Materials Research Express

PAPER

Poly(hexamethylene biguanide) salicylate as a novel ionic liquid with antibacterial properties in the production of poly(vinyl alcohol) films

Anna Słubik®, Iwona Maślowska-Lipowicz, Dorota Wieczorek and Łucja Wyrębska

Łukasiewicz Research Network—Łódź Institute of Technology, Zgierska 73, 91-463 Łódź, Poland

E-mail: anna.słubik@lit.lukasiewicz.gov.pl

Keywords: ionic liquid, PHMB-SA, poly(vinyl alcohol), antibacterial properties

Abstract

The presented work describes the effect of poly(hexamethylene biguanide) salicylate (PHMB-SA) ionic liquid on the properties of poly(vinyl alcohol) film. The ionic liquid synthesized is used as an antimicrobial agent. The ionic liquid consists of the poly(hexamethylene biguanide) cation and the salicylic anion, which exhibit synergistic antibacterial and bacteriostatic properties. The structure of obtained new ionic liquid was confirmed by infrared spectroscopy and nuclear magnetic resonance. Additionally, the article describes a simple method of producing an antibacterial PVA-based film with the addition of collagen and an ionic liquid. The study investigated the effect of the amount of the PHMB-SA on the mechanical properties, microorganism in liquid medium and the antibacterial properties of the PVA film. The obtained results indicate that the mechanical and antibacterial properties of the PVA/Col films depended on the amount of the ionic liquid. The higher value of the tensile strength (27.11 MPa) and the higher degree of microbial growth inhibition (7 mm) was obtained for the sample containing 0.75 g PHMB-SA.

Introduction

At the turn of the 20th and 21st centuries, many scientific and technological centers around the world undertook intensive research on a new group of substances—ionic liquids. Currently, an ionic liquid is defined as chemical compounds with an ionic structure, that show a melting point below 100 °C, low vapor pressure, high resistance to fire, high polarity, high thermal stability, amphiphilicity, surface and interfacial properties, the ability to dissolve inorganic and organic compounds, as well as some polymers [1–3]. Their greatest advantage is the designability associated with the selection of an appropriate cation and anion, which allows to obtain a compound with the desired properties. Ionic liquids are used in many fields: electrochemistry, separation techniques, synthesis, catalysis, medicine, chemistry of surfactants [1–8]. The literature is dominated by studies on ionic liquids with a positive charge located on the quaternary nitrogen atom (like as, alkylimidazolium, alkylpyridinium). There are few studies on polymeric ionic liquids belonging to the derivatives of guanidine [9, 10] and biguanide [11].

Poly(hexamethylene biguanide) (PHMB) is a polymer used as a disinfectant and antiseptic [12]. PHMB is effective against pathogens such as bacteria, amoebas and yeasts [13–16]. Moreover, there is a growing number of reports indicating that PHMB has anti-HIV activity [17–19]. Due to the wide spectrum of activity of PHMB against pathogenic microorganisms, it is used as a disinfectant in human and veterinary medicine. It is used in dermatology for the care and treatment of chronic wounds, disinfection of skin and mucous membranes before and after surgery, for surgical and non-surgical dressings, sterilization of surgical instruments, medical and industrial equipment, it is a component of hand and surface disinfectants, as well as eye drops for the treatment of keratitis [13, 20–22]. Disinfectants with a biocidal effect, the so-called HDPs, to which PHMB belongs, are a new innovative group of biocides competing today with silver preparations, the nanofragmentation of which causes their migration through human cell membranes and, consequently, deposition in internal organs. It has...
been proven that nanosilver from antiseptic dressings, migrates into the blood [23]. PHMB is free of heavy metals and phenols, and is also non-corrosive and non-toxic to humans and animals as it is an environmentally friendly product [24–26]. Salicylic acid or ortho-hydroxybenzoic acid (SA) and related compounds belong to a diverse group of plant phenolic. It belongs to the endogenous plant hormones necessary for plant growth [27, 28]. Salicylic acid is obtained from the white willow (Salix alba), mainly from its roots [29]. It is a safe compound for human health and the environment, additionally exhibits antifungal properties against many types of microorganisms [27, 28].

