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New promising antimicrobial material based on thermoplastic polyurethane modified with polymeric biocide polyhexamethylene guanidine hydrochloride

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ABSTRACT

New antimicrobial polymer composite based on commercial polyurethane (PU) Laripur®LPR9020 and polymeric biocide polyhexamethylene guanidine hydrochloride (PHMG-Cl) has been prepared by solvent casting method. Modified PU films containing from 1 to 4 wt% of PHMG-Cl were characterized in terms of mechanical, thermal and surface properties, biocide release behavior, as well as antibacterial activity against Gram-negative (*Escherichia coli*) and Gram-positive (*Bacillus subtilis*) bacterial strains. The results of mechanical testing indicate that the introduction of PHMG-Cl did not cause noticeable changes in the tensile strength of PU films, but improved their elasticity by at least 20–30%. The surface of PU/PHMG-Cl films was found to possess significantly higher hydrophobicity than neat polymer that is manifested in sharply increased water contact angle value from 72° for neat PU to 95° for PU/PHMG-Cl (2%). According to thermogravimetric analysis data, PU/PHMG-Cl composites are thermally stable to at least 300 °C which indicate their availability for melt processing by common methods. The results of differential scanning calorimetry showed reduction of both glass transition and melting temperatures of PU by more than 7 °C when contained only 1% of PHMG-Cl.

Gradual release of polymeric biocide from modified PU films into water has been detected spectrophotometrically. The total biocide release ratio was found to be in the range from 29% to 48% after 50 hours immersion, depending on its content in polymer composite. PU/PHMG-Cl films showed much lower leaching rate into physiological saline solution compared to pure water (from 10% to 21% after 50 hours), which may be caused by poor solubility of polymeric biocide in sodium chloride solutions.

The study of antibacterial activity of modified PU films using both disc agar diffusion and contact methods indicate high efficacy against tested microorganisms when contained 3% of polymeric biocide. So, this approach,

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based on the modification of PU resin with polymeric biocide PHMG-Cl, has been discussed in terms of potential medical applications when biocide release system or contact active material are necessary.

1. Introduction

Polyurethanes (PUs) are among the most versatile construction materials which are used everywhere in modern life. PUs are segmented polymers, containing soft segments that provides flexibility and a hard segments formed by diisocyanate and chain extender that ensures high tensile strength [1,2]. Thus, they can be divided into several different classes based on the desired material properties: rigid, flexible, thermoplastic, elastomers [1]. Common PUs applications include building insulation, car and household furnishing, manufacture of footwear, coatings, adhesives and sealants, as well as textiles. PUs are popular materials for various medical applications due to the unique combination of toughness and flexibility, good resistance to impact and abrasion, biocompatibility, as well as easy processability by common methods. Melt-processable, or thermoplastic polyurethanes (TPUs) are used extensively in medical devices, such as catheters, tissue adhesives, needle hubs, oxygen masks, gloves, medical tubing, hospital bedding, surgical drapes, as well as wound dressing materials [1–3]. The most frequent medical use of PUs is for short-period implants [1,4]. Thus, PU based central venous catheters (CVCs) are an important component of the rescue and treatment of critically ill patients. An important advantage of PU based catheters is their considerable softening on insertion into the body due to some extent hydrophilicity [4]. However, medical implants are convenient surface for microbial growth, both the short-term devices (urinary catheters) and the long-term implants (artificial joints). Clinical trials on PU based internal medical devices showed that the microbial biofilm formation causes enzymatic biodegradation of the material [4,5]. Moreover, the resulting biofilms cause chronic infections because surface-associated microorganisms exhibit an increased tolerance to both antimicrobial agents and disinfectant chemicals [4–8]. In fact, most of the health care-associated infections were found to be medical-device related, and the common complication of CVC use is catheter-related blood stream infections [4,6,7].

Polyurethane wound-dressing films are utilized to make a coating that is impermeable to fluids and bacteria but allows moisture to permeate. PU foam dressings are able to handle large volumes of wound fluid, with different permeability characteristics [9,10]. Thus, bilayered PU wound dressing composed of microporous top layer and highly porous sponge-like sublayer are known to provide efficacy in preventing dehydration and bacterial penetration [9]. However, pathogenic microbial flora, especially in chronic wounds or surgical wounds, can lead to infection and sepsis, as well as excessive inflammation [4,11]. These complications most often lead to delayed healing, extended hospital stay and increased costs.

The introduction of antimicrobial agents into medical devices is considered the most efficient approach to prevent the growth of biofilms on their surface. A wide variety of organic and inorganic biocides is available, whether synthetic or nature-inspired [12–16]. Inorganic nanoparticles (titanium dioxide, zinc oxide, copper, silver) are widely employed as antimicrobial additives to various medical polymers, including PUs [4,16–20]. Silver in different forms (metallic or salt) is used as typical biocide for control of colonization of medical devices and dressing materials [4,13,14]. Silver ions can be released from the polymer matrix into the wound fluid or exudate and act against a wide spectrum of pathogens such as gram-negative and gram-positive bacteria, yeast and viruses. Because of this, silver-containing foam dressings have been commonly used for healing wounds, including burns, diabetic ulcers, and surgical wounds [21,22]. Silver-based biocides are nevertheless relatively expensive and in the case of nanoparticles it is necessary to implement complicated processes to disperse them homogeneously in the polymer matrix [19]. Moreover, the cellular

toxicity of silver ions has also been reported in several studies as serious side-effect [4,16,21,23] and it should also be noted that some bacterial strains are resistant to silver [4,24,25].

Common approaches to impart antimicrobial activity to CVCs involved impregnation of materials with systemic antibiotics such as minocycline/rifampicin, miconazole/rifampicin, benzalkonium chloride, or with antiseptics such as synergistic combination of silver sulfadiazine/chlorhexidine [4,26,27]. The functionalization of medical devices with the said biocides can be time consuming and costly when it requires additional manufacturing steps and tight processing conditions to achieve uniformity. With surface functionalization, there is always the risk that the coatings have a reduced efficacy due to a lower density of antimicrobial molecules [4] and therefore contribute to increasing the tolerance of bacteria to antibiotics.

Povidone-iodine, or PVP-iodine is a labile complex of elemental iodine with polyvinyl pyrrolidone (PVP), which serves as sustained released reservoir of iodine [28]. PVP-iodine has demonstrated many benefits for wound healing due to its high activity against a broad spectrum of pathogenic microorganisms, and minimal cytotoxicity to host cells [28–30]. PU foam dressing materials containing PVP-iodine can be fabricated by one step bulk polymerization from a reactionary mixture involving polyglycol, diisocyanate and solid particles of PVP-iodine [31]. Recent studies have shown that new commercial PU foam dressing Betafoam impregnated with 3% of povidone-iodine was the most effective dressing in terms of wound healing and re-epithelialization compared to various silver-containing foams and gauze [32,33]. However, several studies reported the cases of povidone-iodide-induced burn that occurs when the substance was not allowed to dry [34]. Moreover, skin antisepsis with common cationic biocide chlorhexidine and alcohol solutions has demonstrated superiority to povidone-iodine in a variety of surgical interventions [35].

