Influence of hydrophilic additives on antimicrobial properties of tungsten trioxide in polypropylene matrix

Dual Master's Degree in Biological Chemistry

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UNIVERSITY LINZ | JKU

Linz
2014

Acknowledgements

I really spent a wonderful time at JKU and SBU, and would like to thank cordially:

- Prof. Dr. Norbert Müller and prof. RNDr. Libor Grubhoffer for their great work to organize and support the Cross Border Master Curriculum of Biological Chemistry.
- My supervisor Univ.Professor Dr. Sabine Hild for giving me the opportunity to work on an inspiring project and for keeping his door always open for discussions at any time.
- Dr. Roman Voloshchuk, Dr, Iryna Voloshchuk and AMiSTec GmbH & Co. KG for materials and help in sample preparation.
- Mgr. Adriana Walnerová, MSc, for a collaboration.
- DI Rudolf Haßlacher, DI Thomas Fischinger, DI Bernhard Jachs, DI Martin Laher, DI Lisa Maria Uiberlacker, DI Matthias Mihalic for their unresting support.
- Our secretary Maria Griffits for her ready help with all administrative issues.
- Laboratory Technician Aloisia Sageder on whom I could always rely for their ready cooperation and availability, and who have also provided an enjoyable working environment.

People who are not explicitly mentioned here but who helped me one way or another.

Abstract

The purpose of this study was to evaluate the inhibition of biofilm formation on newly developed polymeric material and test antimicrobial activity of metal oxides. A series of tungsten (VI) oxide concentrations in a combination with Pluronic PE8100 (PEG non-ionic surfactant) were melt-mixed with polypropylene (PP). This polymer was chosen since it is commonly used material for medical and domestic applications.

In this work we investigate the influence of physical surface properties (roughness, surface tension, crystallinity). The purpose was to establish the influence of the tungsten oxide, wetting agent concentrations and their mixtures to the polymer structure, and to estimate the relationship between structure of polypropylene and antimicrobial activity of the surface. For that we developed a protocol to prepare homogeneous flat polymer samples by a melt-pressing at defined temperature. The morphological changes of polymer compositions were analyzed by Differential Scanning Calorimetry (DSC). The surfaces of materials we analyzed by atomic force microscopy (AFM) and measurement of contact angle.

Biofilm formation on the surface was assessed by agar plates colony counting using *Escherichia coli* mutant. Method of qualitative analysis of attachment density of bacteria on surfaces was developed.

The results indicated that an increase in the concentration of tungsten (VI) oxide in the materials increases some antimicrobial activity, but mixture with Pluronic enhanced it dramatically. Also synergistic effect between tungsten oxide and Pluronic was found. It was concluded that there is no bactericidal effect of composition. It seems that the main antibacterial effect comes out at the step of bacteria adhesion. The composition possessed self-cleaning properties and displayed signs of inhibiting biofilm formation on their surfaces.

Keywords

Antimicrobial active surfaces, tungsten oxide, polypropylene, contact angle measurement, atomic force microscopy

Contents

1. Introduction

Over the last years there was significant increase in using antimicrobial and antifungal additives in polymers, especially in the field of medicine and goods in contact with food. This is obvious from many research publications on this subject; as well as from the reports from scientific and not-for-profit organization responsible for standards development, product certification, auditing and education, such as the American National Sanitation Foundation (NSF) and others. Results show that germ probes taken from doors, handles, ketchup bottles, tables of shops, offices, restaurants, hospitals, and over 30 places an average adult can touch within a minute are contaminated with fecal bacteria and different kind of pathogens, in broad spectra from opportunistic till antibiotic-resistant staphylococcus [1].

Now there are many evidences supporting the role of surfaces in the epidemiology of disease caused by the staphylococci in particular methicillin-resistant *Staphylococcus aureus* (MRSA) [2]. Surfaces may act as reservoirs of microbes which could in turn lead to the spread of infection upon being touched. In general the number of colony forming units (CFU) required to initiate an infection by MRSA lies in the very broad range of between 10 and several million [3]. Still there is no common bacteriological standard for surfaces quality control in hospitals. The task to develop a method to assess the minimum hazard characterization for contaminated surfaces when it starts to be dangerous for health would be very actual.

Once a surface became contaminated the cycle of microorganisms transfer to workers, patients and to other surfaces starts until interrupted by cleaning and disinfection. Antimicrobial surface coating has been developed to prevent bacterial contamination and to interrupt this "vicious circle" from inside. Very often the growth of microorganisms is negligible (no visible staining or discoloration) but results are odor and increases the risk of transmission of infection. The main objective of the antimicrobial additives is to reduce bacterial load in the product and on its surface.

1.1. Biofilm grow prevention

1.1.1. Biofilm formation.

One of the most important mechanisms of Prokaryotes' adaptation to any environment is attachment and aggregation (that is suitable for higher life forms positioning mechanisms such as tissues). Organisms can exist in an environment independently but in many cases they proliferate more effectively by interacting and forming communities. Aggregation enhances cell-cell interaction and yields the additional benefit of the phenotypic versatility of their neighbors [4].

Bacterial communities in nature play a key role in the production and degradation of organic matter and in extreme environment survival: a biofilm is polymorphic and structurally adapted to changes in nutrient availability and outside aggression [5].

Different species form the biofilm under different conditions. Nevertheless, many species have shown distinct developmental steps in biofilm formation, which include:

initial attachment to a surface ⟩ the formation of microcolonies ⟩ mature biofilm.

These basic steps leading to the formation of a single-species biofilm are shown in Figure 1.

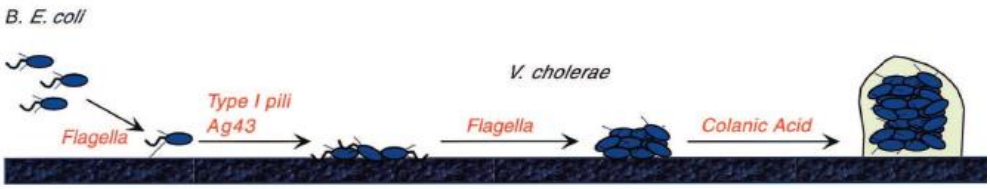


Figure 1. Biofilm development in gram-negative organism *E.coli*. Schematic representation of monolayer and multilayer biofilms steps *(Adopted from ref. [5] Microbiology and molecular biology reviews).*

The following factors play an important role in biofilm formation:

- flagellum-mediated motility of bacteria cell (to initiate the early attachment processes),

- signal transduction pathway (the major phase-variable outer membrane protein, chemotaxis proteins),
- nutrient availability
- surface structures (each organism has adapted the use of surface structures to its own particular needs) [6].

In a process which seems to be random some bacteria remain attached to the surface for extended periods of time and form microcolonies. With time microcolonies are distributed on the surface as a single layer and develop into a monolayer. In the multilayer biofilm composed of multiple layers of bacteria encased in a extracellular polymeric substance (EPS). It is also commonly called an extracellular adhesive matrix. Components of the adhesive matrices synthesized by bacteria may include exopolysaccharides, protein, and DNA. One of the most common and most extensively studied matrix is a polymer of poly-1,6-N-acetyl-D-glucosamine (PNAG) In *E. coli*, it is required for both surface attachment and formation of multilayer biofilms [7]. Mature biofilms are resistant to a wide range of antimicrobial treatments and standard disinfection. Consequently they pose persistent pathogenic threats.

1.1.2. The interaction of microorganisms with plastics

The interaction of microorganisms with plastics can occur in different ways:

1. Direct destruction when microorganisms are used plastic or its components as a nutrient medium:
   - Change in mechanical properties as a result of bacterial consumption of functional additives - plasticizers and stabilizers. This is the most serious demonstration of plastics biodegradation.
   - The increased permeability to gases and solvents also results from damage to the surface of the product.
2. Destruction or alteration of the external appearance of the article under the action of metabolic products of microorganisms (acid, enzymes, pigments, etc.):

- Staining or color change occurs as a result of exposure the intracellular pigments (mainly mold - penicillin and Aspergillus), or the extracellular dye
- Changes in the electrical properties (conductivity) due to produces polysaccharides.
- Odor due to release of metabolic products of microorganisms - amine, ammonia and hydrogen sulphide.

3. The formation of colonies of microorganisms on the surface of the product, without causing visible damage to the product:

- Surface contamination due to the formation of colonies of microorganisms that create micro-roughness which accumulate dust and acts as a source of human infections.

Most of the plastics are damaged by fungi and bacteria mainly feeding on various organic additives contained in the products. Plasticized PVC and foamed polyurethane are the easiest to contaminate. The former contains plasticizer which acts as a powerful source of bacteria while the latter has a large number of pores in where dust, moisture, and fungal spores accumulated.

Polyolefins are generally less susceptible to the action of microorganisms as compared to PVC and polyurethanes. Low molecular weight polyethylene (molecular weight less than 10,000) and the polymers with small amounts of branching (HDPE) are most prone to biodegradation. At the same time the studies of various synthetic fibers and fabrics demonstrated that streptococci can deposit on the surface of polyester, polypropylene and polyamide fibers.

1.1.3. The typical strategies to inhibit microbial colonization and biofilm growth

Functionally antimicrobial additives can be divided into 2 types: biostabilizers and biomodifiers. The former protects plastics from fouling fungi, algae, mold etc. and help to prevent the destruction of plastics by microorganisms. The latter gives plastics

the ability to maintain surface sterility for a long period of time and prevents odor formation. There are two main strategies used [8]:

1) Surfaces, which microbes find hard to attach and form monolayer. The strategy of this technique is to prevent microbial adhesion to the device or surface at the beginning. As such this is a preventative strategy. A great deal of additional work is necessary to establish a direct link between surface functions required to prevent bacterial adhesion and biofilm development.
2) A wide variety of antimicrobial coating technologies, which use diffusible antimicrobials. In polymers product additives migrate to the surface and interact with microorganisms, what kills the microbes during their growth and prevents multilayer formation. In these technologies organic and inorganic antimicrobials are used. But they are non-permanent: gradually washed out from the surface of the product and the protective layer is reduced. Also these techniques have the potential problem of inducing microbial resistance.

1.2. Types of antimicrobial additives

Some of the first biostabilizers were arsenic, sulfur, mercury or copper. These were the biostabilizers used until the 30s of the last century. Then research leading to the production of organic antimicrobial compounds has started.

1.2.1. Polyethylene glycol coatings

This is one of the well-established method for preventing the microorganism adhesion. PEG modified surfaces are often used in microbiological and medical application to inhibit non-specific absorption. PEG-coated surfaces are antifouling because of hydrophilic repulsion with the otherwise hydrophobic microbial cell envelope. The second factor making the attachment to the surface more difficult is the dynamic movement of the PEG chains bound to the surface. The “brush” of PEG molecules keeps microbes at a distance and yields a sevenfold attenuation of the Van

der Waals attraction between the microorganisms and the surface [9]. One of the disadvantage of this approach is multistep synthesis.

1.2.2. Easy clean surfaces—prevention of microbial adhesion

The idea refers to the surface properties of self-cleaning mechanism of lotus plants and other organisms. Either an exceptionally hydrophilic (less than 10°) or a hydrophobic (>140°) surface are required for self-cleaning. From the one side very smooth surfaces are harder to colonize than rough surfaces. From the other side surface roughness is often required to obtain very high contact angles. Contact angle measurement (indicator of the hydrophilicity or hydrophobicity of the surface) can be used as characteristics how easy it is for a microbe to colonize a surface [10].

