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Antimicrobial Activity of Monolayer and Multilayer Films Containing Polyhexamethylene Guanidine Sulphanilate

DOI: 10.5604/01.3001.0011.5742

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Abstract
The aim of this study was to determine the antimicrobial properties of multilayer films containing a PHMG sulphanilate (polyhexamethylene guanidine sulphanilate). Three types of films were selected: monolayer and three-layer films (both containing biocide) and market foil. The antibacterial activity of polyethylene film with PHMG sulphanilate was verified based on the guidelines of ISO 22196: 2007 (E): Plastics – Measurement of antibacterial activity on plastic surfaces. The antimicrobial efficacy of the monolayer film against Escherichia coli and Staphylococcus aureus was very good, equalling 6.25 log (100%) and 6.02 log (100%), respectively. It means that a total reduction in bacteria on the surface tested was achieved. The antimicrobial efficacy of the three-layer film against Escherichia coli was satisfactory and equaled 1.32 log (95.2%). The antimicrobial efficacy of this film against Staphylococcus aureus was very good and equaled 6.02 log (100%). The antifungal activity of polyethylene film with PHMG sulphanilate was verified based on the guidelines of ASTM G21 – 96: Standard practice for determining the resistance of synthetic polymeric materials to fungi. The fungal growth of Aspergillus niger, Chaetomium globosum and Trichoderma viride on the monolayer and three layer films was also inhibited, which means that the biocide in the films also exhibits antifungal activities. For the market foil, poor antibacterial efficacy against the bacteria and no antifungal activity against the fungi tested was observed.

Key words: *polyhexamethylene guanidine sulphanilate, biocide, antibacterial activity, antifungal activity, protection against microorganisms, multi-layer films.*

Introduction

Since 1966, polyethylene has been one of the most widely commercially produced plastics in the world, due to its relatively easy processing, low price, functionality, biocompatibility and aesthetics [1]. This material is primarily used for film production. The most commonly used processing technique is extrusion blow moulding. Polyethylenes of different densities can be processed separately or mixed together. The stability of a blown sleeve may vary depending on the type of material and additives used. During this process, it is possible to modify the film using a variety of substances. In this way films with different physicochemical and biological properties are obtained [2]. Foil additives that are used include UV stabilizers, anti-blocking additives, slip and antifogging agents and electrostatic additives [1, 2]. In addition, a range of substances, including natural and synthetic, organic and inorganic (essential oils, bacteriocin, silver particles) can be used for the production of polyethylene films with biocidal properties [3, 4]. Metal oxides and their compounds were also found to be toxic to different types of microbe [5]. It should be noted that a good antimicrobial agent should be effective against a broad spectrum of bacterial and fungal species. In addition, the antimicrobial agent must also exhibit very low toxicity to the environment and consumers [6].

Guanidine-based cationic polymers show great potential for the development of new materials with biocidal properties [4]. Polyhexamethylene guanidine (PHMG) is becoming increasingly popular due to its broad range of antibacterial activity, relatively low toxicity and high stability in soil and aquatic environments [7-10]. The antimicrobial effectiveness of PHMG was first described in the forties, indicating its high activity against bacteria and moulds [9, 11]. The probable mechanism of action is that PHMG diffuses through the cellular membrane and forms a complex with phospholipid molecules of the lipid bilayer. This destabilises the osmotic equilibrium and destructs the cytoplasmic membrane. As a consequence, PHMG causes a loss of membrane function in bacteria or fungi [12-14]. The extent of membrane disruption is shown to increase with an increase in the polymer length. The antibacterial activity of PHMG also depends on the molecular weight and anion composition [15]. Recently it has been found that PHMG has broad *in vitro* antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi, and viruses [16-19].

Currently PHMG and its derivatives with antibacterial and antifungal activity are mainly used as disinfectants and preservatives for human eye infections, the impregnation of fabrics, water treatments, and the disinfection of various solid surfaces [18]. Guanidine derivatives have also been investigated as medical or crop protection agents as well as antiseptics for industrial products, food [15, 19, 20], and for the textiles and cosmetics industry [20]. Interestingly some PHMG derivatives are stable in heat, which makes them ideal additives for polymer materials produced in the melting process, such as melt spinning and injection [20]. Also it has been confirmed that the introduction of biocides into polymer has proved to reduce microbial growth on its surface [4]. In this paper polyhexamethylene guanidine sulphanilate (PHMG sulphanilate) was synthetised and used to produce multilayer polyethylene films with biocidal properties.

The aim of this study was the development of new antimicrobial polyethylene films by their modification with water resistant polymeric biocide PHMG sulphanilate. In addition, the antimicrobial properties of these films against the following bacteria: *Staphylococcus aureus* and *Escherichia coli*, and moulds: *Aspergillus niger, Chaetomium globosum* and *Trichoderma viride* were also determined.