Nowadays, polymeric biocides comprising guanidinium cations in polymer backbone such as polyhexamethylene biguanide hydrochloride (PHMB-Cl) and polyhexamethylene guanidine hydrochloride (PHMG-Cl) are being considered as valuable and cheap alternatives to common inorganic antimicrobial agents since they demonstrated high efficacy in killing antibiotic-resistant bacteria and fungi, as well as low cytotoxicity [36–40]. The above cationic polymers are already widely used as effective disinfectants in cooling systems, swimming pools and hospitals, in personal hygiene products, as well as in food industry [36,38,39,41]. The preparation of PU/PHMB-Cl electrospun nanofibre membranes with polymeric biocide content 5–35% has been reported [42]. The membranes displayed burst release of PHMB-Cl into PBS solution during the first hour followed by a gradual release over 120 hours. PU films containing 5–15% PHMB-Cl displayed superior antimicrobial activity, as well as minimal human cell toxicity confirming their use as wound dressing material [42].

Another polymeric biocide, PHMG-Cl has broad range of antimicrobial activity, being however much cheaper than PHMB-Cl due to simple one-step synthesis [41]. Recent studies have also shown that PHMG-Cl possesses pronounced anti-inflammatory and wound healing properties and therefore may be used for the treatment of chronic wounds and thermal burns [43]. Thus, PHMG-Cl based composite effectively reduced the level of proinflammatory and increased the level of pro-inflammatory cytokines, as well as normalized the oxidative-antioxidant homeostasis by normalizing the content of markers of free radical oxidation to accelerate the treatment of thermal burns. The antioxidant activity of this polymeric biocide has also been established in Ref. [44].

The acute toxicity studies on rats showed that the median lethal dose (LD_{50}) for PHMG-Cl is significantly higher (600 mg/kg) [45] than for

PHMB-Cl (25.6 mg/kg) [46]. In other study, results of acute oral toxicity showed that PHMG-Cl at 5% presents a lethal dose 50% greater than the 2000 mg/kg [47]. From a practical point of view, PHMG-Cl seems very promising antimicrobial additive for thermoplastic PUs. Indeed, in contrast to PHMB-Cl, which has thermal decomposition point of 205–210 °C without melting [48,49], PHMG-Cl has low melting point of 135–140 °C and is thermally stable up to 313 °C [50]. Such properties make it convenient for joint melt processing with TPU resins by common methods. However, the use of PHMG-Cl as an external additive for bulk modification of TPUs has not yet been reported. An amine terminated PHMG-Cl was prepared and used as curing agent of different epoxy-terminated polyurethane prepolymers. Such internally modified PU dressing membranes showed pronounced contact killing mechanism of antimicrobial activity, as well as excellent cytocompatibility against fibroblast cells [51].

So, in this study, commercial TPU was modified with low content of polymeric biocide PHMG-Cl (1–4 wt%) in order to develop new promising antimicrobial material which has biocide releasing properties. The impact of PHMG-Cl additive on mechanical, surface and thermophysical properties, as well as antibacterial activity of PU, has been studied.

2. Materials and methods

2.1. Synthesis of polymeric biocide PHMG-Cl

PHMG-Cl was synthesized by melt polycondensation of guanidine hydrochloride (98%, Applichem) and hexamethylenediamine (98%, Sigma-Aldrich), according to the method described in Ref. [50] (Scheme 1).

The mixture of guanidine hydrochloride (20 g, 0.21 mol) and hexamethylenediamine (23.1 g, 0.2 mol) was put into a round-bottomed flask (500 ml) equipped with a mechanical stirrer. At first, the mixture was heated to 100 °C and the melt was stirred for 4 h at this temperature. Further, the reaction was carried out for 4 h at 130–140 °C and 4 h at 180 °C to obtain highly viscous melt. The vitreous solid of PHMG-Cl was obtained after cooling to room temperature. It was dissolved in water (200 ml), filtered and precipitated by addition of saturated water solution of sodium chloride (100 ml). The polymer was isolated by decantation of water solution, dried at 140 °C for 24 h and powdered in porcelain mortar. It has melting point of 136–138 °C. Intrinsic viscosity was 0.09 dl/g for PHMG-Cl solution in 0.1 N NaCl at 25 °C.

¹H NMR (400 MHz, DMSO-D₆): δ = 1.32 (m, 4H, CH₂), 1.47 (m, 4H, N-CH₂CH₂), 3.16 (m, 4H, N-CH₂), 7.1–8.1 (br s, 4H, NH).

Elemental analysis: (C₇H₁₆N₃Cl)_x (177.5)_x: found (%): C 46.9, H 9.4, N 24.0, Cl 20.5; calculated (%): C 47.3, H 9.0, N 23.6, Cl 20.0.

2.2. Preparation of PHMG-Cl modified PU films

Thermoplastic adipate ester polyurethane Laripur®LPR9020 was purchased from COIM S.p.A. (Italy). 1 g of polymer was dissolved in 15 ml of N,N-dimethylformamide (Merck). PHMG-Cl was dissolved in ethanol (10 wt%) and added to the PU solution in concentrations from 1 to 4 wt% in a composite and the mixture was stirred for 2 h at 50 °C. The solutions were casted onto glass substrates and kept at 60 °C for 24 h to form polymeric films. They were dried in vacuum 5 mbar at 50 °C for 24 h. The prepared films were about 150 μm thick.

2.3. Characterization of PU/PHMG-Cl composites

In order to evaluate physicochemical interaction between PU and PHMG-Cl, the samples were first placed on the Platinum diamond ATR module and IR spectra were recorded using a Bruker Vertex-70V FTIR spectrometer (all Bruker Optics Inc., Ettlingen, Germany) equipped with a L-alanine-doped deuterated triglycine sulfate (DLADTGS) detector. Spectra were acquired with a resolution of 2 cm⁻¹ in the spectral region from 400 to 4000 cm⁻¹ as the co-addition of 30 scans. Acquisition of these spectra has been done with the Bruker OPUS software (version 6.5).

Mechanical testing of the polyurethane samples was performed using P-50 universal tensile testing machine (Milaform, Russia) at a deformation rate of 10 mm/min. The obtained films were cut into specimens with the size of 40 × 10 × 0.15 mm. An average value (with standard deviation) for the tensile strength was obtained from three samples of each film.

Static contact angle measurements were performed using a Drop Shape Analyzer DSA25E (Krüss, Germany) by the sessile drop method. The contact angle values were estimated, using CAM software, as the tangent normal to the water drop (3 ml) at the intersection between the sessile drop and the polymer surface. All reported contact angles are the average of at least five measurements taken at different locations on the polymer surface.

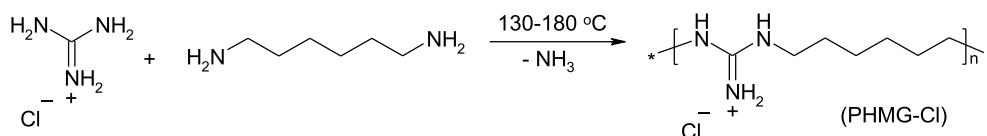
The 3D surface scans of the samples were undertaken using the confocal white light optical imaging S-NEOX profiler (Sensofar-Tech S. L., Barcelona, Spain). The 850 × 709 μm² area were collected using a 20 X EPI Nikon objective (0.45 numerical aperture). The data were analyzed by the open-source Gwyddion software (version 2.48). The average roughness Sa, describing the difference in height of each point compared to the arithmetical mean of the surface, and the root mean square roughness Sq, describing the root mean square average of height deviations taken from the mean data plane, were evaluated for each surface three times over the complete 3D surface respectively.