Hydrophobic materials significantly reduce microbial adhesion. It can solve the problem of microbial contamination of the treated area but it does not address the problem of pathogenic infections distribution, which are simply moved to other areas. Hydrophobic materials need to be combined with other antimicrobial techniques.

1.2.3. Diamond-like carbon films (DLC)

DLC materials exhibit a desirable combination of a low coefficient of friction and high micro-hardness. These coatings are biocompatible and can be used for biomedical devices. They contain no active antimicrobials, but DLC films may be doped with other inorganic species such as Ag or Cu, yielding antimicrobial properties in addition to the anti-adhesive properties.

1.2.4. Zwitterionic polymer biomimetic surfaces

Zwitterionic surfaces mimic the lipid bilayers of biological membranes. This is group of copolymers formed from phosphorylcholine, sulfobetaine and carboxybetaine [11]. The charged zwitterionic head can associate a large amount of water, making the material essentially hydrophilic. These surfaces demonstrate very high biocompatibility and can be very promising for devises used in different biological environment.

1.3. Antimicrobial coatings and surface technologies

These techniques relying on diffusible organic or inorganic antimicrobials, released from the products.

1.3.1. Microbicide-releasing surfaces

Triclosan-incorporated products are the most widely known to suppress bacterial growth within the domestic environments. However ability of pathogens to develop Triclosan-resistance reduces its application within hospitals. In that case other modifications are more promising:

- Silver and silver-containing surfaces and nanoparticles. Silver is known as anti-bacterial agent since ancient times. Nowadays it has been successfully used in cosmetics, wound dressings and as an additive in catheters and other medical devices [12]. Drawbacks of silver are cytotoxicity of Ag ions towards mammalian cells and quite expensive price for industrial application.
- Copper and copper alloy surfaces clearly exert antimicrobial effect. Clinical trial shows copper continuously reduces bacterial burden by 83% and reduces the risk of infection by 58% [13]. Other heavy metals such as cadmium and lead are also under consideration.
- Bacteriophage-modified particles used for many medical purposes, but its application for surfaces is a very recent development. This approach is interesting in particular against antibiotic-resistant bacteria which are not phages resistant. And obviously the phage with inherent specificity for individual bacterial species may leave out potentially harmful organisms.

Despite the initial effectiveness of these existing antimicrobial coatings they have one major drawback—they diffuse into the environment. This makes them non-permanent.

1.3.2. Polycationic antimicrobial surfaces

Microbial cell envelope is hydrophobic and negatively charged. Surfaces treated with hydrophobic polycations electrostatically attract microbes and kill microbes upon contact by causing physical damage to the microbe's cell. The most recent surface coatings of this type are polyethyleneimines (PEIs) [14]. PEIs with a high molar mass and a high degree of branching are of highest interest in pharmaceutical research as polymeric vectors for gene delivery as they can electrostatically interact with negatively charged molecules like DNA and RNA. However, their clinical application is limited due to cytotoxic effects and a low hemocompatibility. Based on its ability to form complexes with anionic species, metal complexes, or metal ions, PEI represents an interesting material for technical applications.

1.3.3. Light-activated antimicrobial agents (LAAAs)

Photodisinfection is a result of the mechanism of photoexcitation and production of radicals such as superoxide and the hydroxyl. There are two principal coating types that produce these reactive species and act as antimicrobial surfaces: a coating comprised of a photosensitizer immobilized antimicrobials and a titanium dioxide based photocatalyst.

The use of a photosensitiser as an antimicrobial agent is a direct refinement of the technique of photodynamic therapy (PDT). PDT is a commonly used therapy to target and destroy cancerous tissues. The key benefits of this antimicrobial surface are the reduction of microbial loads on a surface using visible light and avoiding the problems of microbial resistance.

1.4. Manufacturing

About 20 manufacturers produce about 80 names and antimicrobial additives [16]. Among the basic compounds can be mentioned [15] [17]:

Table 1. ***Biocides in Plastics***

Active compound	Antibacterial efficacy	Used in
10,10'-Oxybisphenoxarsine (OBPA)	It is a broad spectrum antimicrobial, effective against fungi, pink staining organisms, bacteria and algae	Flexible PVC and polyurethane
2,4,4'-trichloro-2'-hydroxydiphenyl ether (Triclosan)	Broad-spectrum antimicrobial agent	Textile, package, medical and some naturally occurring polymers
n- octyl-isothiazolone (OIT)	Microbiocide and fungicide	Vinyl, polyurethane and other polymeric compositions
4,5- dichloro -2-n-octyl-4-izotriazolin -3 -one (DCOIT)	Bactericidal, common mold fungicidal	Wood protection
Mercapto oxide (Pyrithione)	Prevents biodegradation in aqueous functional fluids, control of fungi	Latex paints, adhesives, polymer emultions
N-Butyl-1,2-Benzisothiazolin-3-one (Butyl-BIT)	Inhibits the growth of bacteria, fungi and algae	Paints, wallboard, ceiling tiles, flooring materials etc
N-(Dichlorofluoro-methylthio) phthalimide (Sanitized PL)	Against mold-fungi	PVC plastisols
The metal compounds (compounds of silver, zink, mercury, copper)	Cytotoxicity for Gram-positive, Gram-negative, and antibiotic-resistant bacteria (cationic biocides)	Latex, PP, PVC, PU, SBR and and other polymeric compositions
Polymeric biocides (polyphosphates , poly -N-halo pyridine , poly (styrene- divinylbenzene)-sulfonamide)	According to Monomers Antimicrobial Properties (the polymers are activated by anchoring antimicrobial species)	VBC, MMA, Styrene

Most of these chemicals are organic compounds with low molecular weight, sometimes containing a metal ion. The antimicrobial mechanism of action is usually chelation and enzyme inhibition. Enzymes regulating cellular processes are destroyed.

Also protein destruction and a specific target within the bacterial lipid synthesis pathway can cause cell death.

Currently silver and zinc compounds are mainly used as the inorganic antimicrobial systems. Such compounds are virtually inert and begin to release silver ions under the interaction with moisture. The main advantage of inorganic compounds is the high thermal stability (up to 500 0C). The high thermal stability allows the use of such materials for the manufacture of engineering thermoplastics.

Usually antimicrobial additives are incorporated into polymers through melt-mixing with the aim of providing persistent antibacterial action on the surface of the polymer. But the standard extrusive and molding equipment doesn't allow reaching uniform distribution of additives in a polymer matrix. That's why for production of products with antimicrobic properties it is recommended to use "superconcentrates".

Still there is no universal antimicrobial agent suitable for every application. Every case is special: depending on polymer structure, preparation and conditions of use the same chemical shows different activity or demonstrates no activity at all. Evidently it is very important to understand the role of all parameters affecting the antimicrobial activity of a polymer-antimicrobial agent compounds.

1.5. Parameters to control biofilm grow

Antimicrobial activity of compounds depends on physical, chemical and biological parameters. It has been mentioned that surface chemistry inhibits biofilm growth. Measurements showed that some parameters affecting antimicrobial activity (pH, temperature, surface tension) also affected surface activity in a similar fashion [19]. Thereby, effects of physical surface properties and many other parameters have to be taken into account:

- Concentration of active ingredient.
- Type of polymer. Structure can prevent the possibility of active molecule migration to the surface.

- How the additive was mixed with the polymer and uniformity of its distributions within the matrix.
- Micro- and nanostructures and topography, the mechanical properties of a surface have recently been reported to play a role [21]. This phenomenon was demonstrated using flat surfaces in the Young's modulus range of ~1– ~100 MPa and showed that there is a positive correlation between the density of attached bacteria and the substrate stiffness. Bacterial mechanoselective adhesion also can be exploited to control and inhibit biofilm growth [20].
- Contact time with the resin. Not only rapid efficiency, but also long-lasting protection is very important. Often only some initial slowing of the bacterial growth rates was observed, followed by the absence of an antibacterial effect over extended periods [22].
- Sensitivity of microorganisms is also an important factor should be taken into account. In most cases negative bacteria less susceptible to antimicrobial additives than Gram-positive, as they have an additional membrane, which retards the penetration of the antimicrobial additive.

Strictly a mechanical–structural property does not rely on surface chemical functionalization, it is not susceptible to masking and may be persistent. As a potential new strategy, nanostructures mimicking an extremely compliant flat surface are promised for diverse applications for controlling and inhibiting biofilm accumulation [20]. The effects of topographical features on bacterial adhesion and biofilm formation are still poorly understood.

1.6. Basic requirements to antimicrobial additives

Even when a very active and proper substance can be found it doesn't automatically mean that it can be used in industrial scale. There are some other "human" and economical parameters which have to be optimized.

General requirements to the antimicrobial additives used as biostabilizers and biomodifier are the same:

- High efficiency
- Low toxicity to humans, animals and the environment in the course of processing and using of final products
- Ease in processing and application
- Compatibility with other additives (stabilizers, processing, etc.)
- No negative impact on the physical and mechanical properties on the product or consumer
- Long shelf-life of products

At the moment undisputed leadership in the biostabilizers market belong to the arsenic compounds, specifically 10,10-oksibisfenoksiarsinom (OBPA). This compound has about 70 % of the market share driven by the best quality/price ratio. However there is a tendency to use a minimum of toxic compounds and more and more antimicrobial agents do not contain arsenic - for example, isothiazol (more effective than OBPA), phthalimides or inorganic compounds (mainly zeolites).

1.7. Methods for estimation the effects of antimicrobial additives

The choice of method to study the sensitivity of microorganisms to plastics additives is extremely important. There are some standard methods for evaluating the resistance of the material to biodegrade and resistance to colonization by bacteria on the surface of products. In addition to ASTM, the American Association of Textile Chemists and Colorists (AATCC) has also developed a methodology for assessing of antimicrobial ability of synthetic fibers and fabrics [18]. Also, there are regulations developed by AFNOR (France), DIN (Germany), IEC (International Electrotechnical Commission), SN (Switzerland).

These techniques are generally similar. Here is a description of the main points:

1. Agar Plate Test (test with the agar plate) - suitable only for evaluation bacteriostatic activity. The advantage of this method is speed, ease to use and high reliability.
2. ASTM G21-90 Standard Practice for Determining Resistance of Synthetic Polymetric Materials to Fungi - a sample placed in a sterile solution, which

allows to determine whether a material can serve as a nutrient medium for fungi.

3. In-Use Test (test in real time) by ASTM D3083 for Flexible PVC Plastic Sheeting for Pond, Canal, and Reservoir Lining. Design for purposes in view of the importance of environmental factors. According to this methodology plastic with antimicrobial additive is dug for 90 days in order to determine the susceptibility of biodegradation.
4. EN ISO 846 - internationally recognized test method. Evaluation of the action of microorganisms on treated plastic materials. A recently developed technique, which is a combination of the first three:

   The surface of plastic test pieces placed onto the surface of an agar plate are seeded with test microorganism and then additionally covered with a layer of inoculated molten agar. After incubation, the plates are monitored till 4 weeks and examined for bacterial growth or fungal growth.