Materials and methods

Materials

Three types of films were selected for the tests: monolayer and three-layer films and market foil. Reference film was used as control sample in all the tests. Also sterilised filter paper (Whatman) was used as a control sample in the antifungal test. The following symbols of the foil samples were used in the tests:

- F1 (monolayer film with 0.8% (wt) PHMG sulphanilate),
- F3 (three-layer film with 0.8% (wt) PHMG sulphanilate),
- FREF (reference film without biocide),
- FM (foil from the market without biocide).

The monolayer, three-layer and reference films were produced by a Polish manufacturing company in accordance with a method described later on. A mixture of polyethylene PE and PHMG sulphanilate (100% powdered active substance with a melting point of t= 155-175 °C) was introduced into a twin screw extruder type BTSK 20/40D (Bühler, Germany). A homogeneous concentrate with a concentration of 20% by weight of the bioactive substance in the mixture was obtained, which was then cooled in air at 25±3 °C and then granulated. The granulate obtained was transferred to a manufacturing company that produced a single-layer foil on a Costruzioni Meccaniche line Luigi BANDERA in a standard way, while a three-layer film was made on a Optimex Coex line from Windmoller & Holscher. In the blow moulding process, a flat film (with/without the PHMG derivative) with a mean total thickness of 0.05 mm was obtained. This process was carried out using a Brabender Plasticorder PLV-151 manufactured by Brabender (Germany). In the case of the three layer film, the biocide was introduced into a single outer layer. The market foil was purchased on the Polish market. It was a polyethylene film generally available on the market, used for freezing food products, and, according to the manufacturer, prevents the multiplication of microorganisms. The polyhexamethylene guanidine sulphanilate (PHMG sulphanilate) tested was synthetised at the Institute of Leather Industry according to patent PL225392 [21]. The synthesis was carried out by adding an aqueous solution of polyhexamethylene guanidine hydrochloride to an aqueous solution of p-aminobenzenesulfonic acid at 20-30 °C and pH 6.5-7 with a dispersant. The authors conducted preliminary tests, which resulted in the selection of the lowest concentration of PHMG sulphanilate (equaled 0.8%) inhibiting the growth of microorganisms.

Microorganisms

The effectiveness of the biocide was tested for two selected strains of bacteria and three strains of moulds. *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were used in the antibacterial activity tests because they are recommended by ISO 22196: 2007 (E): Plastics – Measurement of antibacterial activity on plastic surfaces [22]. Bacterial strains came from the Collection of Industrial Microorganisms at the Institute of Agricultural and Food Biotechnology (Poland). It should be noted that *Staphylococcus aureus* is a Gram-positive, round-shaped bacterium, while *Escherichia coli* is Gram-negative bacteria. *Aspergillus niger* (LOCK 0440), *Chaetomium globosum* (LOCK 0570) and *Trichoderma viride* (LOCK 0476) were used in the antifungal activity tests because they are recommended by ASTM G21 – 96: Standard practice for determining resistance of synthetic polymeric materials to fungi [23]. These moulds came from the Pure Culture Collection at the Institute of Fermentation Technology and Microbiology LOCK (Lodz University of Technology, Poland).

Assessment of antibacterial activity

Bactericidal properties of all the samples of polyethylene films were tested by the Institute of Agricultural and Food Biotechnology (IAFB, Poland) in accordance with standard [22]. 50 mm x 50 mm film samples (F1, F3, FM, FREF) were prepared for the tests. In each test 0.4 ml of a suspension of the test organisms (containing approximately 3.1 x 10^5 CFU/ml of *Escherichia coli* and 3.4 x 10^5 CFU/ml of *Staphylococcus aureus*) was placed on the surface of all the samples. The suspension was held in close contact with the test and control surface using a sterile polyethylene film (squares with a side of 40 mm and thickness 0.05 mm). The control samples (FREF) were inoculated and washed off immediately, each in neutralizer solution, and bacteria counts were determined. The remaining samples (F1, F3, FM, FREF) were incubated at 37 °C for 24 h. After 24 hours the test pieces were washed off and bacteria counts determined. The number of microorganisms was determined using the plate method on a PCA medium (incubation at 37 °C for 24-48h). Each sample type was tested in triplicates. Antibacterial activity (R) was calculated using ***Equation (1)***:

$$R = (U_t - U_0) - (A_t - U_0) = U_t - A_t \quad (1)$$

where: R – is the antibacterial activity; U_0 – is the average of the common logarithm of the number of viable bacteria, in CFU/cm^2, recovered from the control test specimens immediately after inoculation; U_t – is the average of the common logarithm of the number of viable bacteria, in CFU/cm^2, recovered from the control test specimens after 24 h; A_t – is the average of the common logarithm of the number of viable bacteria, in CFU/cm^2, recovered from the teste specimens after 24 h.

As antimicrobial efficacy criteria is not defined in the ISO standard, the IAFB laboratory uses the following criteria to comment on the level of antibacterial activity determined (***Table 1***).