The scanning electron microscopy (SEM) images were obtained using a JSM-6490L V microscope (JEOL, Tokyo, Japan) equipped with an Oxford Instruments INCA 350 energy-dispersive X-ray spectroscopy (EDS) system allowing local quantitative elemental microanalysis of polymer surface. The samples were observed, after a platinum sputtering treatment, under an accelerating voltage of 20 kV. SEM images were acquired in secondary electron imaging (SEI) at different magnification of 2000X and 10000X. The lithium-drifted silicon Si(Li) detector is equipped with a thin beryllium window which allows oxygen to be revealed and quantified with good precision. However, the EDS detector cannot quantify with accuracy light elements, like carbon, and only gives relative analyzes.

Thermal gravimetric analysis (TGA) data were obtained using a TGA Q500 (TA Instruments). About 10 mg of each sample was heated from 30 °C to 700 °C with a heating rate of 10 °C/min under an air atmosphere.

Differential scanning calorimetry (DSC) measurements were performed using DSC Q2000 TA Instrument analyzer. The heating process was conducted from -90 to 150 °C at 20 °C/min.

The release of PHMG-Cl from the PU film was investigated by UV-visible spectrophotometric analysis using a Jenway 6850 spectrometer (Great Britain). The calibrating graph was obtained by measuring the absorbance of PHMG-Cl aqueous solutions in a concentration range of 1.5·10⁻⁵ – 1.5·10⁻⁴ mol/l at 194 nm (water) and 204



Scheme 1. Synthesis of polymeric biocide PHMG-Cl.

nm (0.9% NaCl). For the evaluation of the leaching rate of polymeric biocide, 2 g of modified PU/PHMG films were placed into a closed 1 l conical flask containing 1 l of deionized water or 0.9% water solution of NaCl. The samples were kept at 22 °C at constant stirring. 3 ml of each solution was taken periodically and analyzed by measuring the absorbance at mentioned wavelength to determine the concentration of the released biocide. The biocide release ratio was determined as the percentage of PHMG-Cl released into the solution from its total quantity in the film. Each measurement has been repeated three times.

2.4. Antibacterial activity tests

Standard gram-negative *Escherichia coli* strain GM 2163 and gram-positive *Bacillus subtilis* strain 168 were used to test antimicrobial activity of PU/PHMG-Cl films. The overnight culture was cultivated at 37 °C in 5 ml of Luria-Bertani (LB) broth (composition per litre: 5 g yeast extract, 10 g tryptone, 10 g NaCl (pH 7.5)) sterilized by autoclaving on wet cycle 20 min at 15 psi to a concentration of $1.5 \cdot 10^8$ cell forming units (CFU) per ml (determined by measuring optical density at 540 nm).

The antibacterial activity of PU/PHMG-Cl films was studied by both standard agar disc diffusion test [52] and the ISO 22196:2007 method [53] for determining the antimicrobial activity on material surfaces (contact method). Polyurethane films were cut into 10 mm diameter discs and sterilized by immersion into 70% water ethanol followed by ultraviolet irradiation for 30 min on each side.

2.4.1. Agar disc diffusion test (diffusion method)

The overnight bacterial culture (100 µl) was added into 200 ml conical flask containing 50 ml of LB agar followed by shaking (140 rpm) at 37 °C for 20 h to produce end concentration of $1.5 \cdot 10^8$ CFU/ml which was controlled by adjusting the turbidity of bacterial suspension to 0.5 McFarland standard. The bacterial culture was evenly poured onto plates with LB agar with the help of sterile cotton swab. The sterile polymer disc was placed on the culture lawn in the centre of the plate. Clear zone of inhibition (in mm) formed around the disc was measured after 24 h incubation period at 37 °C. The measurements were performed in triplicate for each polymer film.

The normalized width of the antimicrobial “halo” (nw_{halo}) for each disc was determined according to Ref. [54] by using equation:

$nw_{halo} = [(d_{iz} - d)/2]/d$, where d_{iz} - the diameter of the inhibition zone, d - the disc diameter. An average value from three replicates was determined.

2.4.2. Measurement of antimicrobial activity on material surface (contact method)

Each polymer film was placed in a sterile Eppendorf in which 1 ml of bacterial suspension was added (initial colonies concentration 10^6 CFU/ml). The samples were incubated for 24 h at 37 °C. The bacterial culture in each Eppendorf was diluted 10 times (100 µl of the suspension, 900 µl of the LB agar). Each sample (100 µl) was distributed on a plate containing agar LB medium. The plates were incubated for 24 h at 37 °C. The number of viable microorganisms was determined according to Ref. [53] using the following equation:

$N = C \cdot D / A$, where N is the number of viable microorganisms recovered per cm^2 per test specimen, C is the plate count, D is the dilution factor, A is the surface area of the test specimen in cm^2 .

The loss of viability (LV, %) to reflect the inhibition of cell growth was determined by using the equation:

$LV (\%) = [(C - S) / C] \times 100\%$, where C is the average number of viable microorganisms (N) in CFU/ $ml \cdot cm^2$, recovered from the control specimens after 24 h; S is the number of viable microorganisms (N) in CFU/ $ml \cdot cm^2$, recovered from the test specimens after 24 h [54].

3. Results and discussion

3.1. FTIR studies of PU/PHMG-Cl composites

FT-IR measurements were carried out to verify the preparation of the composites by analyzing the specific peak positions. Fig. 1 shows the infrared spectra of PU, the composite PU/PHMG-Cl (4%), and PHMG-Cl. For adipate ester PU, the assignment of the vibration modes was made on the basis of previous published work [55–57].

In Fig. 1a, the IR spectrum of PU presents a broad band at 3326 cm^{-1} and two main peaks located at 2942 and 2852 cm^{-1} commonly assigned to N–H stretching mode and asymmetric and symmetric aliphatic CH_2 stretching modes respectively. In Fig. 1b, the more intense peaks of PU at 1728 and 1700 cm^{-1} are attributed respectively to non-hydrogen bonded and hydrogen bonded urethane C=O stretching vibrational modes. It is also admitted that the asymmetric and intense band centered around 1528 cm^{-1} is attributed to urethane N–H bending + C–N stretching mode, the band at 1220 cm^{-1} to urethane C–N stretching mode and the bands at 1109 and 1078 cm^{-1} to aliphatic asymmetric and symmetric C–O–C stretching modes.