   It allows to comprehensively investigate plastics intended to use in the open air or in the soil.
5. Direct Contamination of the Test Specimen - independent to the velocity of microorganisms migration, suitable for the examination of samples containing insoluble or bad soluble (silver-based inorganic and zinc) an antimicrobial additive.

Usually for laboratorial purposes standard methods are not always suitable. For CFU biofilm quantification assay each laboratory develops methods according to specific scientific interests, for instance using different regimes of bacteria cultivation and fluorescence imaging.

1.8. The main directions in antimicrobial additives development

There is a growing demand for bio modification supplements to help prevent odor and able to work with a wide range of microorganisms. The obvious trend is towards the use of low-toxic anti-microbial additives. Compounds based on arsenic and heavy metals are progressively replaced by less toxic isothiazolines derivatives or

silver and zinc-based compounds. The former show greater effectiveness while the latter has the drawback of lower resistance towards oxidation and discoloration by oxygen.

Another promising direction is using the insoluble polymeric antimicrobial compounds. They are much more slowly leached out of the product and may be regenerated.

For the manufacture of plastic products intended for direct contact with food are developed natural antimicrobial agents (e.g., enzymes peroxidase). Also different forms of inorganics with very low toxicity level for mammal cells, but capable to alter the metabolism of microorganisms, mainly interacts with enzymes. In most cases these natural antimicrobial agents are combined with additives which increase compatibility with the polymer and regulate their migration.

Also, nature provides some hints to preventing microbial colonization of surfaces. Materials, following by this alternative strategy with topographical features mimicking plants and animal's skins, like lotus or sharks at certain scales, have shown increased resistance to bacteria and algae biofouling. Physical structures act over a longer time and may provide more persistent form of inhibitive interaction between bacteria and surfaces.

1.9. Current work motivation

Under a change of environmental conditions a biofilm may become unstable. Bacteria must be able to detect and respond to the unfavorable environmental conditions, such as lack of nutrition, oxygen, interruption of signal transduction pathways, and other factors. Furthermore the influence of many substances on biofilm formation, growth and degradation still has to be investigated. There is significant scientific and practical interest in developing cost efficient and robust antimicrobial agents on commercial scale. Metal oxides are one of the cheapest and efficient class of substances. Transition metal oxides used as antimicrobial agents can provide a long lasting antibacterial effect and are ideal for surfaces which can be used in wet environments. For instance coating surfaces of dental and orthopaedic implants with

antimicrobial nanoparticles of different metal oxides, including tungsten oxide WO_3, should lead to an increased rate of implant success [23]; yet underlying mechanism of their action is not well understood in every case For these reasons molybdenum and tungsten oxides were selected as additives to polymers. The choice of these particular metal oxides was based on preliminary experiments performed at AMiSTec. These experiments have demonstrated the potential Mo and W oxides as non-toxic antimicrobial agents [24]. Moreover they are potentially suitable for use in polymer matrix, and such systems were not described earlier. Polypropylene as matrix was chosen since it is widely used material for medical application.[1]

As was mentioned before (see part 1.5) micro-roughness and wettability are some of the main factors for influence on antimicrobial activity of the surface. Therefore the main purpose of this work was to:

a) Develop a protocol for polymer sample preparation to control surface roughness on microscopic scale and provide define condition for polymer crystallization.
b) Test wettability and roughness and correlate it on microscopic scale.
c) Develop an easy to perform protocol for testing bacterial activity.

[1] Present study was carried out in close collaboration with AMiSTec GmbH & Co. KG. This startup company aims to develop commercially viable non-toxic antimicrobial solutions for medical industry.

2. Results and discussion

The aim of this work was to investigate the influence of tungsten oxide (VI) and wetting agent (Pluronic PE8100, PEG non-ionic surfactant) on microstructure and surface properties of polypropylene. Also the purpose was to explore the bacterial adherence to the given PP composition and to establish the relationship between structure of polypropylene/tungsten oxide/wetting agent compounds and their antimicrobial activity.

To explore the physical-chemical and biological properties different technics were used.

For composites microstructure investigation:

- Differential Scanning Calorimetry (DSC)

For PP surface properties investigation:

- Contact angle measurement (surface free energy- hydrophobicity)
- Scanning force microscopy (elastic modulus of the sample)
- Atomic Force Microscopy (topography, morphology)

Bacteriological grow analysis:

- Bacterial colonies counting

2.1. Materials

Sample preparation

Earlier it was found that the composition containing 2 wt% of WO_3 and Pluronic in polypropylene matrix shows antimicrobial activity towards *E. Coli* and *S. Aureus*. In order to investigate the influence of every individual component and their mixtures on polypropylene structure and its antimicrobial properties the following compositions were prepared by melt extrusion. Concentration of both WO3 and Pluronic PE8100 was varied between 0 and 4 wt%; compounds with only one component as well as the mixture of the two were extruded. After they were re-melted in between two glasses to get identical uniform and flat surface for every sample.

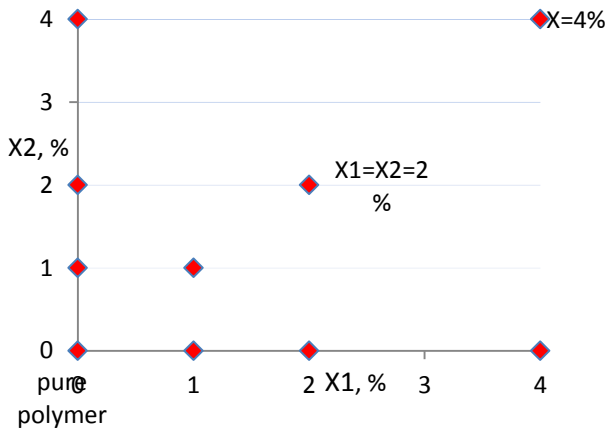


Figure 2. Samples composition.

Where:

X1 - Tungsten oxide (VI) (with size of particles $1 < d \leq 10$ mkm), % of mass

X2 - Pluronic PE8100 (PEG non-ionic surfactant), % of mass

Polymer - PP Domolen 1040 (matrix) till 100 % of mass

General procedure:

All samples were prepared in Brabender® measuring mixer W 50 EHT under atmospheric pressure.

The base material (PP, 37g) was placed in a preheated to 190 °C kneading chamber. Suspension of tungsten oxide (powder) in Pluronic 8100 (liquid) at mass ratio of 1:1 was prepared to ensure uniform distribution of the components in the polymer. Then this suspension was added dropwise into the molted polymer under constant agitation in a mixer. Amount of added suspension was calculated to obtain composition 1%, 2% or 4% (Table 2).

Table 2. **Sample preparation**.

PP, g	WO3, g	Pluronic, g	mix, g
37	0,38	0,38	37,76
37	0,77	0,77	38,54
37	1,60	1,6	40,20

The amount of the base material was constant in all experiments in order to maintain optimal mixing conditions. The loadings of solid and liquid additives were calculated individually for each experiment and their impact on a volume of a sample was neglected.

Mixing was carried out for 5 min at 190°C. After that hot melt was taken out of the kneading chamber, placed between two mold-release foils and pressed under a hot press (160 °C) for several seconds. The sample was obtained in a form of a disk with a diameter ca. 20 cm and thickness 1-1.5 cm. Small part of a sample (about 0.5 g) was placed between 2 glass slides and put into the oven at 190 °C under press for melting during 5 min. After cooling 1-1.5 mm thin layer samples for measurements were obtained.

2.2. Methods

2.2.1.DSC

To study the influence of additives on the bulk properties and crystallinity of polypropylene differential scanning calorimeter DSC8000 was used. The amount of the heat flow as a function of temperature was detected and enthalpy during the melting transition was determined. This energy is associated with phase transition from crystal to the liquid state.

$$\Delta Hm = \int_0^\infty Cp\, dT \quad [\mathrm{J/g}]$$

Crystallinity of the polymer was calculated as ratio of measured enthalpy to the literature data given for ideal polypropylene crystal, taking into account the pure mass of polypropylene in composite material.

$$\alpha = \frac{\Delta Hm}{\Delta Hm0} * 100$$

Procedure.

Used computer program:

Step 1. Hold 3 min at 80 °C

Step 2. Heating to 200 °C with rate 10 °C/min

Step 3. Hold 3 min at 200 °C.

Step 4. Cooling from 200 °C to 80 at 10 °C/min

Step 5. Hold 3 min at 80 °C

Step 6. Heating from 80 to 200 °C with rate 10 °C/min

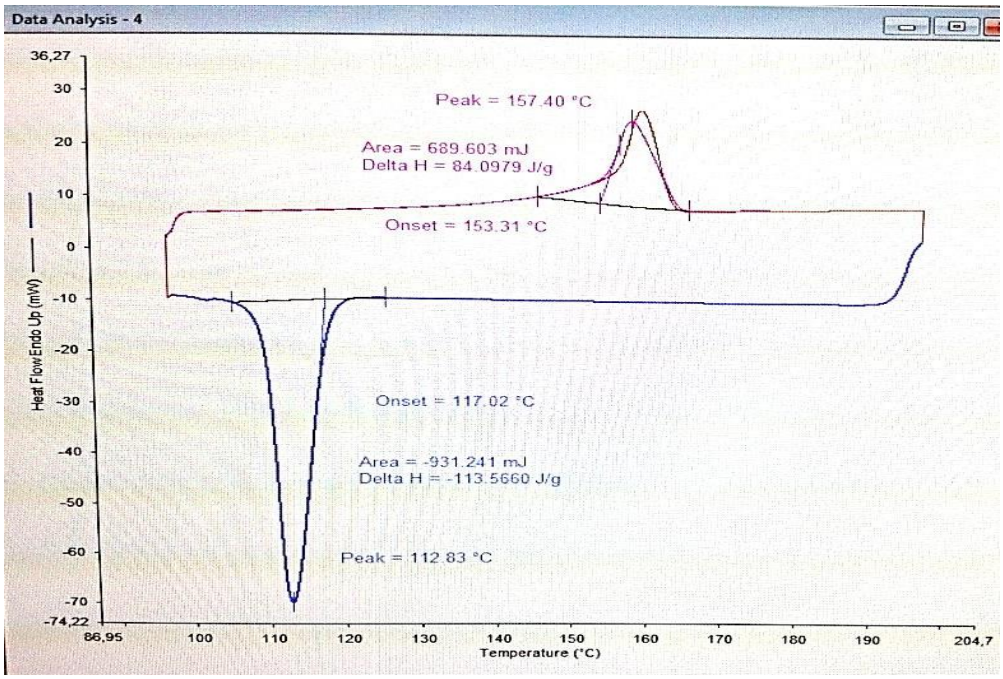


Figure 3. Reresulted DSC thermogram. The first heating-cooling cycle was carried out to erase thermohistory of sample preparation. The result of the second heating was taken for calculation.