The aim of our research was the synthesis of new ionic liquid based on poly(hexamethylene biguanide) and salicylic acid. The designed ionic liquid was used for the production of polyvinyl alcohol and waste collagen film in order to give it antibacterial properties. In the pro-ecological aspect, an important issue of research is the use of waste material from the leather industry—collagen, which is non-toxic and highly biodegradable. Collagen decomposes in the presence of an appropriate content of water, oxygen, carbon dioxide and soil microorganisms, therefore it does not remain in the environment for many years [30, 31]. Our study includes tests mechanical and antimicrobial properties of a new type of polymer film.

**Experimental parts**

**Materials**
The following materials were used to produce PVA/Col/PHMB-SA films:

- poly(vinyl alcohol) (PVA) (product of the Sigma-Aldrich company) with molecular weight 13000–23000 and degree of hydrolysis 87–89,
- collagen hydrolysates obtained from tanning waste [31],
- poly(hexamethylene biguanide) salicylate (PHMB-SA) ionic liquid synthetized at Lukasiewicz Research Network – Lodz Institute of Technology according to Patent Application nr P. 440793 [32],
- glycerin (product of the Eurochem BGD Sp. z.o.o company) with a density 1.263 g cm⁻³,
- SPUMOL (product of the Organika S.A., Poland company) used as a defoamer.

**Synthesis of the ionic liquid PHMB-SA**
The 20% aqueous solution of poly(hexamethylene biguanidine) in the form of hydrochloride and the appropriate amount of ethyl alcohol were placed in a flask equipped with a high-speed stirrer. Then, after stirring for 0.5 h, the solution of 160 g of sodium salicylate (1 mol) in 200 ml of water was added dropwise over 2 h. The contents of the flask were subjected to intensive stirring at the temperature of 20 °C–30 °C for 1 h at the pH value 6.5–7. The two-layer mixture was poured into a separating funnel and left overnight. The lower layer was washed with water and then dried in a vacuum oven at 40 °C for 10 h. The process yield was 80%.

**Preparation of the PVA/Col/PHMB-SA films**
The polymer film were prepared using 10% PVA solution in distilled and deionized water. The viscous and transparent solution of PVA was obtained after mixing PVA powder with water for 4 h at a temperature of about 80 °C–90 °C. Then, 0.5 g of collagen and 0.5 g of glycerin was added to 10 ml of 10% PVA. The mixture was stirred at 50 °C until a clear solution was obtained. Then, 0.25 g of PHMB-salicylate was added with constant stirring at a temperature of 50 °C. The resulting solution was poured into appropriately shaped molds and dried at 40 °C for 6 h. A second film containing 0.75 g of PHMB-salicylate was prepared in an analogous manner as a first film.

**Characterization methods**

**¹H NMR spectroscopy analysis**
The nuclear magnetic resonance (NMR) spectra of the PHMB-SA were measured using a Bruker Avance II Plus apparatus (Bruker, Billerica, MA, USA) at 700 MHz (¹H NMR). The measurements were carried out in d-methanol (CD₂OD), using solvent signals as a reference (¹H NMR, δ = 3.33 ppm and δ = 5.15 ppm (CD₂OD)). Chemical shifts (δ) were presented in ppm. ³¹HNMR (700 MHz; CD₂OD, ppm): δ = 1.307(br s,
PHMB); 1.505 (br s, PHMB); 3.181 (br s, PHMB); 6.796–6.835 (arom. 1H); 6.846–6.847 (arom. 1H); 7.297–7.322 (arom. 1H); 7.853–7.867 (arom. 1H). FTIR (cm$^{-1}$): 3318 (brm), 2934 (s), 2173 (m), 1544 (s), 1483 (m), 1454 (m), 1381 (s), 1338 (w), 1303 (w), 1250 (m), 1141 (w), 1045 (w), 859 (m), 757 (m).

Infrared spectroscopy
The infrared spectra of the ionic liquid was developed with Thermo Scientific Nicolet 6700 FT-IR spectrometer equipped with Smart Orbit ATR (Waltkam, MA, USA) diamond attachment, using the attenuated total reflectance (ATR) method. The spectra were made for the wavenumber range of 3500–500 cm$^{-1}$. Before the spectra of samples were made, background measurement had been carried out, each time including 64 scans.

Mechanical properties
The tensile strength properties were measured in accordance with the PN-ISO 37:2007 standard using a ZWICK machine (model 1435) (Ulm, Germany) connected with the appropriate computer software. The scope of the properties tests was included: stress at 100% of elongation ($S_{100}$), tensile strength ($TS_b$), and elongation at break ($E_b$).