In Fig. 1a, the IR spectrum of PHMG-Cl presents very broad bands in the region 3050 to 3400 cm^{-1} (with maxima at approximately 3152 and 3254 cm^{-1}) resulting of NH_2 and NH stretching modes and two well-defined peaks at 2934 and 2857 cm^{-1} attributed directly to the asymmetric and symmetric stretching vibrations of the methylene groups, respectively [41]. The infrared spectrum presents also strong absorption bands between 1550 and 1700 cm^{-1} , characteristic of both the C=N stretching and the in-plane NH_2 scissoring modes and the band at 1462 cm^{-1} to the bending vibrations of CH_2 groups.

A comparison of the IR spectra of the neat PU film and the PU/PHMG-Cl (4%) composite reveals there is no evident difference between the intensity and the maximum positions of all characteristic bands (Fig. 1). Indeed, despite the polar structure of PHMG-Cl, IR analysis did not detect any strong physicochemical interaction between the guanidine salt moiety and urethane groups. This is probably due to a low content of polymeric biocide in the composite. Nevertheless, for the PU/PHMG-Cl (4%) composite, the main noticeable changes concern the relative intensities of the CH_2 and NH stretching vibration modes. The absorbance of the NH stretching mode decreases that may indicate hydrogen bonding with chloride ions. The absorbance of the CH_2 stretching modes increases and note that the CH_2 symmetric stretching vibrations are shifted from 2852 to 2858 cm^{-1} . Moreover, the peak position of symmetric C–O–C stretching vibrations of PU aliphatic chains has also been changed from 1078 to 1070 cm^{-1} . Overall, this may indicate weak intermolecular interaction between aliphatic chains of PHMG-Cl and PU (Scheme 2).

3.2. Mechanical, surface and thermal properties of PU/PHMG-Cl composites

According to the results of mechanical tests, the introduction of PHMG-Cl did not lead to noticeable changes in tensile strength of PU films (Table 1). At the same time, increased elasticity was observed for PU films containing 1% and 2% of PHMG-Cl (by at least 20–30% compared to neat polymer). However, further increase of PHMG-Cl content returned the elasticity to initial values comparable with neat PU (Table 1).

The results of static water contact angle measurements of PU films are summarized in Fig. 2. For all PU/PHMG-Cl composites, significantly increased hydrophobicity was detected compared to neat PU, with maximum contact angle value for PU/PHMG-Cl (2%).

Fig. 3 shows SEM images of modified PU films. The presence of micropores with pore size around $1 \mu\text{m}$ is observed on the surface of PU/PHMG-Cl (2%) films (Fig. 3, 2), whereas the surface of neat PU is mainly uniform (Fig. 3, 1). SEM images of PU/PHMG-Cl (4%) showed the presence of a significant number of micropores with pore size around

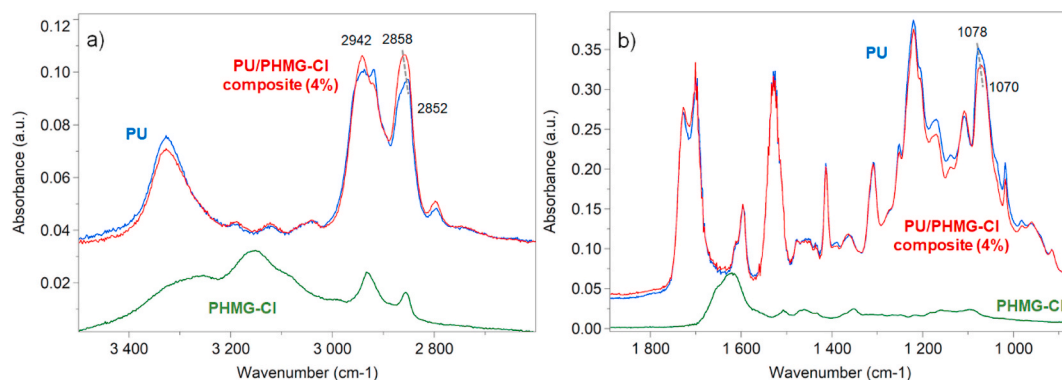
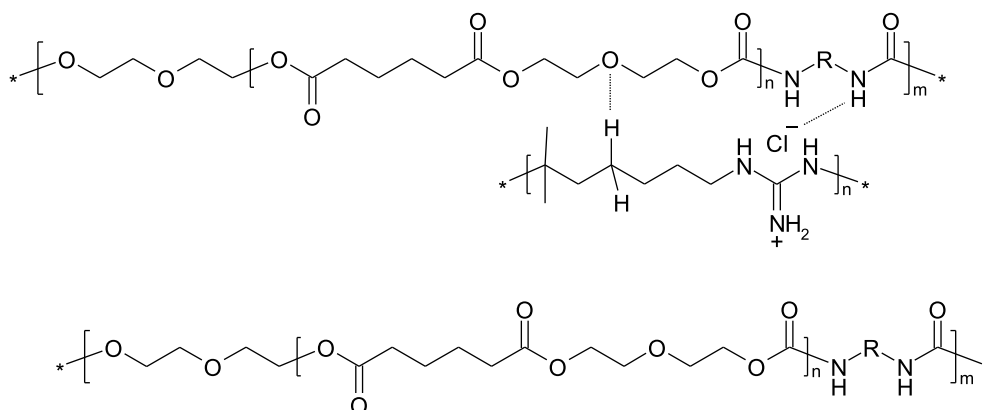


Fig. 1. Infrared spectra of PU, PU/PHMG-Cl (4%) composite and PHMG-Cl.



Scheme 2. Possible physicochemical interaction between PU and PHMG-Cl.

Table 1
Mechanical properties of PU/PHMG-Cl films.

Sample	Tensile strength, MPa	Elongation at break, %
PU control	14.0 ± 1.5	524 ± 10
PU/PHMG-Cl (1%)	14.5 ± 1.2	630 ± 14
PU/PHMG-Cl (2%)	15.0 ± 2.4	701 ± 18
PU/PHMG-Cl (3%)	15.5 ± 2.8	543 ± 10
PU/PHMG-Cl (4%)	16.0 ± 0.5	495 ± 12

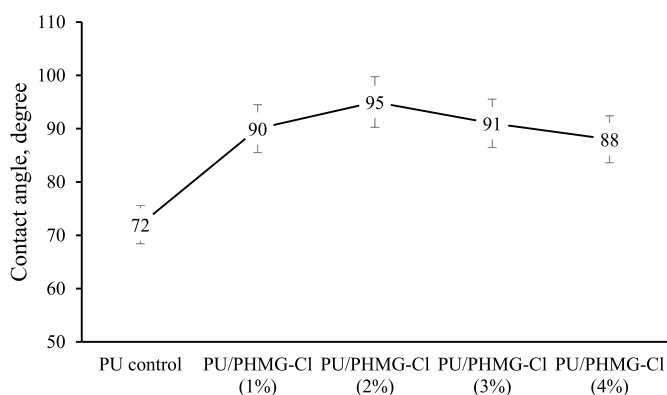


Fig. 2. Contact angle values for modified PU films.

2 μm (Fig. 3, 3). These observations are consistent with the averaged roughness values obtained from the 3D surface scans using the confocal white light optical imaging profiler (Fig. 4). Indeed, the roughness values Sa and Sq of PU/PHMG-Cl films (1%) are similar to those found

for neat PU, then increase by 0.2 μm between 1% and 3% PHMG-Cl and by 0.4 μm between 3% and 4%.

