units/ml). The samples were incubated for 24 h at  $37^\circ \text{C}$  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.

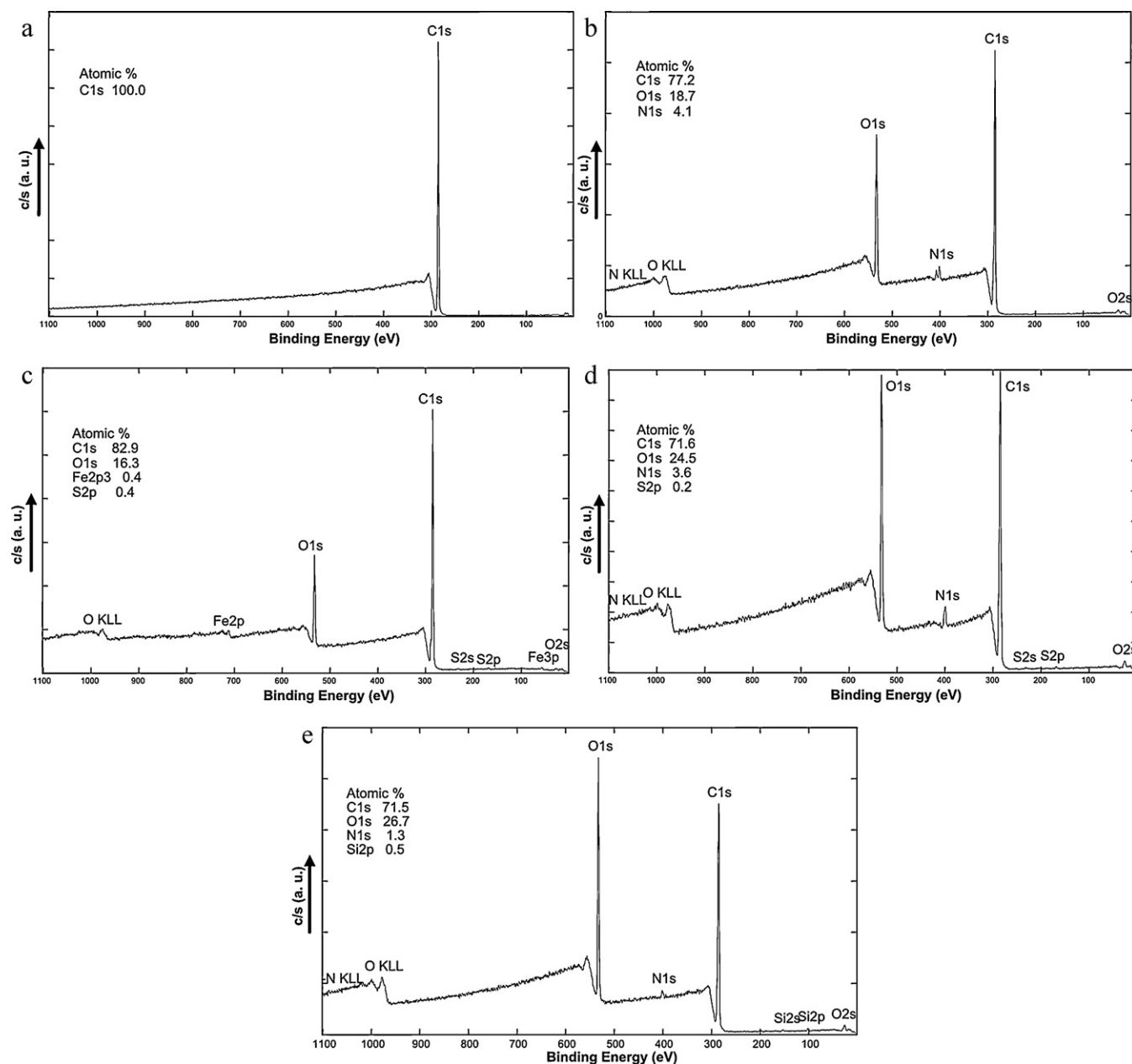
## 3. Results and discussion

### 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 ( $\theta$ ) due to low surface wettability. The significant decrease of  $\theta$  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  $\theta$ , whereas PAA contains polar carboxylic groups. In addition, the chitosan and chitosan/pectin multilayer led to the significant decrease of  $\theta$  due to the presence of characteristic polar functional groups. Accordingly to the measured contact angle values, low values were calculated also for  $\gamma^{\text{tot}}$  of untreated LDPE associated with its hydrophobic nature. The plasma treatment leads to the increase of LDPE  $\gamma^{\text{tot}}$  indicating the surface polarity increase. Even greater increase of LDPE  $\gamma^{\text{tot}}$  was recorded for PAA grafted LDPE and for chitosan immobilized LDPE. The highest increase of  $\gamma^{\text{tot}}$  and  $\gamma^{\text{p}}$  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  $\gamma^{\text{tot}}$ , 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  $\text{cm}^{-1}$ ) and/or other oxygen containing products at the surface of the material (region 1845–1510  $\text{cm}^{-1}$ , 1280  $\text{cm}^{-1}$ , 1126  $\text{cm}^{-1}$ , 1150  $\text{cm}^{-1}$ , 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  $\text{cm}^{-1}$  (carbonyl band, C=O stretching), and also some unresolved peaks in the fingerprint region (1300–1100  $\text{cm}^{-1}$ , C–O stretching and  $\text{CH}_2$  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  $\text{cm}^{-1}$ ), pectin – 1734  $\text{cm}^{-1}$  (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  $\text{cm}^{-1}$  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  $\text{cm}^{-1}$  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– $\text{NH}_3^+$ , – $\text{ONO}_2$ . 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

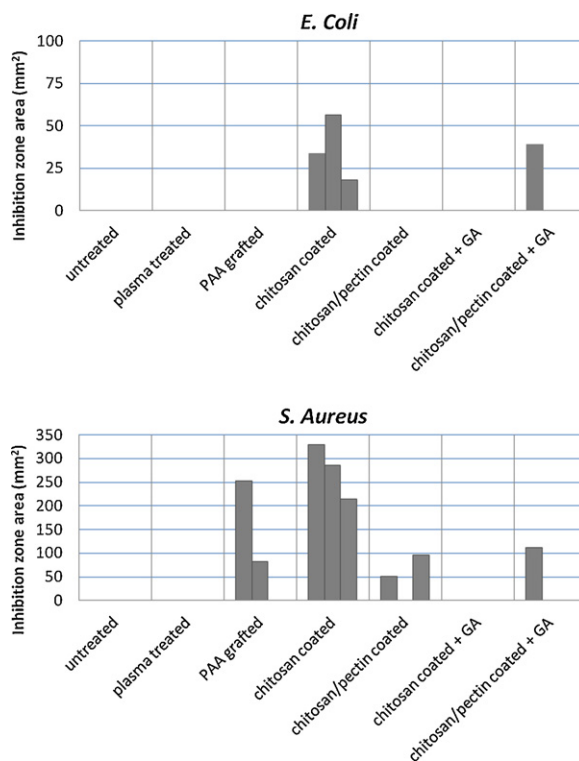


Fig. 7. Inhibition zone area of LDPE samples for *S. aureus* and *E. coli* strains. Each column represents the inhibition zone area for one experiment out of three.

XPS spectrum indicates, that a number of different moieties (e.g. carbonyl, carboxyl, etc.) containing oxygen is present in treated LDPE.

### 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  $\text{mm}^2$ . 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  $\text{mm}^2$  for *E. coli* and 275  $\text{mm}^2$  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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