Antibacterial properties
Two bacterial strains: *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 9144 were used as bacteria to test the antimicrobial properties of the PVA/Col/PHMB-SA films. Antibacterial tests were carried out in accordance with the PN-EN ISO 20645 standard. Samples were placed on plates with two-layer agar. The lower layer contains only agar, the upper layer contains agar inoculated with a suspension of bacteria of a certain density. After the incubation period, the growth of the bacteria in the contact zone between the agar and the working sample and the zone of inhibition of growth around the working sample were assessed. The zone of inhibition of growth was calculated using the following formula (equation (1)):

$$H = \frac{D - d}{2}$$

where: $H$ - width of the braking zone [mm]; $D$ - the total diameter of the working specimen and the width of the braking zones [mm], $d$ - diameter of the working specimen [mm].

Effect of PVA/Col/PHMB-SA film on the microorganism in liquid medium
Yeast strain *Y. lipolitica* IPS21 from the pure culture of collection Lukasiewicz Research Network - Lodz Institute of Technology, were maintained on modified yeast YPG medium (15 g/1 yeast extract, 5 g/1 chloride sodium). Parameters of tap water: pH 7.0, conductivity: 433 ms ml$^{-1}$, Ca: 70 mg ml$^{-1}$, Mg$^{2+}$: 30 mg ml$^{-1}$, Na: 5.61 mg ml$^{-1}$. To prepare the inoculum, the stock suspension over with 10 ml of saline was poured and left after washing the microorganism cells from the slopes. The resulting suspension was diluted with saline until the culture density was OD 600 = 1.000. The use of the optical density measurement method covers the determination of the course of the cultivation of microorganisms as well as obtaining precise information about the influence of factors on the cultivation course. The study of growth kinetics on the basis of density measurement is an important determinant of the viability of microorganisms living in the natural environment. The stock suspension (0.4 ml) was transferred to 100 ml flasks containing 40 ml of sterile medium YPG with PVA or PVA/Col film with PHMB-SA (without agar) and cultivated for 48 h at 30 °C on an orbital shaker (210 rpm). Before process by autoclaving (121 °C, 20 min) was sterilization.

Dehydrogenase activity was determined according to Miksch (1985) with the use of 2,3,5-triphenyltetrazolium chloride (TTC). Under mild reducing conditions, the colorless TTC was converted into colored 1,3,5-triphenyltetrazolium formazan (TF). The test consists of the following stages: (1) addition of the reagents to the supernatant (diluted twice TRIS HCl pH 8) in the proper order: 1 ml solution of sodium sulphite (Na$_2$SO$_3$), 1 ml distilled water (control sample) or the proper solution of the tested, and finally 0.1 ml 3% solution of TTC; (2) 24 h incubation in the dark at room temperature 21 °C ± 0.5; (3) sludge separation; (4) stopping the reaction by the addition of pure 100% ethanol; and (5) measurement of absorbance at 485 nm. TTC solutions in the range from 0.2% to 4% were tested in order to optimize the concentration of this reagent in the dehydrogenase tests. It occurred that 3% solution of TTC was optimal for the supernatants studied. The incubation time equal to 1 h was assumed in all tests. Measurement of the change in pH during cultivation was performed every 24 h using an Elmetron CP404 pH meter.
Results and discussion

Characterization of the structure of the PHMB-SA

As a result of the chemical reaction described in the methodology, the polymeric ionic liquid was obtained with the structure shown in figure 1. The liquid was oily, crystal clear, with a slightly straw shade and high viscosity. The reaction yield was 80%. The obtained ionic liquid, had limited solubility in water (up to 1%), in contrast to PHMB. It is not soluble in acetone, toluene, hexane, but soluble in ethanol, dimethylsulfoxide, N-methylpyrrolidone, dimethylformamide, dimethylacetamide, tetrahydrofuran.

The structure of the polymeric ionic liquid was confirmed spectroscopically by nuclear magnetic resonance $^1$HNMR (figure 2). The spectrum showed three characteristic peaks for the protons of poly(hexamethylene biguanide): $^1$HNMR (CD$_3$OD) 1.307, 1.505, 3.181 ppm and the signals of the four aromatic protons of the salicylate anion 6.796, 6.846, 7.297 and 7.853 ppm.