Assessment of antifungal activity

Determination of synthetic polymeric material's resistance to fungi was carried out by the Institute of Leather Industry (Poland) in accordance with standard [23]. Samples were tested using Petri dishes containing sterile nutrient salts – agar (pH 6.5) and one 50 mm x 50 mm piece of each foil (F1, F3, FM, FREF). Each replicate was inoculated with a fungal suspension that consisted of equal volumes (40.0 ml) of 3 mould suspensions that were at a concentrations of 1,0 x 10^6 ± 2,0 x 10^5 spores per ml. The fungal species tested included *Aspergillus niger, Chaetomium globosum* and *Trichoderma viride*. Three pieces of inoculated sterilised 25 mm x 25 mm filter paper (Whatman) were included as controls. The Petri dishes with inoculated samples (F1, F3, FM, FREF, control) were incubated at 25±2 °C (tempera-

ture), maintained at 85.0% (humidity) for 4 weeks, and analysed after 7, 14, 21 and 28 days. Each sample type was tested in triplicate. Viability controls produced heavy fungal growth within 7-14 days, confirming the viability of the spore suspension. The grading scale for this test is shown in ***Table 2***.

Results and discussion

In this study ISO Standard 22196:2007 [22] was used to evaluate the antimicrobial activity and antibacterial efficiency of multilayer polyethylene films containing a PHMG sulphanilate. The bacterial counts obtained together with antibacterial activity R (shown as a log reduction) and the antimicrobial efficacy, are given in ***Table 3*** for *Escherichia coli* and in ***Table 4*** for *Staphylococcus aureus*. It was found that the monolayer film, marked as F1, was characterised by the highest antibacterial activity, and thus the highest antimicrobial efficacy of all the samples tested. The antimicrobial efficacy of the monolayer film against *Escherichia coli* and *Staphylococcus aureus* was very good, equalling 6.25 log (100%) and 6.02 log (100%), respectively. It means that the total reduction of bacteria on the surface tested was achieved within 24 hours of testing. The antimicrobial efficacy of the three-layer film against *Staphylococcus aureus* was also highly satisfactory and equaled 6.02 log (100%). Moreover a satisfactory level of antibacterial efficacy (1.32 log) of this film against *Escherichia coli* was also obtained. In this case, a reduction in the number of microorganisms down to 95.2% was observed. Referring to ***Tables 3*** and ***4***, the number of bacterial cells (in the case of *Escherichia coli* and *Staphylococcus aureus*) on FM after 24 hours of contact with the film remained at the same level as at the beginning of the test (0 hours, FREF – control sample). Therefore the antibacterial activity of the market foil (FM) was less than 1 and the antibacterial efficacy was assessed as "poor".

It can be concluded that the addition of a PHMG sulphanilate to a film gives it antibacterial properties. In the F3 case antibacterial properties were stronger against Gram-positive bacteria and slightly weaker against Gram-negative ones. A similar observation was made by Walczak et al. [4], whose results indicate that a PHMG derivative (e.g. PHMG salt of sulfanilic acid) introduced into biodegradable polylactide polymer inhibits

Table 1. *Antimicrobial efficacy criteria.*

Antibacterial activity R, log	Decrease in the number of microorganisms, %	Antimicrobial efficacy
<1.0	<90.0	poor
1.0-2.0	>90.0-99.00	satisfactory
2.0-3.0	>99.00-99.9	good
>3.0	>99.9	very good

Table 2. *Grading scale for visible effects.*

Growth observed on specimens	Percentage coverage of sample, %	Rating
None	0%	0
Traces of growth	Less than 10%	1
Light growth	10 to 30%	2
Medium growth	30 to 60%	3
Heavy growth	60% to complete coverage	4

Table 3. *Antimicrobial activity against Escherichia coli.* ***Note:*** *(–) not determined.*

Sample ID	Mean bacterial count, CFU/cm²		Antibacterial activity, log	Decrease in the number of microorganisms, %	Antimicrobial efficacy, –
	Initial 0 h	After 24 h			
FREF (Control)	1.8×10^4	1.8×10^6	–	–	–
F1	–	0	6.25	100	very good
F3	–	8.6×10^4	1.32	95.2	satisfactory
FM	–	1.7×10^6	0.01	5.56	poor

Table 4. *Antimicrobial activity against Staphylococcus aureus.* ***Note:*** *(–) not determined.*

Sample ID	Mean bacterial count, CFU/cm²		Antibacterial activity, log	Decrease in the number of microorganisms, %	Antimicrobial efficacy, [–]
	Initial 0 h	After 24 h			
FREF (Control)	1.9×10^4	1.1×10^6	–	–	–
F1	–	0	6.02	100	very good
F3	–	0	6.02	100	very good
FM	–	1.1×10^6	0.00	0.00	poor