Table 3.**. Analysis of Differential scanning calorimetry data**

Differential scanning calorimetry							
Composition #	WO3, %	Pluronic, %	T_m, °C	Peak area (mJ)	ΔH (J/g)	ΔH corrected	Crystallinity, %
1	1		158,13	485	59,87	60,5	28,9
2	2		158,14	650,6	70,72	72,2	34,2
3	4		156,57	660	91,66	95,5	44,3
4	**1**	**1**	157,4	664,3	81,01	82,7	39,1
5	**2**	**2**	156,36	474,07	83,17	86,6	40,2
6	0	1	159,79	556,49	83,06	83,9	40,1
7	0	2	156,19	468,7	83,7	85,4	40,4
8	0	4	159,7	538,2	78	81,3	37,7
9	**4**	**4**	157,5	642,5	72,19	78,5	34,9
0	**0**	**0**	**157,35**	**936**	**96,49**	**96,5**	**46,6**

Table 3 shows DSC data of samples compositions PP / WO_3/ Pluronic at various contents WO_3 and Pluronic. Important feature is crystallinity. Interestingly addition of tungsten oxide has influence the crystallinity, hence morphology: it leads to increases in crystallinity of the matrix approximately linearly. But Pluronic inhibits the effect of WO_3, as shown on the Figure 4.

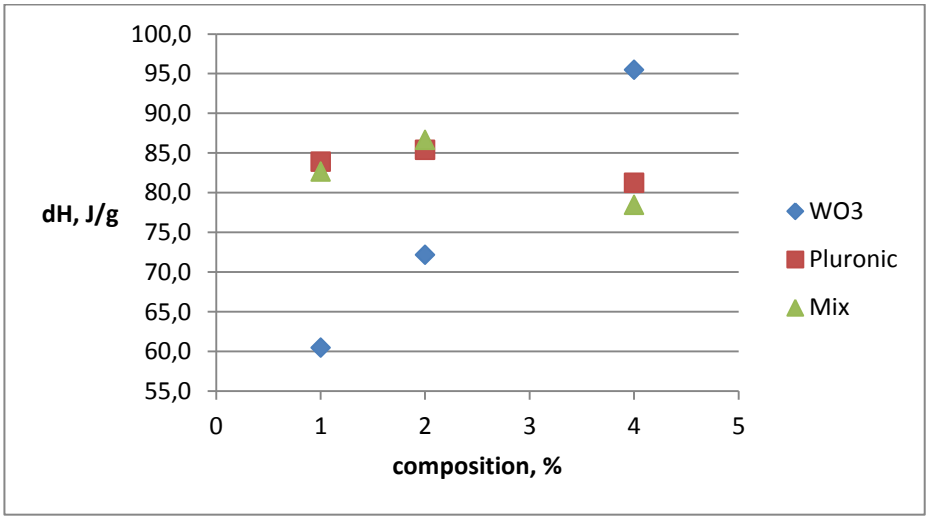


Figure 4. Melting enthalpy as the function of wt% of individual components.

Pluronic does not result in any significant enthalpy change irrespective of its concentration in polypropylene. However the mixture of both WO_3 and Pluronic yields the most interesting results. Instead of having an intermediate enthalpy value for the mixtures containing equal amounts of WO3 and Pluronic we observe that compounds' enthalpy is exactly equal to that of the pluronic-PP compound. In a way the influence of WO_3 on polypropylene crystallinity is removed by Pluronic!

We can see no significant influence the composition on the melting points T_m of samples. Characteristic peak of pure PP 157.3 °C barely shifts by 1-2 °C. But at the low content (<5%) the changes can be very minor. To study this process better to use composites with >10% of fillers. Usually fillers play a role of structure builder. Modifying effect of WO_3 may be associated with changes in the supramolecular structure of the polymer.

2.2.2. Contact angle measurement

Contact angle ***θ*** is a quantitative measure of the wetting of a solid by a liquid. This analysis involves the interfacial free energies between the liquid, gas and solid phases and the contact angles were estimated using by Laplase-Young's equation:

$$\gamma(L) * cos\theta = \gamma(S) - \gamma(SL)$$

a) b)

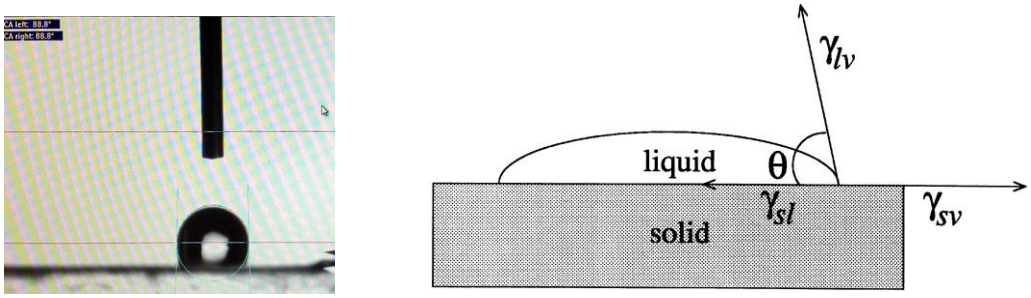


Figure 5. Snapshots of water droplet on the layer formed by water and polypropylene (a). Contact angle formed by a liquid at the three phase boundary where a liquid, gas and solid intersect (b).

Deionized water was used for contact angle measurements. Static (constant drop volume) and advanced dynamic (drop-growing) contact angles were measured with optical tensiometry by the sessile drop method using a conventional drop shape analysis technique equipped with a high-speed video camera (CSA20, Hamburg, Germany).

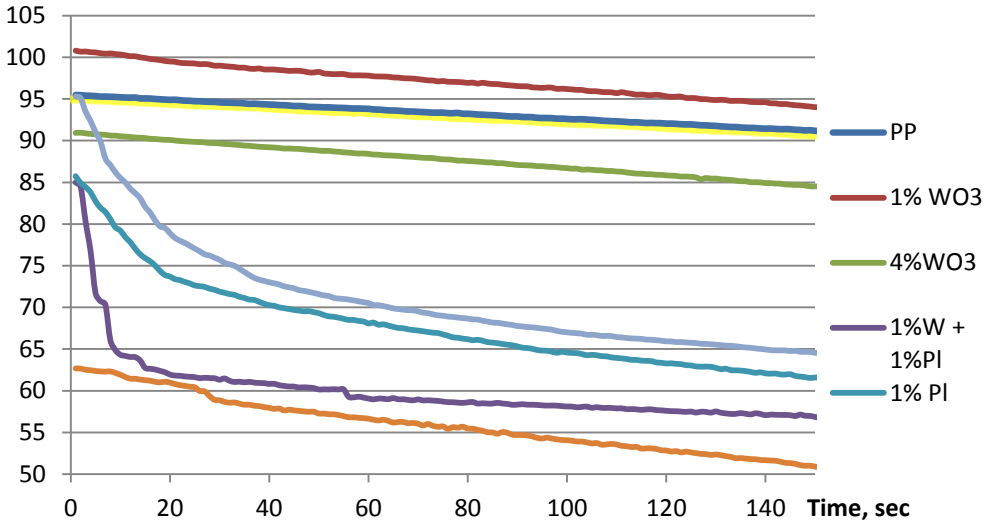
To obtain reliable contact angle data, extreme care in specimen preparation and storage and experimental proceedings are important. Contaminant molecules and particles readily adsorb spontaneously onto any surface, and therefore the contact angle data often may characterize surface properties of the principal contaminants rather than of the material in study.

Technical experiment

Liquid droplets were dropped carefully onto the sample surface, and the average value of 5 measurements, made at different positions of the same sample, was adopted as the average values of contact angles of water/substrate. Static angles were measured at the constant drop volume 2 μl. Advanced angles were measured by supplying the

water into the drop at constant velocity. (0.1 µl/sec), starting from 2 µl. When equilibrium established data from 20 till 50 seconds were taken for analysis. The error of the mean contact angle values, calculated as the standard deviation, did not exceed 1-2 deg. All measurements of contact angle were carried out at 22±1 °C and constant relative humidity.

a) Static contact angle in time:



b) Advanced contact angle in time:

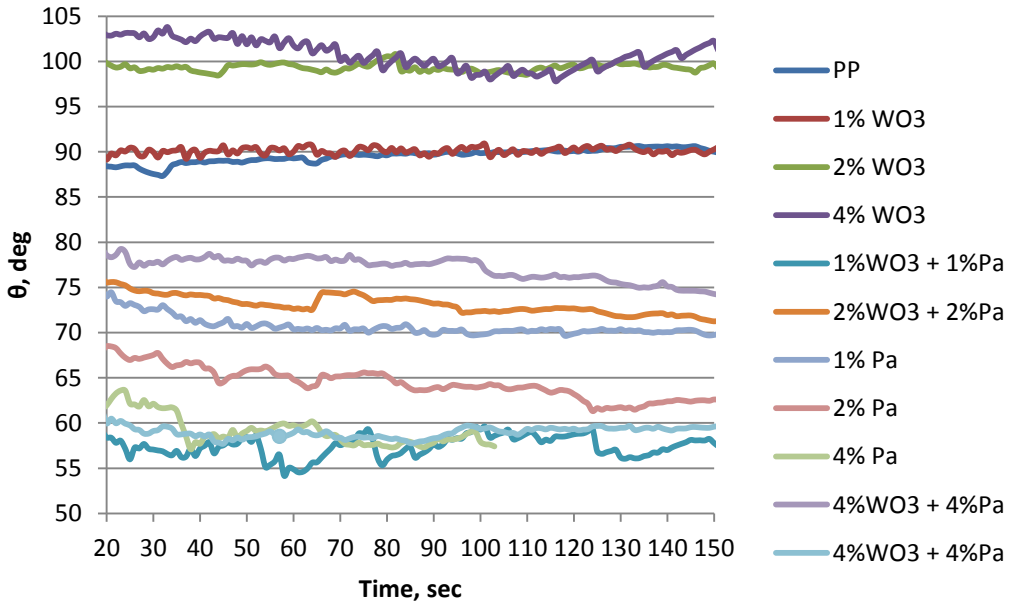
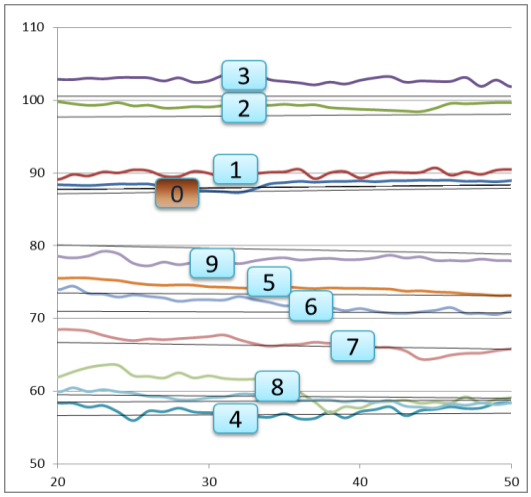


Figure 6. Wetting properties of polypropylene modified with WO3, Pluronic and the mixture of both. Static angle of 2 µl water drops (lower upper panel) on the samples and advancing (0.1 µl/sec) water contact angle (lower panel).