The structure of the polymeric ionic liquid was confirmed spectroscopically by nuclear magnetic resonance $^1$HNMR (figure 2). The spectrum showed three characteristic peaks for the protons of poly(hexamethylene biguanide): $^1$HNMR (CD$_3$OD) 1.307, 1.505, 3.181 ppm and the signals of the four aromatic protons of the salicylate anion 6.796, 6.846, 7.297 and 7.853 ppm.

The structure of the resulting ionic liquid was also confirmed on the basis of the FTIR spectrum (figure 3). In the FTIR spectrum, absorption bands attributed to the N-H stretching vibration at the wavenumber of 3318 cm$^{-1}$ and the N-H deformation vibration at the 1544 cm$^{-1}$ wavenumber present in poly(hexamethylene biguanide) were observed. Also, the bands at the wavenumber of 2173 cm$^{-1}$ was related to the stretching vibration of the C=\(N\) group present in poly(hexamethylene biguanidine). There was also an apparent
carboxylate anion that absorbs in a different frequency region than other carbonyl compounds. It is caused by mesomerism, as a result of which both C–O bonds become equal bonds with an order intermediate between the order of the C=O and C–O bond and the intermediate value of the force constant. The vibrations of these bonds were also strongly mechanically coupled, therefore, a band of asymmetric C–O stretching vibrations at wavenumber of 1483 cm\(^{-1}\) and a band of symmetrical stretching vibrations at wavenumber of 1454 cm\(^{-1}\) were observed in the FTIR spectrum. Moreover, absorption bands from C–H deformation vibrations in the two-substituted aromatic ring at the wavenumber of 757 cm\(^{-1}\) were visible\[32, 33\].

Mechanical properties
The obtained PHMB-SA ionic liquid was used as a new antibacterial substance for the production of film based on poly(vinyl alcohol) and waste collagen. The films were prepared using the pouring method, and then their strength properties were tested. The tensile strength test simulates one of the most important performance parameters of polymer materials- mechanical strength, which has a key impact on the service life of material in a specific application environment. The mechanical properties of PVA/Col films with different amount of PHMB-SA are given in Table 1. The amount of ionic liquid to obtain PVA/Col films affected the parameters such as the stress at an elongation of 100%, the tensile strength, the elongation at break and maximum strength at break. In the case of the stress at an elongation of 100%, the incorporation of more amount of the PHMB-SA into the PVA/Col film led to a decrease in the value of this parameter. The tensile strength depends on the nature and type of cross-links, cross-link densities, and chemical structure of the used polymer. Higher tensile strength (TS\(_b\) = 27.11 MPa) and higher maximum strength at break (63.87 MPa) was observed for the sample with the

| PHMB-SA (g) | S\(_{100}\) (MPa) | TS\(_b\) (MPa) | E\(_b\) (%) | F\(_{max}\) (MPa) |
|-------------|-----------------|--------------|------------|---------------|
| 0.25        | 18.88 ± 1.35    | 25.61 ± 0.69 | 203 ± 5    | 56.46 ± 1.13  |
| 0.75        | 16.39 ± 0.76    | 27.11 ± 0.44 | 260 ± 13   | 63.87 ± 1.50  |

S\(_{100}\) — stress at elongation of 100%, TS\(_b\) — tensile strength, E\(_b\) — elongation at break, F\(_{max}\) — maximum strength at break.

Figure 3. FTIR spectra of the PHMB-SA ionic liquid.

Table 1. Effect of the amount of the PHMB-SA on the mechanical properties of the PVA/Col films.
content of 0.75 g ionic liquid. Additionally, the sample with more ionic liquid was characterized by higher elongation at break (260%).

**Antibacterial properties**

Determination of the antibacterial activity of the PVA/Col films with addition of the PHMB-SA was carried out against two strains of bacteria: *E. coli* ATCC 8739, *S. aureus* ATCC 9144. The growth of bacteria was assessed on the basis of the designated zone of inhibition of growth around the test sample (table 2).