Table 5. *Visual rating of fungal growth observed (in triplicate).*

Sample ID	Rating											
	Week 1			Week 2			Week 3			Week 4		
Strain	***Aspergillus niger***											
F1	0	0	0	0	0	0	0	0	0	0	0	0
F3	0	0	0	0	0	0	0	0	0	0	0	0
FM	0	0	0	0	0	0	1	1	1	1	1	1
FREF	0	0	0	0	0	0	1	1	1	1	1	1
Control	1	1	1	3	3	3	4	4	4	4	4	4
Strain	***Chaetomium globosum***											
F1	0	0	0	0	0	0	0	0	0	0	0	0
F3	0	0	0	0	0	0	0	0	0	0	0	0
FM	0	0	0	0	0	0	1	1	1	1	1	1
FREF	0	0	0	0	0	0	1	1	1	1	1	1
Control	3	3	3	4	4	4	4	4	4	4	4	4
Strain	***Trichoderma viride***											
F1	0	0	0	0	0	0	0	0	0	0	0	0
F3	0	0	0	0	0	0	0	0	0	0	0	0
FM	0	0	0	0	0	0	0	0	0	1	1	1
FREF	0	0	0	0	0	0	0	0	0	1	1	1
Control	4	4	4	4	4	4	4	4	4	4	4	4

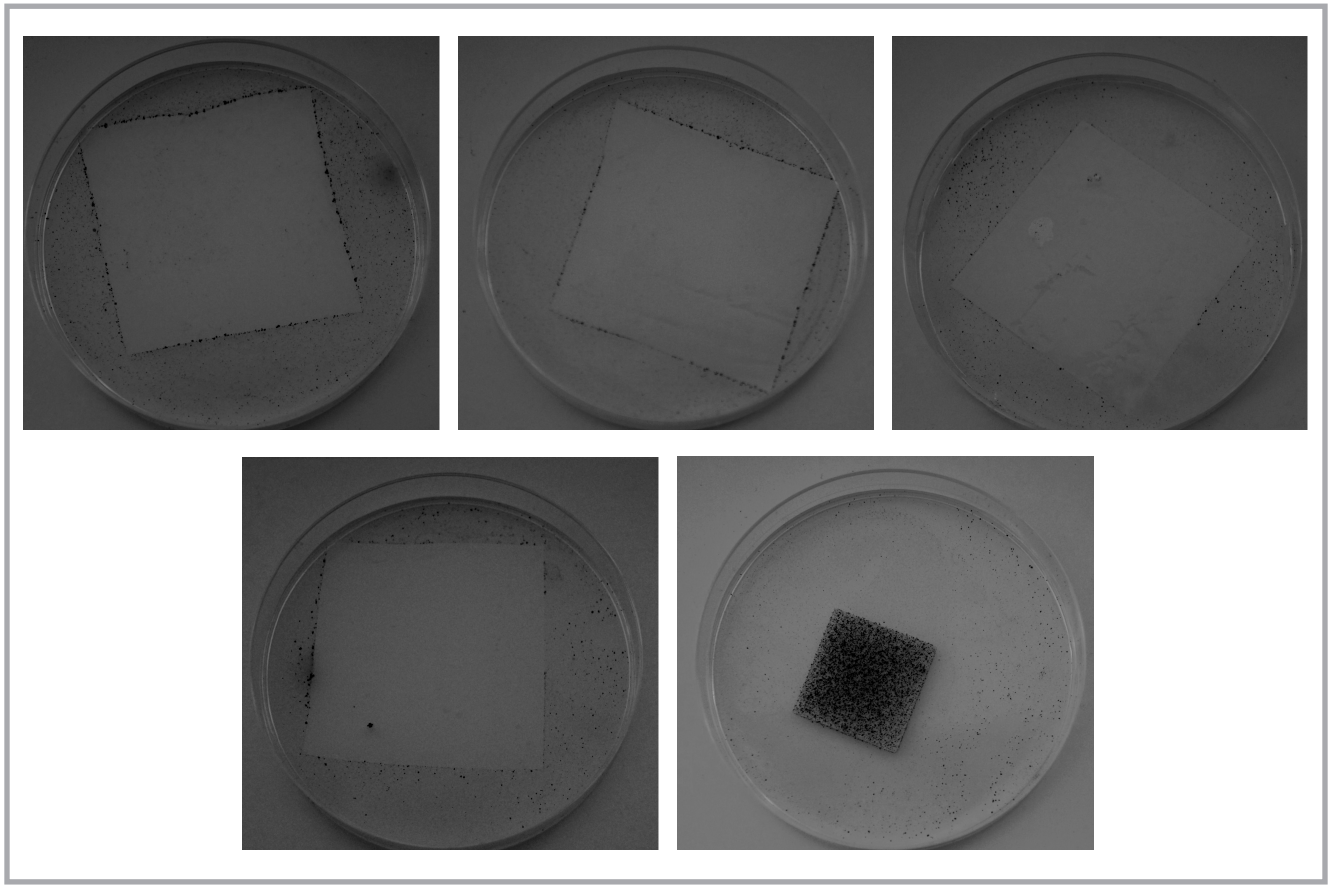

Figure 1. *Antifungal activity of test samples against Aspergillus niger after 28 days, from left to right: F1, F3, FREF, FM, control.*