In view of the difference between static and low-rate dynamic contact angles, it seems that the surface roughness and drop relaxation take place.

a)



b)

№	WO3 %	Pl.ac. %	adv.θ
3	4	-	100,5
2	2	-	97,5
1	1	-	87,3
0	-	-	86,7
9	4	4	80,9
5	2	2	73,8
6	-	1	71,2
7	-	2	67,3
8	-	4	59,8
4	1	1	56,4

c)

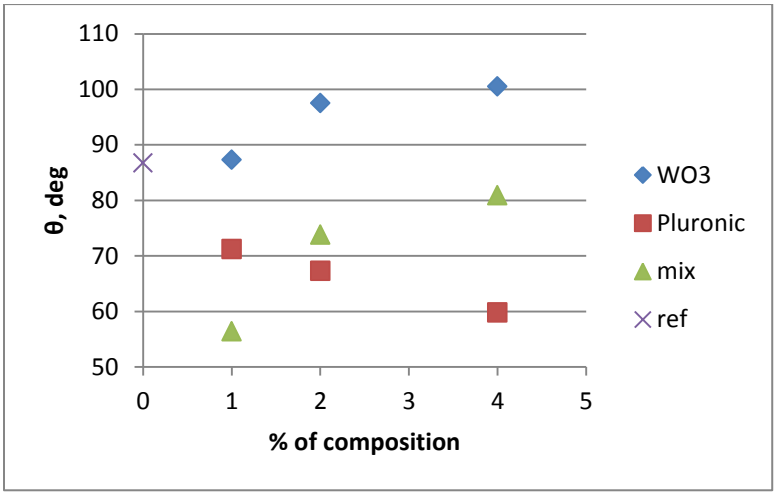


Figure 7. Experimental results representation:

(a) Dynamic contact angle representated in time 20>t>50 sec; (b) Mean contact angles for the different treatments; (c) Contact angle versus sample composition.

Conclusion.

There is a clear effect of given additives at least on the advancing angle:

$$\Theta(WO3)>\theta(PP)>\theta(mix)>\theta(Pluronic)$$

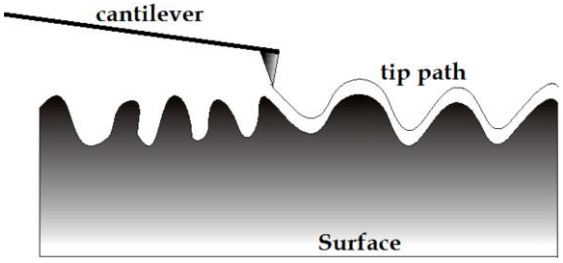
A subsequent removal of tungsten oxide reduces the average surface hydrophobicity, and primarily further increases the roughness (Figure 7). On the other

hand higher wetting agent content results in more hydrophilic surface. The mix of two components gives the average effect, but more hydrophilic then initial polypropylene. The result of the mixture number 4 (1%:1%) was not stable and clear.

2.2.3. Atomic Force Microscopy

Surface texture is an important issue when the main interest is to understand the nature of material surfaces and it plays an important role in the functional performance of many polymeric components.

The AFM provides a 3D profile on a nanoscale, by measuring forces between a probe and a flexible cantilever at very short distance (0.2-10nm). The AFM tip gently interacts with the surface and records the small force between the probe and the surface (Figure 8).



This force can be described using Hooke`s law:

$$F = kx$$

Figure 8. Contact Mode (adopted from http://www.intechopen.com)

a) Surface texture: Roughness

The polypropylene samples of different composition were examined by using environmental scanning force microscopy The EasyScan 2 AFM (Nanosurf, Switzerland).

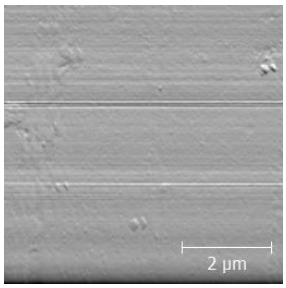
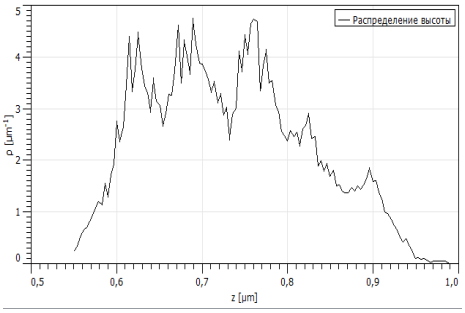
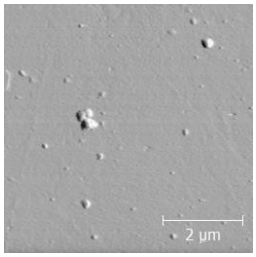
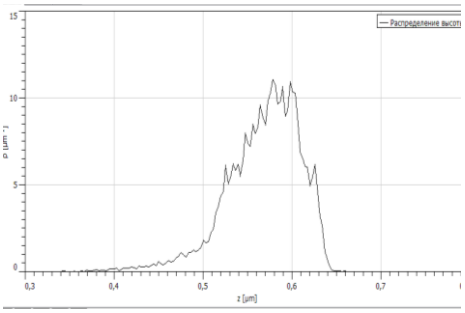
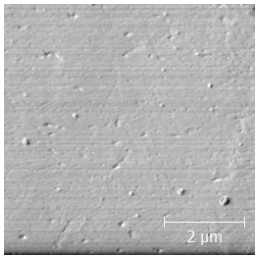
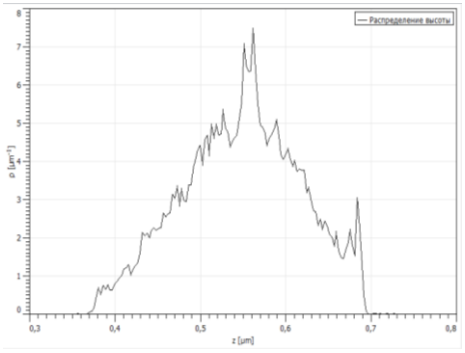
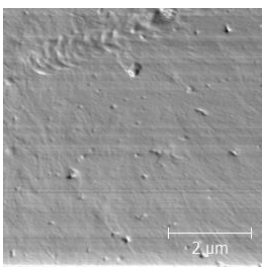
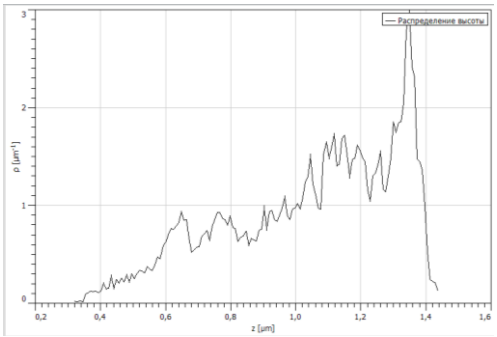
Si-tips ACLA, R<10 nm*, f:* 145-230 kHz.

Protocol.

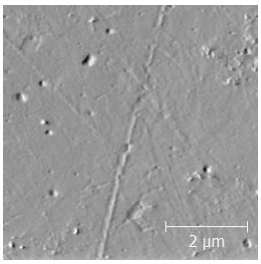
The roughness characteristics were obtained from 6x6 μm scale images by the EasyScan 2 AFM in tapping mode. The resolution of each image taken was 256x256 lines. The average value of dimension was calculated from Gwiddion software.

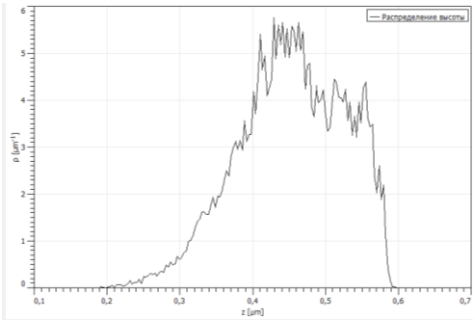
The samples were prepared by melting polymer compositions between two glasses to ensure flat specimen surface and to allow comparison between all compositions.

Results of measurements of surface roughness by atomic force microscopy are represented on the Fig.7 below.

Sample image	Histogramm of hight	Av. height.
0		
		0.75 µm
1		
		0.57 µm
2		
		0.55 µm
3		
		1 µm