The results of the antibacterial activity of the tested films against *E. coli* and *S. aureus* are presented in the table 3. The applied antibacterial substance—PHMB-SA showed a very good antibacterial effect in relation to the test microorganisms used. A good effect of the ionic liquid against both gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria was observed. After the incubation period of the plates with the tested samples, zones of inhibition around the tested samples and no growth directly on the samples were observed for both strains. In the case of the reference sample (pure PVA film), the strong growth of microorganisms and no inhibition zone was observed around the PVA film, indicating that PVA film did not have antibacterial activity. When PHMB was incorporated in the formulations, the films were found to have an antimicrobial effect against both species. There was increased inhibition of bacteria growth with increasing PHMB content. The inhibition zone for the sample containing 0.75 g of PHMB-SA were 7 and 6 for *E. coli* and *S. aureus*, respectively.

**Effect of PVA/Col/PHMB-SA film on the microorganism in liquid medium**

The ability of microorganisms to decompose organic compounds in the tested environment was assessed by determining the activity of dehydrogenases (figure 4). Dehydrogenases are enzymes from the group of oxidoreductases that catalyze the oxidation of organic substances. These enzymes detach electrons from the substrate and attach them to protons. The donation of electrons to oxygen takes place via electron carriers, e.g. NAD, NADP, flavoproteins, ubiquinone, cytochromes. The activity of dehydrogenases can be an indicator of the biochemical activity of microorganisms both in the soil and in the water environment. This determination allows the assessment of the ability of microorganisms to decompose organic compounds present in the tested environment [34]. In this research, the highest activity of dehydrogenases was determined in 48 h for the PVA/Col (1 g) sample. This activity was higher than for the PVA sample. This result indicates that the presence of collagen increases the activity of enzymes in the yeast *Y. lipolytica* IPS12 and has a positive effect on the biochemical activity of the strain. The lowest dehydrogenase activity in the trials with PHMB-SA was observed. PHMB is an antiseptic with antiviral and antibacterial properties [35]. The lowest dehydrogenase activity was observed in the samples of PVA/Col/PHMB-SA because the PHMB can lead to inhibition of the growth of microorganisms, which was also confirmed in antibacterial tests (table 3).

*Y. lipolytica* yeasts are aerobic organisms and do not conduct fermentation processes. The optimal temperature for their growth is in the range of 20 °C–28 °C, although the multiplication of these organisms is also observed in a wider temperature range: 5 °C–37 °C. *Y. lipolytica* grows in the pH range from 2 to 8 with a water activity of 0.85–0.89 [36]. The starting pH of the culture medium after sterilization was 6.02. Whereas, the highest pH value (8.89 at 48 h) was recorded in the PVA/Col (1 g) sample (figure 5). The significant increase in pH in the medium was probably due to the intensive metabolism of yeast and the absorption of organic nitrogen from the medium and collagen and/or the ongoing consumption of carboxylic acids, which may be a product of PVA decomposition. In the work of Larking et al an similar phenomenon was observed. The initial increase in pH was related to the use of PVA-derived carboxylic acids by the fungus [37]. As other studies show, the pH value has a significant influence on the biodegradation process of PVA. Zhang et al showed that the maximum rate of biodegradation in the presence of *Curtobacterium* sp. and *Bacillus* sp. occurs at an initial pH of 8.0 [38]. In the sample containing PHMB-SA, the lowest pH value was found. The pH in the aqueous solution of PHMB-SA was

| Table 2. Assessment of the bacteria growth on the tested sample. |
|---------------------------------------------------------------|
| Inhibition zone | Growth on the surface on the sample | Description | Assessment |
| >1 | lack | Inhibition zone above 1 mm, lack of growth | Good effect |
| 1−0 | lack | Inhibition zone up to 1 mm, lack of growth | |
| 0 | lack | No inhibition zone, lack of growth | |
| 0 | low | No inhibition zone, only some colonies limited, growth stopped almost completely | Limited efficiency |
| 0 | medium | No inhibition zone, half the increase compared to the control | |
| 0 | strong | No inhibition zone, no reduction in growth compared to the control, or only a slight reduction in growth | No effect |


Table 3. The results of the antibacterial activity of the tested films against *E. coli* and *S. aureus*.