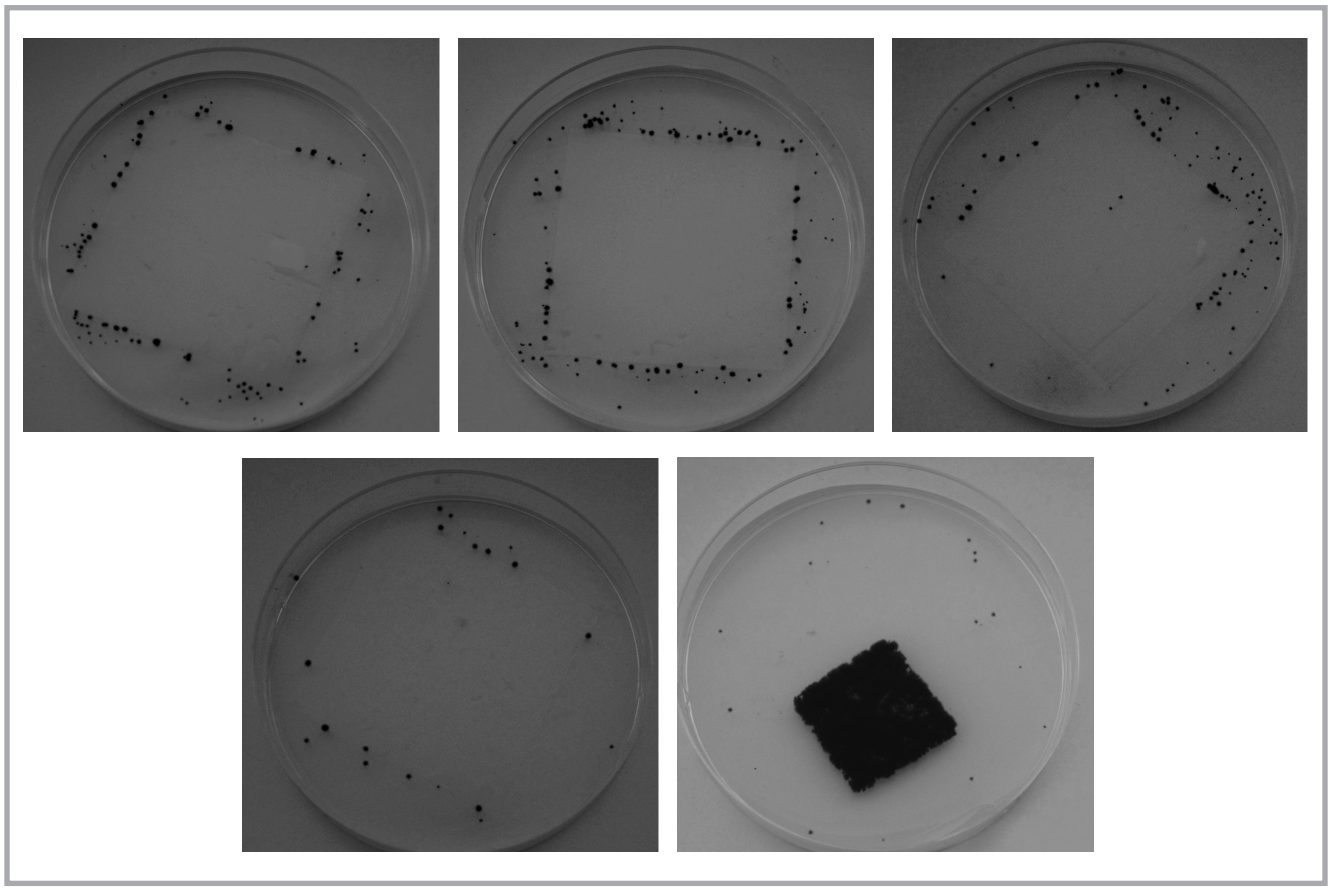

Figure 2. *Antifungal activity of test samples against Chaetomium globosum after 28 days, from left to right: F1, F3, FREF, FM, control.*