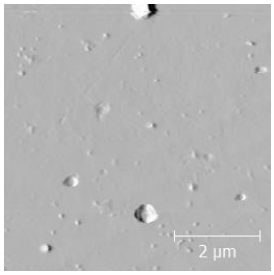
4

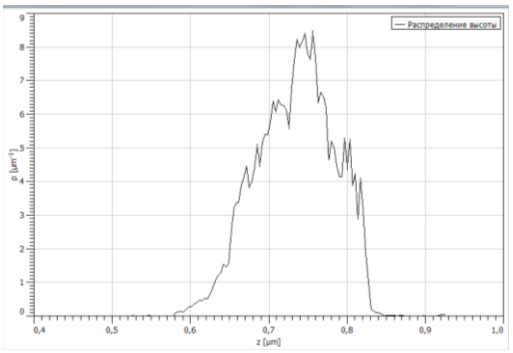




0.45 µm

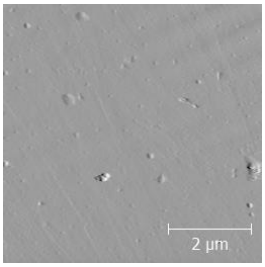
5

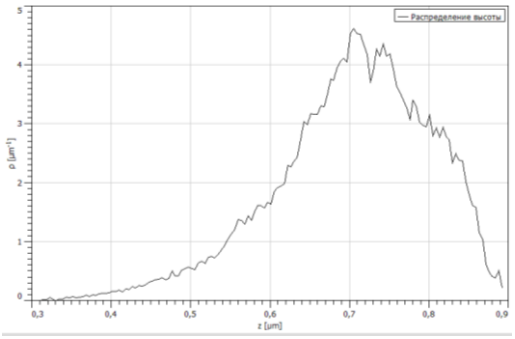




0.74 µm

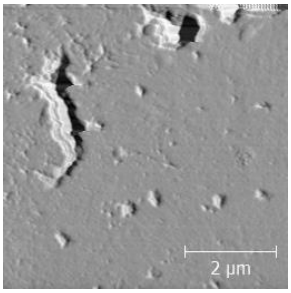
6

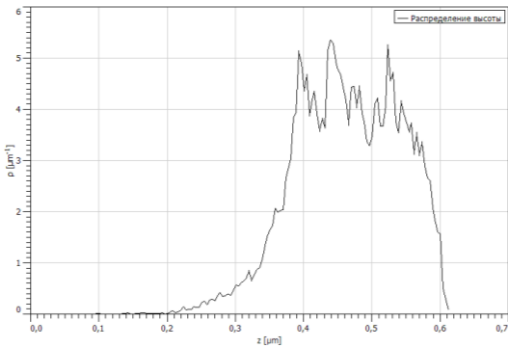




0.74 µm

7





0.46 µm

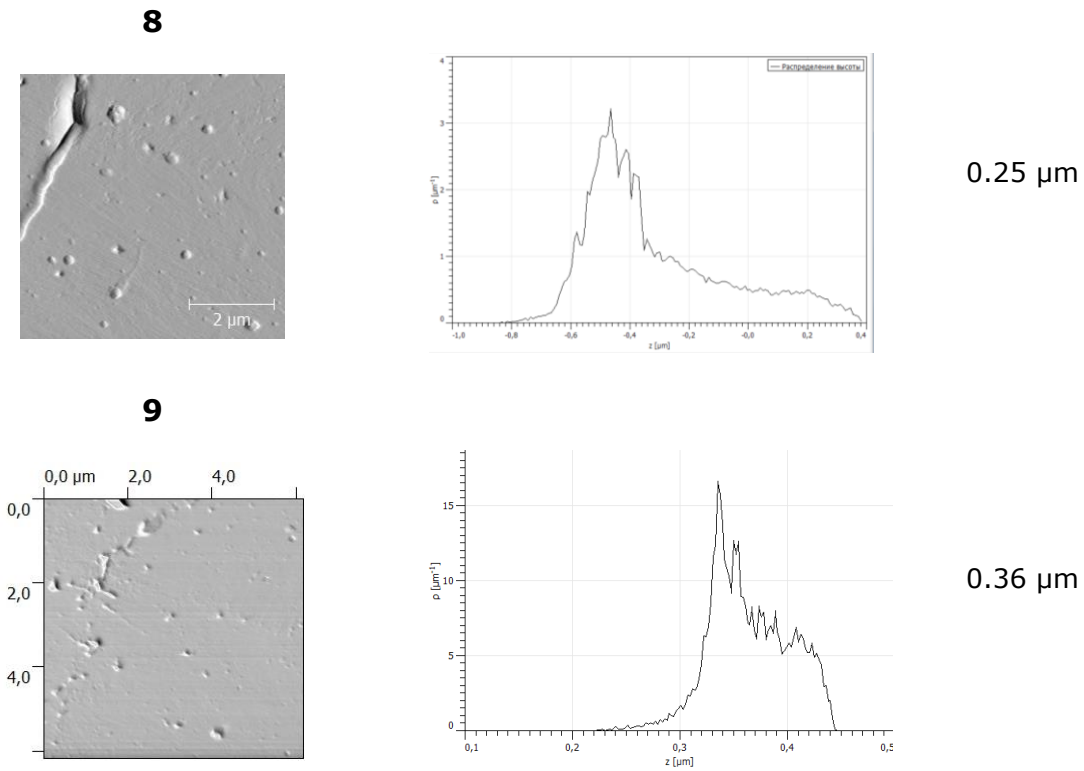


Figure 9. AFM images of PP samples and average roughness (profile amplitude) calculated from AFM

The study gave similar topography of all samples. Usually roughness increases if the scan is taken over a larger area [25]. At the same time one may observe the influence of components on the topography (**Figure 10**).

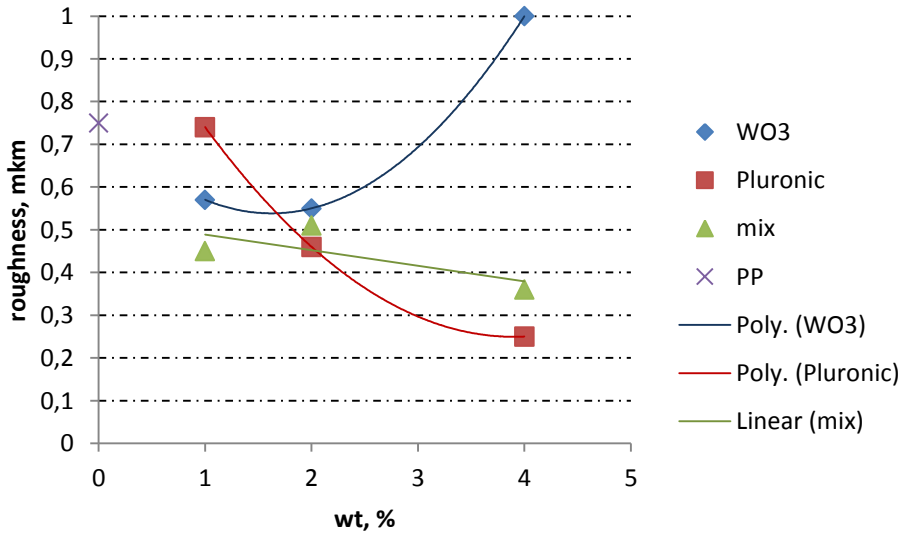


Figure 10. The influence of components on the surface roughness.

Tungsten oxide particles make the surface rougher, wetting agent gives smoothness. The mixture of the two yields no significant roughness change with increase in components concentration.

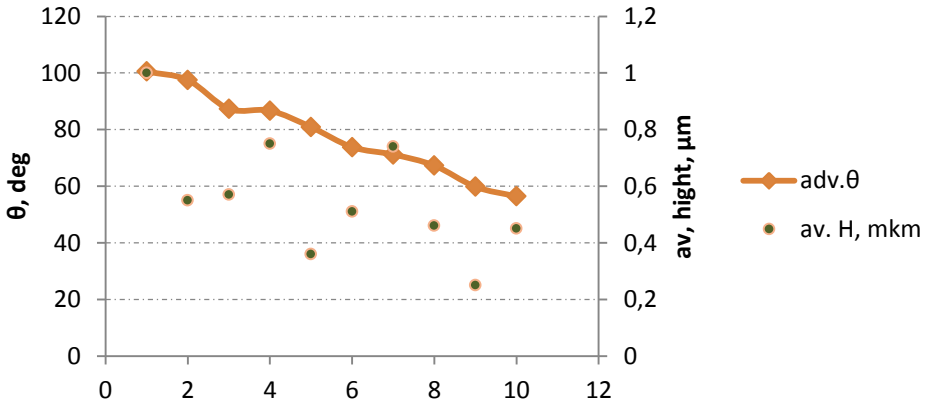


Figure 11. Decreasing of contact angle compare to the surface roughness.

There is some minor trend: less roughness – less hydrophobicity. Seems to be no clear correlation because of broad values deviation. It may be a question of statistics and more detailed roughness analysis. At the same time, this may be due to the simultaneous independent effect of composition both on hydrophilicity and roughness of the surface.

b) Material characteristics: Force Spectroscopy

The deflection of the cantilever is directly proportional to the tip – sample interaction force. Tip deflection was measured in contact mode and mechanical properties of the surface were determined (see Table 4).

Table 4**. Coefficients calculated from the experimental data.**

Composition #	WO3, %	Pluronic, %	K
1	1	0	1 544 059,39
2	2	0	1 778 434,37
3	4	0	1 830 085,35
4	1	1	1 313 685,33
5	2	2	1 382 563,64
6	0	1	1 698 393,32
7	0	2	1 625 866,86
8	0	4	1 781 859,23
9	4	4	1 494 083,33
0	**0**	**0**	1 627 012,61

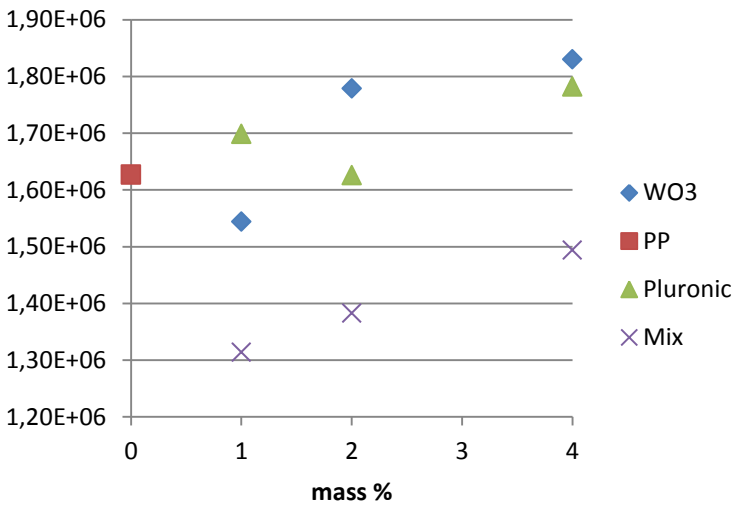


Figure 12. Compliance coefficients as a function of polymer compositions.

Tungsten oxide again gives the increasing rigidity of the samples. And Pluronic has no significant influence. But we observe the synergetic effect of the mixture: the elastic modulus of mixture is less than modulus of each of components and increases linearly with the oxide content increasing.

The content of additives in polymer of our samples is low. Hence in our case observed effect may be consequence of wettability rather than rigidity, because of thin layer of water on the surface. And one may observe tip-surface interaction due to capillary forces instead of Wan-der-Waals forces. Chosen method is not reliable for characterization of whole surface since the scanning is measured only in randomly selected points. It's better to use alternative technics where one can carry out the scanning of the whole surface at nano-scale.

2.2.4. Bacteriological analysis

To described antimicrobial activity of polymeric surface and understand the polymer behavior in wet hospital environment the bacteriological analysis was developed.

Also the purpose was to predict the influence of composition on the working mechanism. The influence of surface topology on antimicrobial activity can be neglected because all samples were prepared under identical conditions. Based on the above shown results it can be assumed to say about similar roughness.

Sample preparation:

The mixing of low-density PP with additives was prepared by extrusion. 0.5 g of the sample was re-melted in owen at 200 °C during 5 minutes in between of two glasses and cooled at the room temperature. Chips with approximately size 5x5 mm were cut off from the resulting flat specimen and used for measurements.

It is very important that surface topology of all samples is very similar. Then we can neglect surface properties and assume that only composition influences antimicrobial properties. If the surface structure is different the influence of roughness of the surfaces will be unknown factor in the result of bacterial counting.

Mediums and solutions

1. Bacterial suspension

E. coli (strain BL21DE3) are maintained in glycerol at -18°C. For E.coli growth 10µl of frozen culture is inoculate in 1 ml of LB medium and incubated overnight (18h) at 37 °C in shaker (use eppendorf for 1.5 ml and shaker for it). Cell concentration should be around 10^{8-9} CFU/ml. A working culture is prepared by dilution till ~$5*10^6$ (10 µl of obtained cells in 1 ml of fresh LB).

Cell concentration can be estimated with optical spectroscopy at 580 nm, D_{580}=0.10-0.15 will be fine. For 10^8 CFU optical density D_{580}=3

2. Cell culture medium LB (for 1L)

Yeast extract	5 g	Mix together in a flask (better use bottle 250-500ml with screw cap for storage). Autoclave 10 min (1.5 bar, 120°C) for sterilization*
NaCl	10 g	
Casein	10 g	
Kanamycin (stock solution 30mg/ml)	1 ml (to the final concentration 30 mkg/ml)	Add after autoclaving and cooling till 50°C at room temp.

* expenditure of LP for 1 day experiment (10 samples) of surface properties (drop method) is about 5 ml usually.

3. Phosphate buffer solution PBS (pH 7.2 for 500 mL)

	Mr(g/mol)	**m (g)**	**M (mol/l)**
NaCl	58.44	4.082	0.1397 M
$Na_2HPO_4*2H_2O$	177.99	0.89	10 mM
KH_2PO_4	136.09	0.122	1.8 mM

- No pH adjustment should be necessary in this formula (but it's better to check if possible)
- If exchange one salt to another hydrophosphate, dissolve in 400 ml of deionized water, adjust pH with HCl or NaOH to 7.2, fill up to 500 ml.
- Sterilize by filtration or autoclaving

4. Agar plate medium

Yeast extract	5 g	Mix together in a flask (better use 2x500ml*). Autoclave 10 min (1.5 bar, 120°C) for sterilization
NaCl	10 g	
Casein	10 g	
agar	20 g	
Kanamycin (stock solution 30mg/ml)	1 ml (to 30 mkg/ml)	Add after autoclaving and cooling till 50-55°C at room temp. Be aware of over-cooling - agar solidifies!