| Type of bacteria | Sample                      | Growth | Inhibition zone (mm) | Description                                                                 | Assessment       |
|------------------|-----------------------------|--------|----------------------|-----------------------------------------------------------------------------|------------------|
| *E. coli*        | PVA                         | strong | 0                    | No inhibition zone, no reduction in growth compared to the control          | No effect        |
|                  | PVA/Col/PHMB-SA (0.25 g)    | lack   | 5                    | Inhibition zone above 1 mm, lack of growth                                  | Good effect      |
|                  | PVA/Col/PHMB-SA (0.75 g)    | lack   | 7                    | Inhibition zone above 1 mm, lack of growth                                  | Good effect      |
| *S. aureus*      | PVA                         | strong | 0                    | No inhibition zone, no reduction in growth compared to the control          | No effect        |
|                  | PVA/Col/PHMB-SA (0.25 g)    | lack   | 5                    | Inhibition zone above 1 mm, lack of growth                                  | Good effect      |
|                  | PVA/Col/PHMB-SA (0.75 g)    | lack   | 6                    | Inhibition zone above 1 mm, lack of growth                                  | Good effect      |
approximately 5.6, regardless of the amount of the antibacterial substance used. Only in these samples, no increase in pH was observed after 48 h, which is probably due to the inhibited growth of yeast (figure 6). Additionally, the reason for the decrease in pH could be the presence of acid decomposition product of PHMB-SA.

**Figure 4.** Effect of the amount of the PHMB-SA on the activity of dehydrogenase.

**Figure 5.** Effect of the amount of the PHMB-SA on the pH.
The obtained results of optical density (figure 6) indicate a large diversification of yeast growth, depending on the composition of the film added to the culture medium. The lowest values were noted when PHMB-SA was added to the PVA/Col film, both in the amount of 0.25 or 0.75 g. The biomass for this sample increase in 24 h was only 0.808 for the PVA/Col/PHMB-SA (0.75 g) sample. Surprisingly, in 48 h the optical density was lower, which meant that the biomass entered the death phase. PHMB-SA in the amount of 0.25 g or 0.75 g had a significant effect on the growth of yeast biomass. This confirms the strong antibacterial and antifungal effect of the tested ionic liquid. PHMB is highly adsorptive to anionic surfaces such as bacterial or yeast cell walls, and indeed its mechanism of biocidal action is considered to be damage to the cytoplasmic membrane [39]. On the other hand, in the case of introducing collagen into the PVA film, the stimulation of yeast growth was observed. This is due to the presence of collagen proteins, which are easily absorbed by microorganisms [40, 41], especially by the yeast Y. lipolitica, which are known for the production of alkaline proteases.

**Conclusions**

The obtained PHMB-SA ionic liquid as a result of the ion exchange reaction was confirmed by analyzing the NMR and IR spectra. The ionic liquid showing antimicrobial properties was used to obtain a film based on the PVA and collagen. The mechanical properties of the obtained films were influenced by the amount of the antibacterial substance used. Better tensile strength ($T_S = 27.11$ MPa) was obtained for the sample containing 0.75 g PHMB-SA. Also, along with the increase of PHMB-SA content, the zone of inhibition of bacterial growth in both *E. coli* and *S. aureus* increased. Additionally, dehydrogenase activity, optical density 600 nm and pH reached the lowest value in the case of samples containing PHMB-SA.

**Acknowledgments**

The study presented in this paper was carried out as part of research: „Self-cleaning surfaces with synergistic, photocatalytic and hydrophobic, removal of impurities mechanism with antimicrobial function” (nr 00/BCS/01/00/1/3/0024) supported by the Ministry of Education and Science.
Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

Declaration of interest statement

The authors report there are no competing interests to declare.