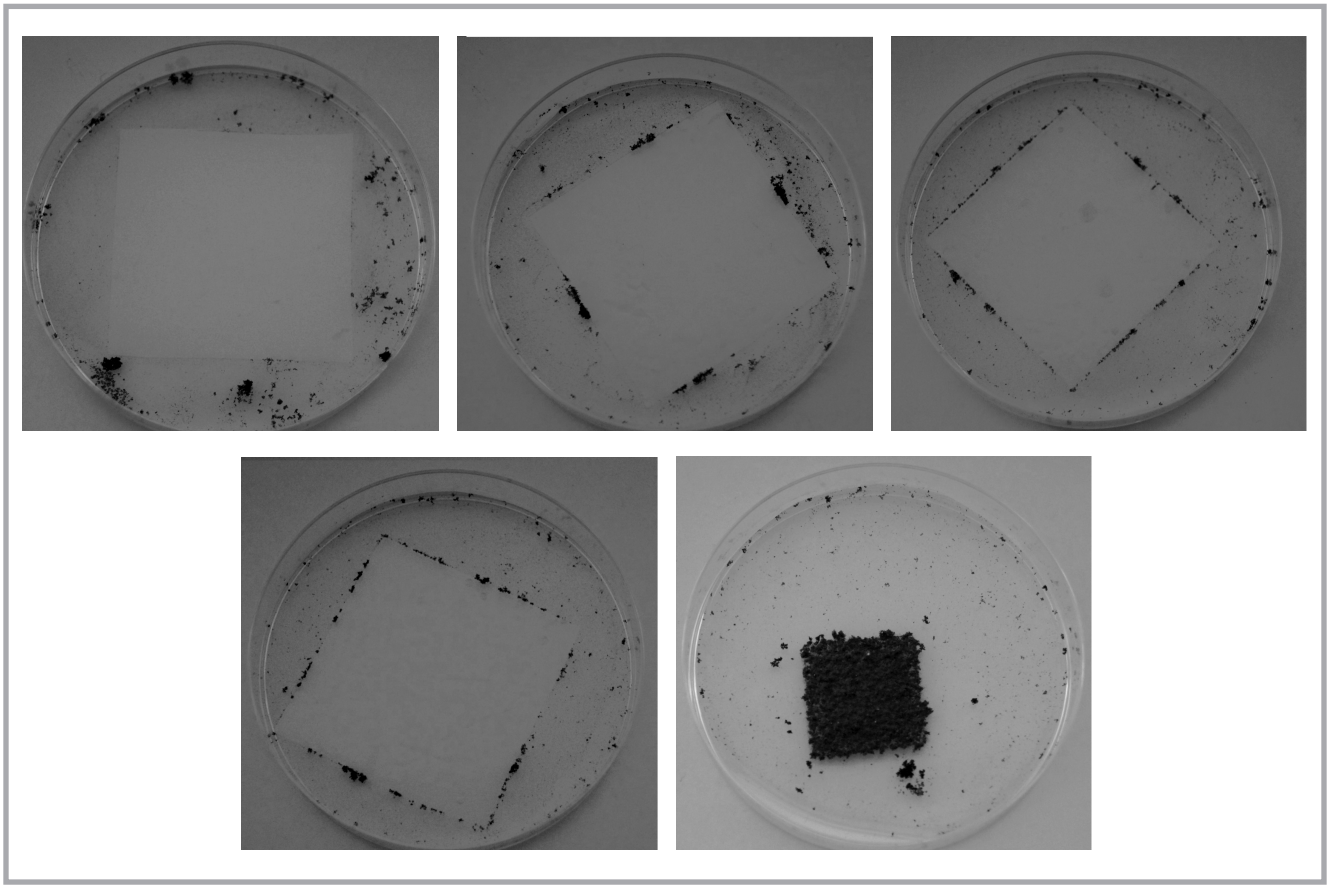

Figure 3. *Antifungal activity of test samples against Trichoderma viride after 28 days, from left to right: F1, F3, FREF, FM, control.*

microbial growth on its surface. Moreover they found that this compound had a slightly stronger bactericidal effect on *Staphylococcus aureus* than on *Escherichia coli.* This observation could be explained by the fact that Gram-negative bacteria have more complex cell envelopes, which are mostly composed of outer and inner membranes. There are large hydrophilic lipopolysaccharide molecules on the outside of the membrane, which can block easy access of these polymers to phospholipids and thence to the cell interior [24]. Brzezińska et al. [14], who indicate that polycaprolactone containing PHMG salt of sulfanilic acid inhibited biofilm formation, stated that PHMG could affect different metabolic pathways and cell elements.

All the above-mentioned reports and observations from this study lead to the conclusion that the antimicrobial activity of PHMG salts may consists in contact with a given material rather than the release of the biocide. This is also confirmed by the limited water solubility of PHMG sulphanilate. It can be assumed that the mechanism of action of the PHMG sulphanilate in the polyethylene film is probably similar to that of sterile-surface polymers in which one end of a long-chained hydrophobic polycation with antimicrobial monomers is attached covalently to the surface of a material (cotton or plastic) [25]. Hydrophobic cations, such as PHMG and its derivatives, are believed to kill cells by disrupting the membrane. It was found that bacterial membrane damage is also the mechanism of action of sterile-surface polymers [25]. Results of the antifungal activity test after one, two, three and four weeks can be found in ***Table 5***. ***Figures 1-3*** show the antifungal activity for the films tested (F1, F3, FREF, FM) and for the control (sterile filter paper) after 28 days of sample incubation. Direct photography does not show small fungal growth clearly. The rating of trace or no growth was confirmed using microscopic observation.