*500 ml of LB-agar should be enough to prepare 22-25 petri dishes. 1 Petri is used for 1 sample (3 points).

After kanamycin adding agar-medium has to be distributed to Petri dishes in sterile conditions.

Attachment of cells

Adherence of Escherichia coli (BL21) on PP composition chips was evaluated.

The chips were first immersed in ethanol for 1 h and let them dry on air (or rinse with sterile distilled water) (or if it's possible, sterilization can be done at 120°C for 10-15 min or UV exposure for 1 h). The sterile clean chips were put to clean plate with cover (to avoid contamination and dust from air, and LB drop evaporation). At the zero moment 20 µl of working bacterial suspension (10^6 CFU/ml) was drop on each chip, in appliance with

1 chip = 1 drop = 1 time point.

Incubate in chamber at room conditions. The number of adhered cells on the different surfaces is evaluated after 2, 4 and 6 h of contact time. The results are expressed in CFU/ml (or in CFU/cm^2 of surface).

For each period of time one chip of each composition is taken (**Figure 13**) and rinsed well twice in sterile PBS to wash out unattached cells together with culture medium.

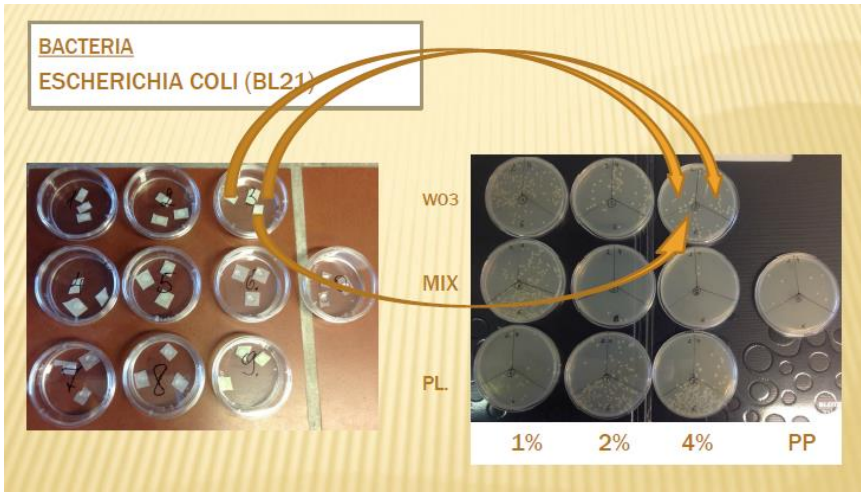


Figure 13. Incubation of bacteria on polymer surface for 2, 4 and 6 h of contact time.

After that each chip is placed into sterile tube (eppendorf) containing 1 ml of sterile PBS. Then tubes containing a chip, is swirled with a vortex mixer for 1 min at

2000 min^{-1}. To release all adhered cells from the surface also possible to add Ultrasound bath for 3 min and vortex again.

Enumeration of adhered cells (plate counting)

10 µl of PBS from each eppendorf is pipetted to the agar plate for bacterial grow (1 agar plate = 1 sample = 3 contact time points) and spread the drop on agar carefully. After all 3 points of each samples are sieving, agar plates incubate at 37°C overnight. Each bacterial colony forming units (CFU) – individual alive bacteria - will reproduce one visible colony into agar. Colonies are counted and compare with corresponding dilution factor.

For the first experiment it also will be good to have positive and negative controls, to be sure that one avoids contamination from environment or bacteria don't die due to other reasons. Also microbial cells can be killed in solution, which thus supports by control the bacterial growth in the drop LB medium on the surface, as shown on the Figure 14.

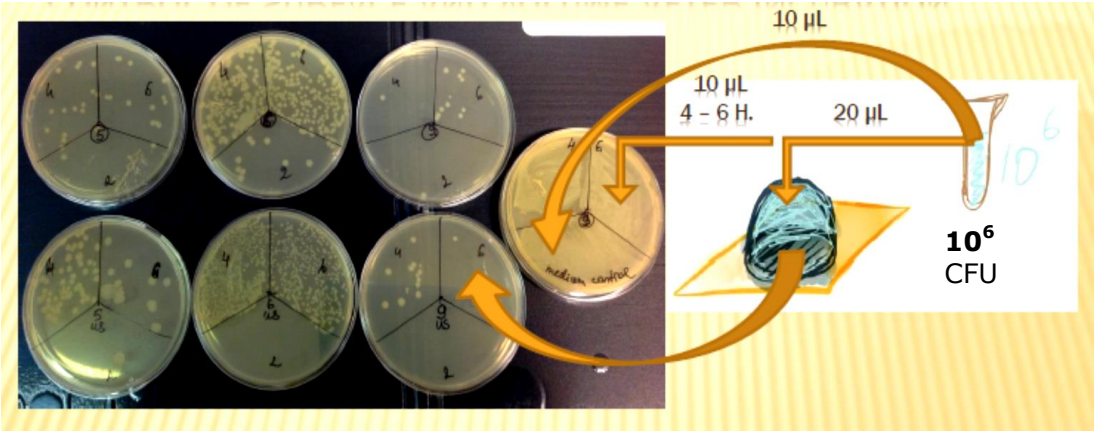


Figure 14. This experiment shows no bactericidal activity of composition in the bacterial suspension in contact with polymer surface. Number of bacteria cells counted from 10 µl of the medium cultivated 6h on the sample №9 is the same as in initial medium, cultivated in flask during the same time. However the same sample composition №9 prevents the bacteria cells attachment to the polymer surface (bacteria survive, but not form a monolayer).

One of the resulting bacterial grow is shown on the Figure 15. Results of 5 experiments were statistically calculated, in this way we can observe the effect of the composition of the polymer on the bacterial growth on its surface (Figure 16).

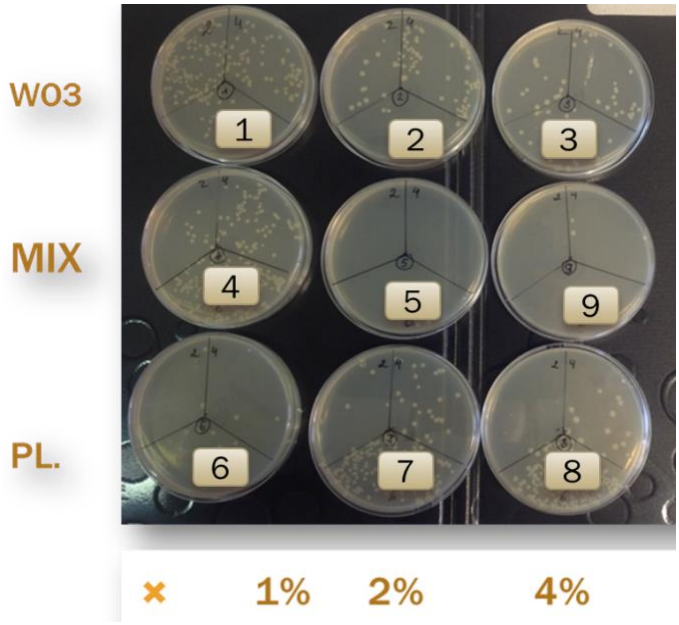


Figure 15. Bacteria colonies counting

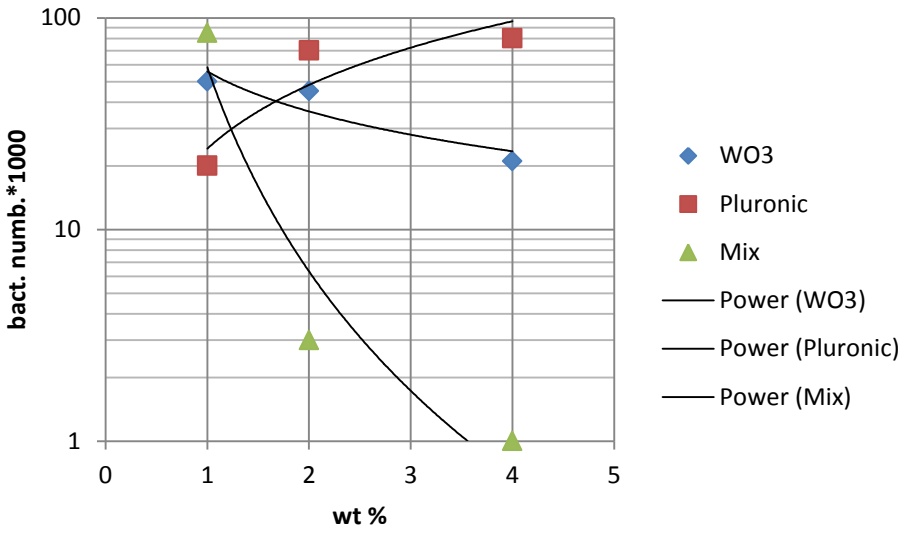


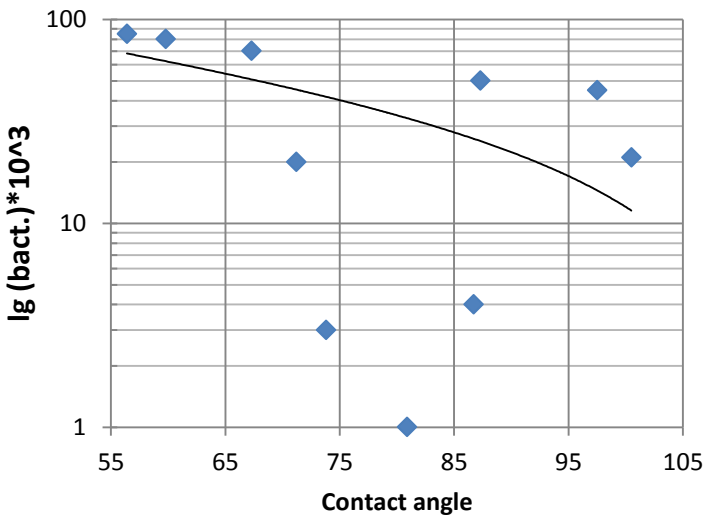
Figure 16. . Number of bacteria colonies vs. composition

The chart on the Figure 16 shows that tungsten oxide has activity, but Pluronic has not. Interestingly the mixture of oxide with Pluronic gives significantly higher antimicrobial activity. The latter increases with increasing amount of additives in the mixture.

Figure 17 also demonstrates the minimum of cells on the surface dependence on hydrophobicity.

Probe №	WO3 %	Pl.ac. %	meas. θ
3 (-)	4	-	100,5
2 (-)	2	-	97,5
1 (-)	1	-	87,3
0	-	-	86,7
9 (+)	4	4	80,9
5 (+)	2	2	73,8
6 (+/-)	-	1	71,2
7 (-)	-	2	67,3
8 (-)	-	4	59,8
4 (+/-)	1	1	56,4

a)



b)

Figure 17. Measured contact angle (a). Number of bacteria cells adhered on different surfaces as a function of contact angle and composition (b).

A short qualitative examination of the experimental data depicts the correlation between the number of bacteria attached to sample's surface and contact angles of the surface:

First, one can observe known dependence between less bacterial attachment and high surface hydrophobicity – lotus effect.