ORCID iDs

Anna Shubik  https://orcid.org/0000-0002-6037-6179

References

[1] Osada I, de Vries H, Scrosati B and Passerini S 2015 Ionic–liquid-based polymer electrolytes for battery applications Anorg. Chem. Int. Ed. 55 500–13
[2] Zhang S-Y, Zhuang Q, Zhang M, Wang H, Gao Z, Sun J-K and Yuan J 2020 Poly(ionic liquid) composites Chem. Soc. Rev. 49 1726–55
[3] Nordness O and Brennecke F 2020 Chem. Rev. 123 12873–902
[4] Feder-Kubis J, Geppert-Rybczyńska M, Musial M, Talik E and Guzik A 2017 Exploring the surface activity of a homologues series of functionalized ionic liquids with a natural chiral substituent: (+)-menthol in a cation Colloids Surf. A 529 725–32
[5] Wu H, Fang F, Zheng L, Ji W, Qi M, Hong M and Ren G 2020 Ionic liquid form of donepezil: preparation, characterization and formulation development J. Mol. Liq. 300 112308
[6] Dong K, Liu X, Dong H, Zhang X and Zhang S 2017 Multiscale studies on ionic liquids Chem. Rev. 117 6636–95
[7] Coutinho J A P, Carvalho P J and Oliveira N M C 2012 Predictive methods for the estimation of thermophysical properties of ionic liquids RSC Adv. 2 7322–46
[8] Paduszyńska K and Domafska U 2014 Viscosity of ionic liquids: an extensive database and a new group contribution model based on a feed-forward artificial neural network J. Chem. Inf. Model. 54 1311–24
[9] Ramkumar V, Das I and Gardas R L 2019 Structural arrangement and computational exploration of guanidinium-based ionic liquids with benzoic acid derivatives as anions Angew. Chem. Int. Ed. 58 2642–57
[10] Moshynets O, Bardeau J F, Makhno S, Cherniavska T, Dzhuzha O, Potters G and Rogalsky S 2019 Antibiofilm activity of polyamide 11 modified with thermally stable polymeric biocide, polyhexamethylene guanidine 2-naphthalenesulfonate Int. J. Mol. Sci. 20 548
[11] Zhang Y, Jiang J and Chen Y 1999 Synthesis and antimicrobial activity of polymeric guanidine and biguanidine salts Polymer 40 6189–98
[12] Vereshchagin A N, Frolov N A, Egorova K S, Seitalieva N M and Ananikov V P 2021 Quaternary ammonium compounds (QACs) and ionic liquids (ILs) as biocides: from simple antiseptics to tunable antimicrobials Int. J. Mol. Sci. 22 6793
[13] Ni Y, Qian Z, Yin Y, Yuan W, Wu F and Jia T 2020 Polyvinyl alcohol/chitosan/polyhexamethylene biguanide phase separation system: a potential topical antibacterial formulation with enhanced antimicrobial effect Molecules 25 1334
[14] Creppy E E, Diabat A, Moukha S, Eku-Gadegbeku C and Cross D 2014 Study of energetic properties of poly(HexaMethylene Biguanide) hydrochloride (PHMB) Int. J. Environ. Res. Public Health. 11 8069–92
[15] Müller G, Koburger T and Kramer A 2013 Interaction of poly(hexamethylenbiguanide) hydrochloride (PHMB) with phospholipidylcholine containing o/w emulsion and consequences for microbial efficacy and cytotoxicity Chem. Biol. Interact. 201 58–64
[16] Gendaszewska D, Szuster L, Wyrzebska and Piotrowska M 2018 Antimicrobial activity of monolayer and multilayer films coating polyhexamethylene guanidine sulphhanilate FIBRES TEXT. East Eur. 26 73–8
[17] Cho Y K, Cho P and Boost M V 2013 Cytotoxicity of rigid gas-permeable lens care solutions Clin. Exp. Optom. 96 667–71
[18] Passic S R, Ferguson M L, Catalone B J, Kish–Catalone T, Khodopyshch V, Zhu W, Welch W, Rando R and Howett M K 2010 Structure–activity relationships of polybiguanides with activity against human immunodeficiency virus type 1. Biomed. Pharmacother. 64 763–32
[19] Forbes S, McRae A J, Felton-Smith J, Sowitt A T, Birchmough H L and Dobson C B 2013 Comparative surface antimicrobial properties of synthetic biocides and novel human apolipoprotein E derived antimicrobial peptides Biomaterials 34 5453–64
[20] Bueno C Z and Moraes A M 2018 Influence of the incorporation of the antimicrobial agent polyhexamethylene biguanide on the properties of dense and porous chitosan–alginat membranes Mater. Sci. Eng. C 93 671–8
