FIBRES & TEXTILES in Eastern Europe
sciendo
FIBRES & TEXTILES in Eastern Europe
Research Article • DOI: 10.2478/ftee-2023-0002 FTEE • 31(1) • 2023 • 9–14

New ionic liquids based on the biguanide cation with antimicrobial properties for applications in the textile and polymer materials industries

Anna Słubik[1]*, Iwona Masłowska-Lipowicz[1], Łucja Wyrębska[1], Bogusław Królikowski[2], Rafał Malinowski[2], Krzysztof Bajer[2]

[1] *Łukasiewicz Research Network – Lodz Institute of Technology, 73 Zgierska Str., 91-463 Lodz, Poland*
[2] *Łukasiewicz Research Network - Institute for Engineering of Polymer Materials and Dyes, 55 Maria Skłodowska-Curie Str., 87-100 Toruń, Poland*
* *Corresponding author. E-mail: anna.slubik@lit.lukasiewicz.gov.pl*

Abstract
The presented article describes the method of synthesizing new ionic liquid based on a biguanide cation and a tiocyanate or benzoate anion with antimicrobial properties. The structure of obtained new ionic liquid was confirmed by infrared spectroscopy. Additionally, the article describes a simple method of producing an antibacterial textile materials and PE-based film with the addition of an ionic liquid.

Keywords
ionic liquid, PHMB, polyethylene, textile materials, antibacterial properties.

1. Introduction

Most antimicrobial substances are active only against a narrow group of microorganisms, and due to their physicochemical properties, they can only be used in selected materials. A specific group are polymeric materials which, due to high-temperature processing, can only be modified with substances stable at high temperatures, without changing the properties of the original material. Therefore, the search for substances with a broader spectrum of antimicrobial activity, without changing the properties of the products, is still ongoing.

The mechanism of action of bioactive substances is discussed extensively by Denyer, describe cationic polymeric compounds with antimicrobial properties [1]. A specific group of biocides are synthetic polymers with biochemical activity that mimic natural proteins (bacteriocins), the so-called Host Defense Peptides (HDPs) [2-4]. Disinfecting preparations showing a bioactive effect, the so-called HDPs are a new, innovative group of biocides that competes with silver preparations, the nano fragmentation of which causes their migration through human cell membranes, and consequently deposition in internal organs [5]. Among the many antibacterial macromolecules, a group called oligoguanidines, i.e. there is a group of derivatives formed by the condensation of guanidine salts with diamines [6, 7]. An example of a polymeric biguanide derivative is polyhexamethylene biguanide (PHMB) in the form of an aqueous hydrochloride solution. Research shows that PHMB and its salts exhibit a wide spectrum of microbial activity against both Gramm positive, Gramm aerobic and non-aerobic bacteria, some viruses, yeasts, fungi, unicellular algae [8-10]. Application of PHMB in the form of a hydrochloride in polymeric materials is not possible due to its excellent solubility in water and, consequently, migration from the polymer. Recently, the attention of scientists has been focused on the use of the guanidine moiety in the synthesis of ionic liquids. For several years, the chemical industry has seen a growing interest in ionic liquids. These are organic compounds with an ionic structure, composed of an organic cation and an organic or inorganic anion. Their ionic structure carries a number of unique properties, thanks to which they can be widely used. Due to their ionic nature, these liquids show thermal stability, do not emit vapors, and thus are practically non-volatile compounds, thus minimizing the problems associated with the adverse impact of the chemical industry on the natural environment [11-13]. The article by Mateus et al. describes the synthesis and properties of tetraalkyldimethylguanidinium salts [14]. The authors of the work presented the synthesized compounds as a new generation of ionic liquids, showing physicochemical properties comparable and, in some cases, complementary to the widely used ionic liquids. In the work of Carrera et al., new guanidine-based ionic liquids containing allyl functional groups were developed and synthesized. Several new guanidinium-based ionic liquids showed high density, low surface tension, and no toxicity [15]. Butschies et al. performed the synthesis of an ionic liquid composed of guanidine-sulfonimide ions, in which both the anion and the cation contained mesogenic units. The replacement of the spherical halide ion with the sulfonimide anion led to a decrease in the melting point of the ionic liquid [16].

The aim of our study was to synthesize two ionic liquids based on the biguanide cation and the thiocyanate and benzoate anion [17]. The structure of the resulting compounds was confirmed by infrared spectroscopy. Additionally, new ionic liquids were applied to cotton fabric and polyethylene film, and then the antibacterial properties of the obtained products were tested.