All the control samples had copious fungal growth after 2 weeks (from rating 3 to 4), confirming the validity of the test. The first slight increase in fungus growth (1 rating) was observed after 3 weeks for *Aspergillus niger* and *Chaetomium globosum* on the FREF and FM samples (without biocide). After 4 weeks trace amounts of fungal growth were observed on FREF and FM samples for every strain tested. The growth intensity for these samples was evaluated at 1. Significantly no fungal growth on the F1 and F3 films was observed during the whole test (0 rating), which is interesting because these samples were enriched with the PHMG sulphanilate. In all cases sporulation around the samples was observed. When analysing the results it should be taken into account that synthetic polymer does not provide a carbon source that supports mould growth [23]. For this reason, limited growth of all the fungi tested was observed on all type of samples. However, the total inhibition of fungal growth only on samples F1 and F3 suggest that the use of a PHMG sulphanilate for film production provides protection against the growth of fungi on the films tested. A similar observation was made by Rogalsky et al. [26], whose results indicate that polyamide films containing 2% (wt) of PHMG-DBS biocide (PHMG dodecylbenzenesulfonate) are found to be highly resistant to *Trychophyton mentagrophytes* fungus. Moreover, Kondratyuk et al. [27] found that

poliamide films have pronounced antifungal properties when modified with 5% (wt) of polymeric biocide PHMG-DBS. The effectiveness of the biocide was tested against a mixture of test-cultures of *Aureobasidium pullulans, Aspergillus terreus, A. niger, Chaetomium globosum, Paecilomyces variotii, Penicillium funiculosum, P. ochrochloron, Scopulariopsis brevicaulis*, and *Trichoderma viride* [27]. The antimicrobial activity of PHMG salts is caused by the presence of guanidinium cations [24]. However, the mechanism of the antifungal action of a PHMG sulphanilate has not been described in the literature.

According to the data obtained in the present study, a PHMG sulphanilate imparts good resistance against microbial growth to polyethylene films. The introduction of a biocide to polyethylene films makes them very interesting for a number of practical applications, such as the production of packaging for food or cosmetics.

Conslusions

1. The use of a polyhexamethylene guanidine sulphanilate for film production provides effective protection against the growth of undesirable microorganisms on polyethylene films.
2. The antimicrobial efficacy of the monolayer film with a PHMG sulphanilate against *Escherichia coli* and *Staphylococcus aureus* was very good, because the total reduction of bacteria on the test surface in a 24 hour test was observed.
3. The antimicrobial efficacy of the three-layer film with a PHMG sulphanilate against *Staphylococcus aureus* was also very good (100% of bacterial reduction), and against *Escherichia coli* – at a satisfactory level (95.2% of bacterial reduction).
4. The monolayer and three-layer film with a PHMG sulphanilate showed no growth of inoculated fungi (antifungal activity). The films containing biocide were found to be resistant to the fungi tested i.e. *Aspergillus niger, Chaetomium globosum, Trichoderma viride*.
5. For the market foil, poor antibacterial efficacy against the bacteria and no antifungal activity against the fungi tested was observed.