EDS microanalysis showed the presence of chlorine ($1.75 \pm 0.25\%$) in all areas of the surface of PU/PHMG-Cl (4%) sample (Fig. 3, 3). Since the chlorine content in PHMG-Cl is 20 wt%, PU/PHMG-Cl (4%) composites should contain 0.8% of Cl in the volume. The excess chlorine content on the surface of PU films is obviously due to the orientation of hydrophilic ionic groups outward of the polymer matrix. The presence of hydrophilic polymeric biocide on the surface may thus explain the reduction of contact angle value reported in Fig. 2. Let us noticed that EDS measurements didn't detect any chlorine on the surface of PU/PHMG-Cl films containing 1% and 2% of polymeric biocide (Fig. 3, 2). Therefore, the increase in the hydrophobicity of these PU/PHMG-Cl composite films (Fig. 2) is probably due to the physicochemical interactions of the polymeric biocide with polar groups of PU, thus reducing the wetting of the surface polymer with the water.

According to TGA data, neat PU has thermal decomposition point (5% weight loss) 314 °C (Fig. 5a). This value is very similar to those for polymeric biocide PHMG-Cl which is thermally stable to at least 313 °C [50]. The thermo-oxidative degradation of neat PU takes place in successive decomposition steps occurring between 300 and 650 °C (Fig. 5a). The first one is related to the decomposition of the hard segments, whereas the second step is due to the composition of the soft segments [16]. The introduction of PHMG-Cl did not cause significant changes of thermal stability of PU (Fig. 5b). The results of TGA for PU/PHMG-Cl composites containing from 1 to 4 wt% of polymeric biocide are summarized in Table 2. From these data it can be supposed that PHMG-Cl is acceptable modifying additive for joint melt processing with PU resin.

According to DSC data, polymeric biocide PHMG-Cl has glass transition temperature (T_g) of 6.3 °C and melting point (T_m) of 137 °C (Fig. 6, curve 1). Neat polyurethane melts at 134 °C and has T_g value of -37.4 °C

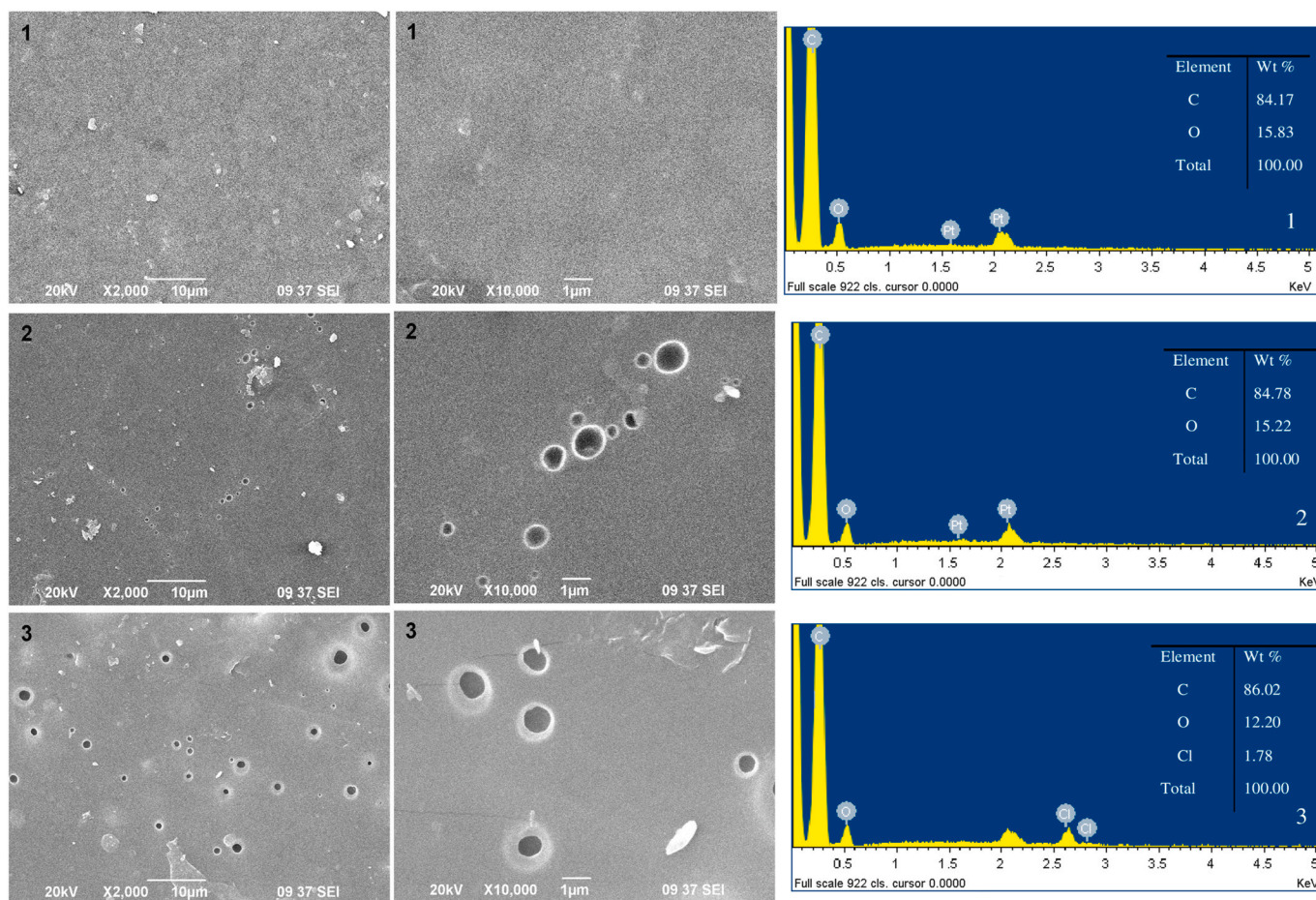


Fig. 3. SEM and EDS images of polymer films: 1 – PU, 2 – PU/PHMG-Cl (2%), 3 – PU/PHMG-Cl (4%)

*SEM images captions from left to right: accelerating voltage, magnification, scale label, focal length of an electron lens forming an electron beam, electron beam current in relative units, image acquisition mode (secondary electron imaging – SEI).

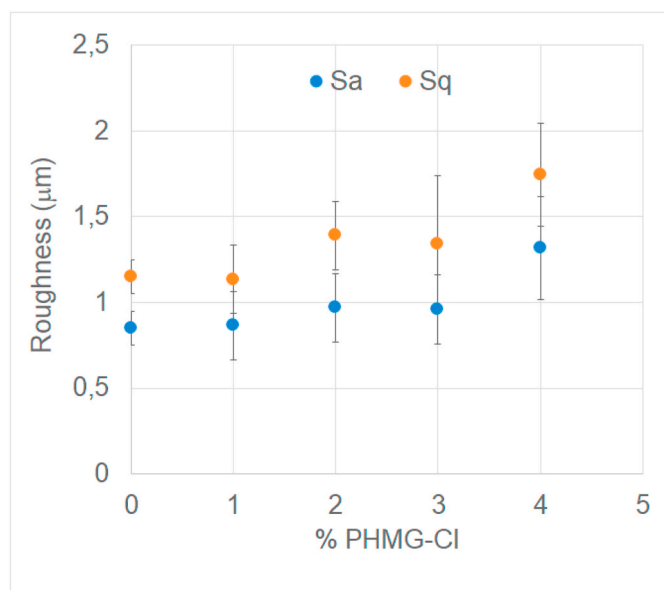


Fig. 4. Surface roughness of PU/PHMG-Cl films.