[21] Siripri R and Jayarat S S 2018 PHMB–an efficient synthetic polymer for wound healing Int. J. Eng. Sci. Invention. 7 2319–6734
[22] Alkharashi M, Lindsey K, Law H A and Siddiq S 2015 Medical interventions for acanthamoeba keratitis Cochrane Database Syst. Rev. CD010792
[23] Akter M, Siddiq T, Rahman M, Ullah A K M A, Hossain K F B, Banik S, Hosokawa T, Saito T and Kurasaki M A 2018 A systematic review on silver nanoparticles-induced cytotoxicity: physicochemical properties and perspectives J. Adv. Res. 9 1–16
[24] Britz J, Meyer W H and Wegner G 2018 Poly(alkylene biguanides) as proton conductors for high-temperature PEMFCs Adv. Mater. 22 72–6
[25] Schmich A, Geier J, Brasch J, Fuchs T and Pirker C 2000 Polyhexamethylenebiguanide: a relevant contact allergen? Contact Derm. 42 302–3
[26] Schmich A, Geier J, Utter W, Basketter D A and Jowsey I R 2007 The biocide polyhexamethylene biguanide remains an uncommon contact allergen Contact Derm. 56 235–59
[27] Fang Y, Fu J, Tao C, Liu P and Cui B 2020 Mechanical properties and antibacterial activities of novel starch-based composite films incorporated with salicylic acid Int. J. Biol. Macromol. 155 1350–8
[28] Hu F, Sun T, Xie J, Xue B, Li X, Gao J, Li L, Bian X and Shao Z 2021 Functional properties of chitosan films with conjugated or incorporated salicylic acid J. Mol. Struct. 1223 129373
[29] Shara M and Stohs S J 2015 Efficacy and safety of white willow bark (Salix alba) extracts Phytotherapy Res. 29 1112–6
[30] Ławińska K, Modrzewski R and Serweta W 2019 Tannery shavings and mineral additives as a basis of new composite materials Fibres Text. East. Eur. 27 89–93
[31] Ławińska K, Lasoń-Rydel M, Gendaszewska D, Grzesiak E, Sierczyńska K, Gaidau C, Epure D G and Obraniak A A 2019 Coating of seeds with collagen hydrolysates from leather waste Fibres Text. East. Eur. 27 59–64
[32] Zang Y, Yang L, Dong Q and Li L 2021 Fabrication of antibacterial fibrous films by electrospinning and their application for Japanese sea bass (Lateolabrax japonicus) preservation Food Sci. Technol. 149 111870
[33] Ramasamy S, Muthusamy S, Nagarajan S, Nath A V, Savarimuthu J S, Jayaprakash J and Gurunadhan R M 2022 Fabrication of collagen with polyhexamethylene biguanide: a potential scaffold for infected wounds J. Biomed. Mater. Res. - B Appl. Biomater. 3 535–46
[34] Phale P S, Sharma A and Gautam K 2019 Microbial degradation of xenobiotics like aromatic pollutants from the terrestrial environments Pharmaceuticals and Personal Care Products: Waste Management and Treatment Technology. Emerging Contaminants and Micro Pollutants 259–78
[35] Asiedu-Gyekye I, Abdulai S M, Awortwe C and Nyarko A K 2015 Toxicological assessment of polyhexamethylene biguanide for water treatment Interdiscip. Toxicol. 1 193–202
[36] Kryczkowska J and Fabiszewska A U 2015 Yarrowia lipolytica - unconventional yeast in biotechnology Adv. Microbiol. 1 33–43
[37] Larking D M, Crawford R J, Christie G B and Lonergan G T 1999 Enhanced degradation of polyvinyl alcohol by Pycnoporus cinnabarinus after pretreatment with Fenton’s reagent Appl. Environ. Microbiol. 4 1798–800
[38] O’Malley L P, Shaw C H and Collins A N 2007 Microbial degradation of the biocide polyhexamethylene biguanide: isolation and characterization of enrichment consortia and determination of degradation by measurement of stable isotope incorporation into DNA J. Appl. Microbiol. 4 1158–69
[39] Małowska-Lipowicz I, Wyrębska L, Lasoń-Rydel M, Shubik A and Ławińska K 2022 New polymeric ionic liquids, their preparation and application Polish Patent Application P 440793
[40] Zhong W, Gu T, Wang W, Zhang B, Lin X, Huang Q and Shen W 2009 The effects of mineral fertilizer and organic manure on soil microbial community and diversity Plant Soil. 326 511–22
[41] de Albuquerque Wanderley M C, Wanderley Duarte Neto J M, de Lima Filho J L, de Albuquerque Lima C, Couto Teixeira J A and Figueredo Porto A L 2017 Collagenolytic enzymes produced by fungi: a systematic review Braz. J. Microbiol. 1 13–24