References

1. Gibas E. Polyethylene films – raw materials and necessary additives. *Plas Proc* (in Polish) 2014; 3: 224-232.
2. Richert A. Bacteriostatic properties of polyethylene composites. *Chem Ind* (in Polish) 2017; 96 (7): 1528-1530.
3. Scannell AGM, Hill C, Ross RP, Marx S, Hartmeier W, Arendt EK. Development of bioactive food packaging materials using immobilised bacteriocins Lacticin 3147 and Nisaplin®. *Int J Food Microbiol* 2000; 60: 241-249.
4. Walczak M, Richert A, Burkowska-But A. The effect of polyhexamethylene guanidine hydrochloride (PHMG) derivatives introduced into polylactide (PLA) on the activity of bacterial enzymes. *J Ind Microbiol Biotechnol* 2014; 41:1719-1724.
5. Ramamurthy P, Chellamani KP, Dhurai B, Rajan SPT, Subramanian B, Santhini E. Antimicrobial characteristics of pulsed laser deposited metal oxides on polypropylene hydroentangled nonwovens for medical textiles. *FIBRES & TEXTILES in Eastern Europe* 2017; 25, 2(122): 112-119. DOI: 10.5604/12303666.1228192.
6. Tawiah B, Badoe W, Fu S. Advances in the Development of Antimicrobial Agents for Textiles: The Quest for Natural Products. Review. *FIBRES & TEXTILES in Eastern Europe* 2016; 24, 3(117): 136-149. DOI: 10.5604/12303666.1196624
7. Lucas AD. Environmental fate of polyhexamethylene biguanide. Bull Environ Contam *Toxicol* 2012; 88: 322-325.
8. Pinto F, Maillard JY, Denyer SP, McGeechan PM. Polyhexamethylene biguanide exposure leads to viral aggregation. *J Appl Microbiol* 2010; 108: 1880-1888.
9. Curd FHS, Hendry H, Kenny TS, Murray AG, Rose FL. Synthetic antimalarials. Part XXVIII. An alternative route to N1-aryl-N5-alkyldiguanides. *J Chem Soc* 1948; 1630-1636.
10. Lysytsya AV. Research on the impact of polyhexamethyleneguanidine on the plant component of biocenoses. *Biosyst Divers* 2017; 25 (2): 89-95.
11. Byrde RJ, Clifford DR, Woodcock D. Fungicidal activity and chemical constitution XI*. The activity of n-alkyl guanidine acetates. *Ann Appl Biol* 1962; 50 (2): 189-377.
12. Broxton P, Woodcock PM, Heatley F, Gilbert P. Interaction of some polyhexamethylene biguanides and membrane phospholipids in *Escherichia coli*. *J Appl Bacteriol* 1984; 57: 115-124.
13. Allen MJ, White GF, Morby AP. The response of *Escherichia coli* to exposure to the biocide polyhexamethylene biguanide. *Microbiology* 2006; 152: 989-1000.
14. Brzezinska MS, Walczak M, Jankiewicz U, Pejchalová M. Antimicrobial activity of polyhexamethylene guanidine derivatives introduced into polycaprolactone. *J Polym Environ* 2017; https://doi.org/10.1007/s10924-017-0974-9.
15. Lysytsya A, Lyco S, Portuhaj O. The polyhexamethyleneguanidine stimulation of seeds growing and cell proliferation. *Mater Sci Eng B* 2013; 3 (10): 653-660.
16. Kratzer C, Tobudic S, Graninger W, Buxbaum A, Georgopoulos A. In vitro antimicrobial activity of the novel polymeric guanidine Akacid plus. *J Hosp Infect* 2006; 63: 316-322.
17. Razzaghi-Abyaneh M, Shams-Ghahfarokhi M, Kawachi M, Eslamifar A, Schmidt OJ, Schmidt A, Allameh A, Yoshinari T. Ultrastructural evidences of growth inhibitory effects of a novel biocide. Akacid-plus on an aflatoxigenic *Aspergillus parasiticus*. *Toxicon* 2006; 48: 1075-1082.
18. Feng L, Wu F, Li J, Jiang Y, Duan X. Antifungal activities of polyhexamethylene biguanide and polyhexamethylene guanide against the citrus sour rot *pathogen Geotrichum citri-aurantii* in vitro and in vivo. *Postharvest Biol Technol* 2011; 61: 160-164.
19. Mathurin YK, Koffi-Nevry R, Guéhi ST, Tano K, Oule´ MK. Antimicrobial activities of polyhexamethylene guanidine hydrochloride–based disinfectant against fungi isolated from cocoa beans and reference strains of bacteria. *J Food Prot* 2012; 75 (6): 1167-1171.
20. Zhang Y, Jiang J, Chen Y. Synthesis and antimicrobial activity of polymeric guanidine and biguanidine salts. *Polymer* 1999; 40: 6189-6198.
21. PL225392 B1 – Process for the production of an aminobenzenesulfonic derivative of polyhexamethylene guanidine (in Polish).
22. ISO 22196: 2007 (E): Plastics – Measurement of antibacterial activity on plastics surfaces.
23. ASTM G21 – 96: Standard practice for determining resistance of synthetic polymeric materials to fungi. West Conshohocken, PA: American Society for Testing and Materials (ASTM);1996.
24. Zhou Z, Wei D, Guan Y, Zheng A, Zhong J-J. Extensive in vitro activity of guanidine hydrochloride polymer analogs against antibiotics-resistant clinically isolated strains. *Mater Sci Eng C* 2011; 31: 1836-1843.
25. Lewis K, Klibanov AM. Surpassing nature: rational design of sterile-surface materials. *Trends Biotechnol* 2005; 23 (7): 343-348.
26. Rogalsky S, Bardeau J-F, Tarasyuk O, Fatyeyeva K. Fabrication of new antifungal polyamide-12 material. *Polym Int* 2012; 61 (5): 686-691.
27. Kondratyuk TO, Bardeau J-F, Sobko VM, Tarasyuk OP, Makhno SM, Sheludko EV, Kyselov YuV, Rogalsky SP. Antifungal activity of polyamide 12 films modified with polyhexamethylene guanidine dodecylbenzenesulfonate. Poster session presented at: *Modern Problems of Surface Chemistry, International Conference*; 2014 May 19-23; Kyiv, Ukraine.

Received 17.11.2017 Reviewed 12.02.2018