(Fig. 6, curve 2). For PU/PHMG-Cl composites, noticeable shift of both T_g and T_m to lower values was detected. Thus, introduction of 4% of PHMG-Cl into PU led to reduction of glass transition temperature of the

polymer by 8.5 °C, as well as its melting temperature by 7 °C (Fig. 6, curve 4). For lower PHMG-Cl content, similar decrease of T_g and T_m values were observed (Fig. 6, curve 3, Table 3). Overall, the obtained results indicate plasticizing effect of PHMG-Cl on PU that is confirmed by significantly increased elongation at break of PU/PHMG-Cl films (Table 1).

3.3. Biocide release behavior of PU/PHMG-Cl films

UV-visible absorption spectrum of PHMG-Cl in water solutions (Fig. 7, curve 1) presents a maximum peak at 192 nm that corresponds to the adsorption of C=N groups [58]. In 0.9% saline solution, the maximum peak is shifted to 204 nm (Fig. 7, curve 2). The release properties of the films were tested by keeping PU/PHMG-Cl samples in contact with deionized water and saline solutions for 10 h. Both solutions were analyzed by UV-Vis spectroscopy and the observation of maximum peaks around 192 nm and 204 nm for water and saline solution respectively confirms the release behavior of polymeric biocide into water from PU/PHMG-Cl films.

In order to determine the release properties of the film as function of PHMG-Cl content, we measured the absorbance at 192 nm as function of time and calculate the percentage of PHMG-Cl released into water from its total quantity in the film. For PU sample containing 1% of PHMG-Cl, fast biocide release (almost 16%) was observed after 8 h followed by more gradual release reaching the value 29% after 50 h (Fig. 8, curve 1). For PU films with higher PHMG-Cl content (from 2 to 4%), biocide release curves contain three distinct regions (Fig. 8, curves 2–4). The

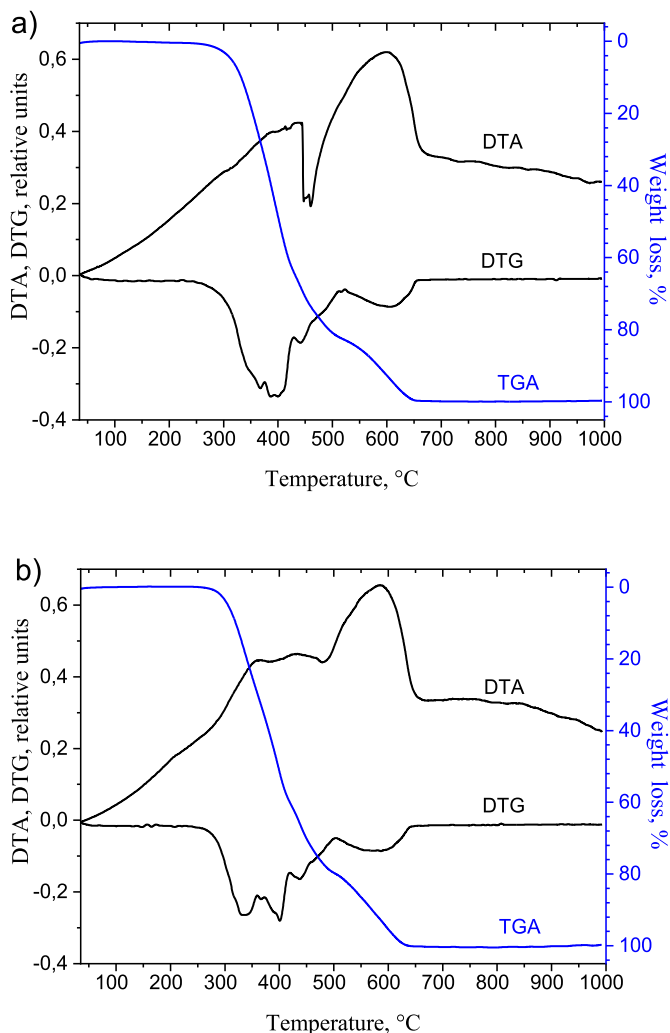


Fig. 5. TGA curves of PU (a) and composite PU/PHMG-Cl (4%) (b).

Table 2
TGA data for modified PU samples.

Sample	$T_{\Delta m = 5\%}$, °C	$T_{\Delta m = 10\%}$, °C	$T_{\Delta m = 20\%}$, °C	$T_{\Delta m = 50\%}$, °C
PU control	314	332	353	402
PHMG-Cl	313	347	358	389
PU/PHMG-Cl (1%)	306	321	341	400
PU/PHMG-Cl (2%)	305	320	340	397
PU/PHMG-Cl (3%)	305	318	338	397
PU/PHMG-Cl (4%)	303	317	337	395

fastest releases of PHMG-Cl were observed during the first hour of immersion: 12.7% for PU samples with 2% biocide content (curve 2) and more than 22% for polymer films with 3 and 4% PHMG-Cl (curves 3, 4). From 2 to 8 h a fast biocide release for all these samples was also detected followed by more gradual constant release in the next stage. After 50 h immersion, PU films containing 2, 3 and 4% of PHMG-Cl showed relatively similar biocide release ratio: 41.6%, 45.4% and 48.7%, respectively (Fig. 8). Spectrophotometric control of PHMG-Cl release from PU films have shown that this process continued for at least 14 days reaching the release ratio of 57% for PU/PHMG-Cl (3%) and 70% for PU/PHMG-Cl (4%) composites (not shown in Figure).

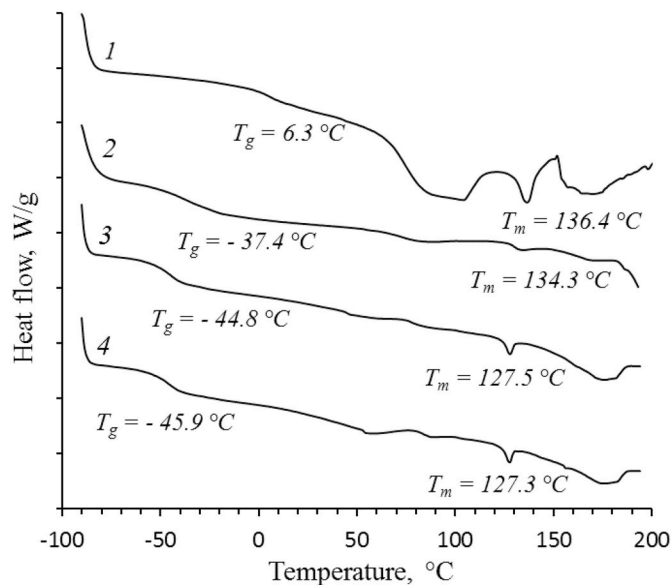


Fig. 6. DSC thermograms: 1 – PHMG-Cl, 2 – PU, 3 – PU/PHMG-Cl (1%), 4 – PU/PHMG-Cl (4%)

Table 3
DSC data for modified PU samples.