Second, unexpected minimum of bacteria attachment in the range of contact angles in between 73-80 degrees, that corresponds to composition numbers 5 and 9 (+).

For compositions № 6 and 4 results are not stable (+/-).

There is no any bactericidal effect of composition.

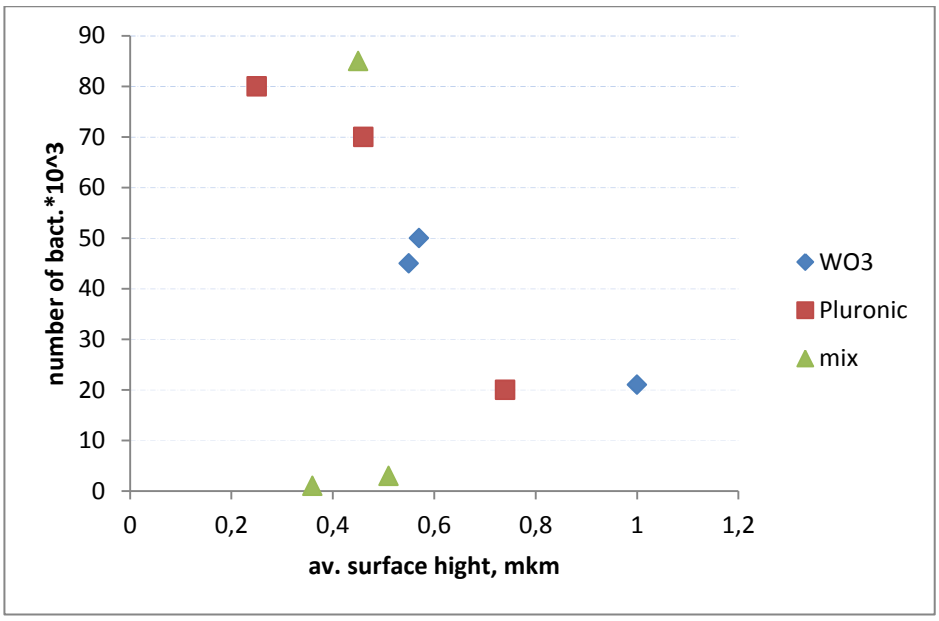


Figure 18. Number of bacteria adhered cells as a function of roughness of different surfaces. There no visible influence of surface roughness on antimicrobial activity. Presented data support earlier statement that topology of surfaces can be neglected.

As we had demonstrated no influence of surface topology, therefor in our case resulting influence both composition and surface tension take place.

3. Conclusion

Simple and quick method to measure antimicrobial properties of surfaces was developed. The influence of polymer composition on the surface structure and antimicrobial activity of the surface was investigated (Table 5). Synergistic effect between tungsten oxide and Pluronic was found.

Table 5. **Summary of the effects. Correlation between compositions and observed features.**

composition	*Structure*	*Surface properties*	*Contact angle*	*Bacteria attachment*
Tungsten oxide(VI)	Lineally increases crystallinity	Lineally increases rigidity	hydrophobicity increase	Some prevention
Pluronic 8100	No effect	No significant effect	Hydrophilicity increase	No effect
Mixture	No effect (Pluronic removes WO3 effect)	Synergistic effect	intermediate	Strong prevention

- The method of sample preparation was developed to control topology of the surface: influence of surface roughness on microscopic scale can be neglected. But nanostructure can be important in that case.
- There is definitely some interaction between oxide and Pluronic on polymer surface and bulk properties. This interaction causes also the enhancement of the antimicrobial effect of the WO_3 in mixture with Pluronic compared to the same amount of pure oxide in polypropylene.
- It was shown that mixture 2% and 4t% of components clearly prevent bacteria attachment on the surface, but the result of 1% mixture was not so obvious. That activity correlates to the contact angle in the range between 73-80°. Also would be interesting to check influence of local pH of drops, it can change the contact angle.
- Easy method to control antibacterial activity of surfaces was developed: toxic influence of composition on the bacteria not observed. Number of attached bacteria is not decreasing during the experiment. They also were not killed in medium on the surface. But surface keeps clean during first period of time. It

seems that the main antibacterial effect comes out at the step of bacteria adhesion that protects the surface against biofilm formation:

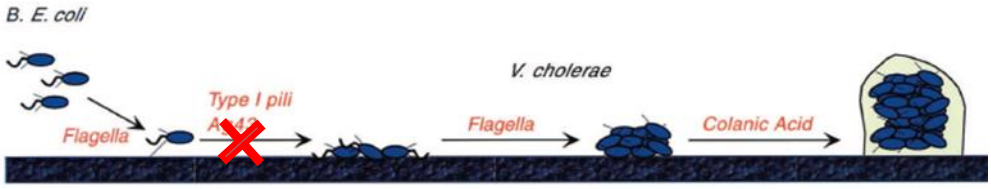


Figure 19. Proposed working concept of bioactivity of polypropylene composition with Pluronic and tungsten oxide (VI) (in experiment with E.coli).

For that type of systems investigated in this work no steric, electrostatic or hydrophobic surface repulsion is expected to prevent bacteria attachment. Therefor this study opens the questions for future development and optimization of antimicrobial surfaces with tungsten oxide (VI). It seems desirable to understand why the combination of different parameters is effective. Investigations should focus on better understanding surface properties on the mechanism of bacteria attachment. We believe that in our case multiple factors might have contributed to the observed effect, not only properties of tungsten oxide are involved into the process.

- Physical-chemical aspect: If there is some multifunctional effect between free surface energy and acidity of surface caused by amphoteric properties of oxide? Two separate low-effective intermediate properties can make a strong combined effect. Local pH as well as surface energy can influence on contact angle of water. Nonpolar component of surface free energy also have to be studied.
- Structural aspect: How Pluronic can improve properties of tungsten oxide in the PP? It might be improvement of proton migration and releasing to the surface in wet conditions. Also one needs to study the surface at nonstructural level.
- Biological factor: different kinds of bacteria should be examined.

It is interesting to control an effective combination of factors to prevent bacteria attachment to the surface and maturation of bacterial multilayer. Even if these factors combination not possess a bacteriostatic or bactericidal activity, surface still stay easy-to-clean by usual antimicrobial treatment. Also the advantage is non-toxicity of such material for human and environment: no releasing of harmful substance or excess of bactericidal liquids is necessary.

It enables researchers to design a cheap and effective surface without it losing its antimicrobial activity that prevent biofilm formation during a long time by safe and simple way.

References

[1] Flores GE, Bates ST, Knights D, Lauber CL, Stombaugh J, et al. (2011) Microbial Biogeography of Public Restroom Surfaces. PLoS ONE 6(11)

[2] [electronic resource] http://www.nhs.uk/Conditions/MRSA/Pages/ Introduction.aspx

[3] Dancer S.J., Importance of the environment in meticillin-resistant Staphylococcus aureus acquisition: the case for hospital cleaning, Lancet Infectious Diseases, 2008, 8(2), 101–113.

[4] Amann R.I., Ludwig W., Schleifer K.-H. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Rev. 59:143–169.

[5] Davey M. E., George A. O'toole. Microbial Biofilms: from Ecology to Molecular Genetics. Microbiol. Mol. Biol. Rev. 2000, 64(4):847.

[6] Karatan E., Watnick P. Materials That Build and Break Bacterial Signals, Regulatory Networks, and biofilms. Microbiol. Mol. Biol. Rev. 2009, 73(2)

[7] Wang, X., J. F. Preston III, and T. Romeo. 2004. The pgaABCD locus of Escherichia coli promotes the synthesis of a polysaccharide adhesin required for biofilm formation. J. Bacteriol. 186:2724–2734.

[8] Page K., Wilson M., Ivan P. Parkin. Antimicrobial surfaces and their potential in reducing the role of the inanimate environment in the incidence of hospital-acquired infections. J. Mater. Chem., 2009, 19, 3819–3831

[9] Roosjen A., Kaper H. J., Henny C. van der Mei, Norde W., Henk J. Busscher. Inhibition of adhesion of yeasts and bacteria by poly(ethylene oxide)-brushes on glass in a parallel plate flow chamber. Microbiology November 2003 vol. 149 no. 11 3239-3246

[10] Okada A., Nikaido T., Ikeda M., Okada K., Yamauchi J., Foxton R. M., Sawada H., J. Tagami, and K. Matin, Inhibition of Biofilm Formation using Newly Developed Coating Materials with Self-cleaning Properties. Dental Materials Journal, 2008. 27: p. 256–272.

[11] Lin Wu, Jacek Jasinski, Sitaraman Krishnan, Carboxybetaine, sulfobetaine, and cationic block copolymer coatings: A comparison of the surface properties and antibiofouling behavior. Journal of Applied Polymer Science, 2012. Vol. 124, Issue 3, p. 2154–2170

[12] Epple M., Chernousova S. Silver as Antibacterial Agent: Ion, Nanoparticle, and Metal. Angew. Chem. Int. Ed. 2013, 52, 1636 – 1653

[13] [electronic resource] http://www.antimicrobialcopper.com/uk/scientific-proof.aspx

[14] Tauhardt L., Kempe K., Knop K., Altuntas E., Dr. M. Jager, Prof. U. S. Schubert. Linear Polyethyleneimine: Optimized Synthesis and Characterization – On the Way to ''Pharmagrade'' Batches. Macromol. Chem. Phys. 2011, 212, 1918–1924

[15] Anthony Gordon Fane "Nanofiltration: Principles and Applications" Elsevier Advanced Technology, 2005

[16] [electronic resource] http://www.freedoniagroup.com/industry-study/3043/disinfectant-antimicrobial-chemicals.htm

[17] [electronic resource] Biocides in plastics / D. Nichols. Publisher: Shawbury, U.K.: Rapra Technology Ltd., 2004.

[18] [electronic resource] http://www.aatcc.org/testing/index.htm

[19] . Kugela A. J, . Ebertb S. M, Stafslienb S. J., Hevusa I., . Kohuta A, Voronova A., Chisholm B.J. Synthesis and characterization of novel antimicrobial polymers containing pendent triclosan moieties. Reactive and Functional Polymers, 2012. Vol. 72, p. 69–76

[20] Epstein AK, Hochbaum A I, Kim P., Aizenberg J. Control of bacterial biofilm growth on surfaces by nanostructural mechanics and geometry, Nanotechnology 22 (2011) 494007

[21] Lichter J et al 2008 Substrata mechanical stiffness can regulate adhesion of viable bacteria Biomacromolecules 9 1571–8

[22] Kalyon BD, Olgun U. Antibacterial efficacy of triclosan-incorporated polymers. American Journal of Infection Control. 2001 Apr;29(2):124-5.

[23] Vargas-Reus MA, Memarzadeh K, Huang J, Ren GG, Allaker RP. Antimicrobial activity of nanoparticulate metal oxides against peri-implantitis pathogens. Int J Antimicrob Agents. 2012 Aug;40(2):135-9.

[24] Zollfrank, C., Gutbrod, K., Wechsler, P., & Guggenbichler, J. P. (2012). Antimicrobial activity of transition metal acid MoO3 prevents microbial growth on material surfaces. Materials Science and Engineering: C, 32(1), 47–54.

[25] [electronic resource] Applied Surface Science 133 _1998. 293–297