Sample	T_g , °C	ΔC_p , J/(g·°C)	T_m , °C
PU control	-37.4	0.26	134.3
PHMG-Cl	6.3	0.17	136.4
PU/PHMG-Cl (1%)	-44.8	0.23	127.5
PU/PHMG-Cl (2%)	-45.3	0.22	128.3
PU/PHMG-Cl (3%)	-45.2	0.24	127.7
PU/PHMG-Cl (4%)	-45.9	0.21	127.3

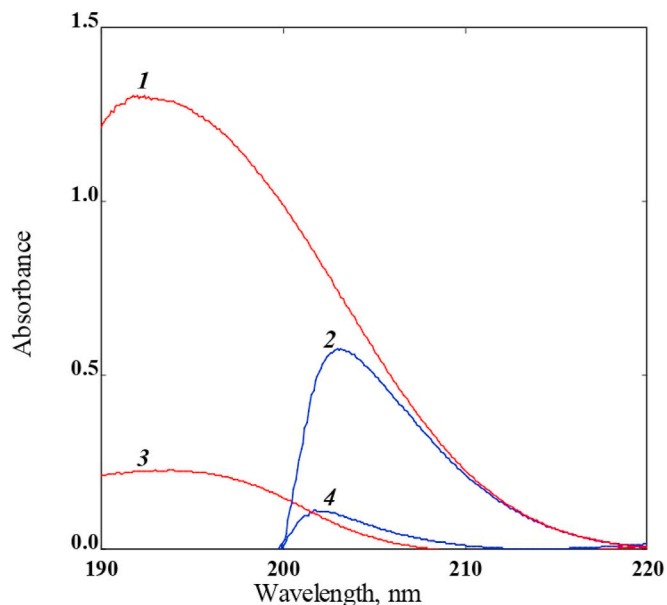


Fig. 7. UV-visible absorption spectra of PHMG-Cl: 1 - $C = 1.1 \cdot 10^{-4}$ mol/l (water), 2 - $C = 1.1 \cdot 10^{-4}$ mol/l (0.9% saline), 3 - water solution after 10 h contact with PU/PHMG-Cl (1%) film, 4-0.9% saline after 10 h contact with PU/PHMG-Cl (1%) film.

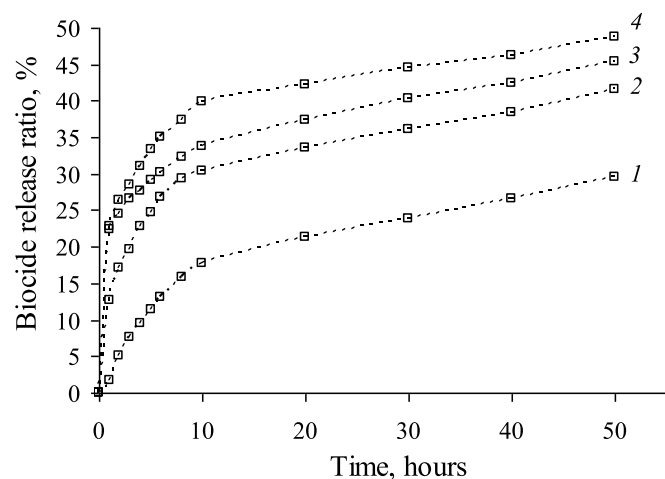


Fig. 8. The release behavior of polymeric biocide into water from PU/PHMG-Cl films containing 1% (1), 2% (2), 3% (3) and 4% (4) of PHMG-Cl.

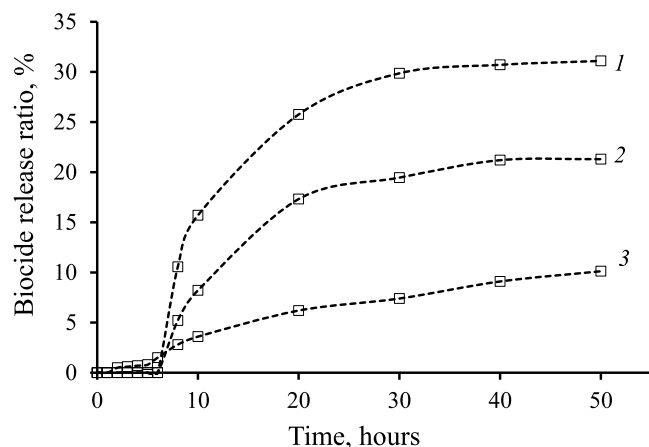


Fig. 9. The release behavior of polymeric biocide into 0.9% saline from PU/PHMG-Cl films containing 1% (1), 2% (2) and 3% (3) of PHMG-Cl.

Taking into account poor solubility of PHMG-Cl in sodium chloride water solutions [50], delayed release rate of polymeric biocide from PU films into physiological saline could be expected. Indeed, the analysis of biocide release curves for polymer films containing 1–3% PHMG-Cl indicated the presence of induction period for at least 6 h (Fig. 9). For PU/PHMG-Cl (1%) samples, a fast biocide release was observed over the next 4 h followed by more gradual release rate (Fig. 9, curve 1). However, the total percentage of PHMG-Cl release was found to be similar for both water and saline after 50 h, 29% and 31%, respectively. For higher biocide content in PU films (2% and 3%), much lower total percentage of its release was detected: 21.3% and 10.1%, respectively, compared to pure water (Fig. 9, curves 2 and 3). At highest PHMG-Cl content in PU films of 4%, biocide release behavior curve had similar view (not shown).

It should be noted that the prepared PU/PHMG-Cl composites showed similar biocide release profile as electrospun nanofibre PU/PHMB membranes reported in Ref. [42]. Overall, the results of this study indicate that PU/PHMG-Cl composites may be efficient controlled biocide release systems and therefore are promising for use as medical dressings.

3.4. Antibacterial activity of PU/PHMG-Cl films

The antibacterial activity of PU/PHMG-Cl films was tested using disc diffusion assays (Fig. 10). Control PU films, as well as PU/PHMG-Cl

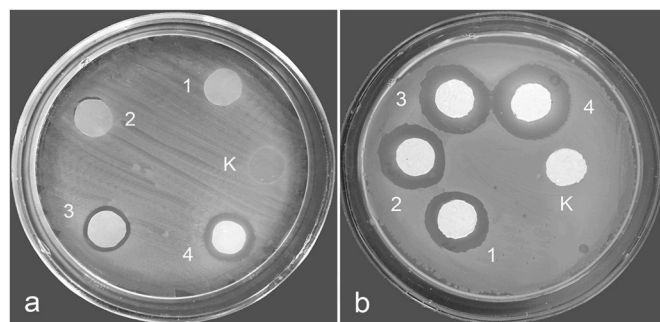


Fig. 10. PU discs on the surface of LB agar inoculated with *E. coli* (a) and *B. subtilis* (b) after 24 h incubation (Agar Disc Diffusion Test): K – neat PU (control), 1–4 – PU films containing from 1 to 4% of PHMG-Cl, respectively.

composites containing 1% and 2% of polymeric biocide did not show detectable zones of inhibition of bacterial growth (*E. coli*) around sample discs (Fig. 10, a). The appearance of clear zone of inhibition was detected for PU/PHMG-Cl (3%) sample, and this zone significantly increased for PU/PHMG-Cl (4%) (Fig. 10). The obtained data indicate low antibacterial activity of modified PU films, probably caused by slow release of polymeric biocide into agar medium. Our data are in good agreement with those previously reported on antibacterial activity of PU/PHMB composite membranes [42]. Indeed, PU films containing from 5 to 15% of polymeric biocide PHMB-Cl showed small diameters of zones of inhibition (2–6 mm) in liquid *S. aureus* bacterial cultures [42]. Significant values of inhibition zone diameter above 10 mm were observed only for polymer films with much higher PHMB content (25–35%).

In LB agar inoculated with *B. subtilis*, modified PU films form much larger inhibition halos even at lower PHMG-Cl content (Fig. 10, b, Table 4). This indicates higher sensitivity of gram-positive bacteria (*B. subtilis*) to PHMG-Cl, compared to gram-negative (*E. coli*).

The antibacterial activity of PU/PHMG-Cl films were also tested by contact method (Fig. 11 and Table 4). Significant reduction of bacterial colonies (*E. coli*) grown in agar medium after 24 h contact with polymer films was detected for PU/PHMG-Cl samples containing 2% of polymeric biocide (Fig. 11, b). Further increase of PHMG-Cl content to 3% led to total inhibition of bacterial growth (Fig. 11, c). Modified PU films were also found to have higher activity against gram-positive bacteria *B. subtilis*. Thus, total inhibition of test-culture was detected for PU/PHMG-Cl (2%) (not shown in Figure).

Overall, the obtained data indicate that PU/PHMG-Cl composites possess pronounced antibacterial activity at biocide content starting from 3% and combine properties of both biocide release system and contact active antimicrobial material. At lower PHMG-Cl content of 2%, the antibacterial activity of modified PU films is probably realized mainly by contact active mechanism. It is worth noting that similar content (5 wt%) of commonly used biocides such as silver nanoparticles and PHMB-Cl were required to inhibit the growth of common hospital pathogenic bacteria on the surface of PU based dressing materials [42, 59].

Table 4
Antibacterial activity of PU/PHMG-Cl composite films.

Sample	nw halo		LV, %	
	<i>E.coli</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>B. subtilis</i>
PU/PHMG-Cl (1%)	0	0.27	90	99
PU/PHMG-Cl (2%)	0	0.33	99.97	99.999
PU/PHMG-Cl (3%)	0.11	0.38	99.999	-
PU/PHMG-Cl (4%)	0.28	0.44	-	-

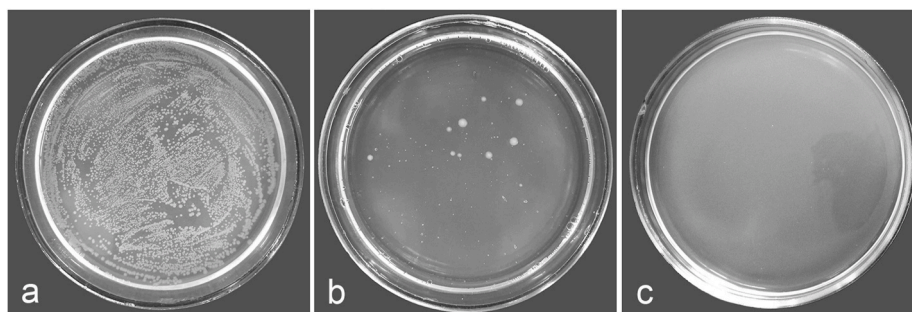


Fig. 11. Bacterial colonies (*E. coli*) grown on LB agar medium after 24 h contact with PU films (Antibacterial Contact Method): a – neat PU, b – PU/PHMG-Cl (2%), c – PU/PHMG-Cl (3%).

4. Conclusion

Commercial polyurethane Laripur®LPR9020 was blended with polymeric biocide PHMG-Cl using solvent casting method to prepare antibacterial polymer composites containing from 1 to 4 wt% of PHMG-Cl. The modified polymer films showed similar tensile strength and noticeably improved elasticity (by at least 20–30%), compared to neat PU. The surface of PU/PHMG-Cl films was found to possess higher hydrophobicity than neat polymer that is manifested in significantly increased water contact angle values. This effect is probably caused by physicochemical interaction between polymeric biocide and PU which was detected by IR analysis. According to the results of TGA, PU/PHMG-Cl composites have thermal decomposition points above 300 °C which are only slightly lower than those for pure polymer. The results of DSC measurements showed noticeable reduction of both glass transition temperature and melting temperature of PU (by more than 7 °C) when contained only 1% of PHMG-Cl. However, further increase of additive content had little or no effect on thermophysical properties of PU which may indicate limited plasticizing effect of PHMG-Cl.

The results of spectrophotometric study showed gradual release of polymeric biocide from PU films into water for at least 14 days. At the first stage (8–10 h) fast biocide release was detected for all PU/PHMG-Cl composites followed by more gradual stage. The total biocide release ratio was found to be from 29% to 48% after 50 h immersion, depending on its content in polymer composite. PU films containing 2% and 3% of PHMG-Cl showed much lower biocide release rate into physiological saline solution which may be caused by poor solubility of polymeric biocide in sodium chloride solutions. Thus, only 21% and 10% of PHMG-Cl released from PU films after 50 h immersion in 0.9% NaCl solution.

The results of microbiological study of modified PU films indicate their high antibacterial activity when contained 2% and 3% of polymeric biocide. The material can be considered both biocide release and contact active system. The latter (contact active) mechanism of antibacterial activity probably dominates, especially at low PHMG-Cl content of 2% and 3%.

Thus, the modification of PU resin with polymeric biocide PHMG-Cl seems efficient approach to prepare new promising antimicrobial materials with controlled biocide release which may find different medical applications. Particularly, PU/PHMG-Cl composites can be used to fabricate dressings for healing of dermal or burn wounds, as well as surgical drapes, where local antimicrobial activity is required.

CRediT authorship contribution statement

Sergiy Rogalsky: synthesized polymeric biocide, analyzed data, and wrote the paper. **Jean-François Bardeau:** wrote the paper. performed infrared analysis, surface analysis, and wrote the paper. **Lyudmila Lyoshina:** designed and performed antibacterial experiments. **Oksana Tarasyuk:** prepared polymer composites and performed spectrophotometric studies. **Olga Bulko:** performed antibacterial experiments. **Oleg Dzhuzha:** performed elemental analysis and surface analysis. **Tetiana**

Cherniavska: performed thermal gravimetric analysis. **Valeriy Kremenitsky:** performed SEM and EDS analysis. **Larisa Kobrina:** performed DSC analysis. **Sergii Riabov:** conceived and designed the experiments, and analyzed the data.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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