2. Experimental parts

2.1. Materials

The following materials were used to synthesis novel ionic liquid:

- poly(hexamethylene biguanide) hydrochloride as a 20% aqueuos solution was purchased from Lonza AG , Switzerland,
- disodium salt of ethylenediaminetetraacetic acid (EDTA-2Na) , 99%, was obtained from Merck KGaA, Germany,
- ammonium thiocyanate (ammonium rhodanide), powder, 98%, and sodium benzoate, powder, 99%, were purchased from Chempur, Poland.

In addition, the cotton fabric (product of the company Antex, Poland) was used. Low-density polyethylene of the FABS 23 D022 type, manufactured by Basell Orlen was used to produce the film.

2.1.1. Synthesis of the ionic liquid poly(hexamethylene biguanide) thiocyanate (PHMB-SCN)

In a flask equipped with a high-speed stirrer, 915 parts by weight of a 20% (1 mol) aqueous solution of poly(hexamethylene biguanide) in the form of hydrochloride and 2.5 parts by weight of ethylenediaminetetraacetic acid disodium salt (EDTA-2Na) were added. Then 76 g of ammonium thiocyanate (1 mol) in the form of a powder was added. The contents of the flask were intensively stirred at the temperature of 20-30℃ for 60 minutes at the pH value = 6.5-7. The two-layer mixture was poured into a separating funnel and left overnight. The lower product layer was washed with water. The obtained product was then dried in a vacuum oven at 40℃ for 10 hours.

230 g of polymeric ionic liquid was obtained in the form of an oily, crystal clear, slightly straw-colored and highly viscous. The yield of the reaction was 95%.

2.1.2. Synthesis of the ionic liquid poly(hexamethylene biguanide) benzoate (PHMB-BS)

In a flask equipped with a high-speed stirrer, 915 parts by weight of a 20% (1 mol) aqueous solution of poly(hexamethylene biguanide) in the form of hydrochloride then a solution of 144 g of sodium benzoate (1 mol) in 280 ml of water was added dropwise. The contents of the flask were intensively stirred at the temperature of 20-30℃ for 60 minutes at the pH value = 6.5-7. The two-layer mixture was poured into a separating funnel and left overnight. The lower product layer was washed with water. The obtained product was then dried in a vacuum oven at 40℃ for 10 hours.

245 g of polymeric ionic liquid was obtained in the form of an oily, crystal clear, slightly straw-colored and highly viscous. The yield of the reaction was 80%.

2.2. Characterization methods

2.2.1. Infrared spectroscopy

The infrared spectra of the PHMB-SCN and PHMB-BS ionic liquid were developed with Thermo Scientific Nicolet 6700 FT-IR spectrometer equipped with Smart Orbit ATR (Waltkam, MA, USA) diamond attachment, using the attenuated total reflectance method (ATR). 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.

2.2.2. Thermogravimetry Analysis and First Derivative Thermogravimetry

The thermogravimetry analysis (TGA) and first derivative thermogravimetry (DTG) analyses were performed using a thermal analyzer (type SDT650 TA Instruments, USA). First, samples of composition were heated in the temperature range of 0–600°C in a nitrogen atmosphere with a heating rate of 10°C/min.

2.2.3. Antibacterial properties of the cotton textile and PE foil with the addition of an ionic liquid

Two bacterial strains: *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 9144 were used as bacteria to test the antimicrobial properties of the cotton textile and PE film with addition of the PHMB-SCN or PHMB-BS ionic liquid. Antibacterial tests of the cotton textile were carried out in accordance with the PN-EN ISO 20743:2013 standard, whereas the antibacterial tests of the PE foil were carried out in accordance with the PN-EN ISO 22196:2007 standard. The antibacterial activity value was assessed according to the following formula 1:

$$A= (lgC_t-lgC_0)-(lgT_t-lgT_0)=F-G \quad (1)$$

where: F – is the growth value on the control specimen; G – is growth value on the antibacterial testing specimen; lgC_t – is the common logarithm of arithmetic average of the numbers of bacteria obtained from three control specimens after an 18h to 24h incubation; lgC_0 – is the common logarithm of arithmetic average of the numbers of bacteria obtained from three control specimen immediately after incubation; lgT_t – is the common logarithm of arithmetic average of the numbers of bacteria obtained from three testing specimens after an 18h to 24h incubation; lgT_0 – is the common logarithm of arithmetic average of the numbers of bacteria obtained from three testing specimen immediately after incubation.

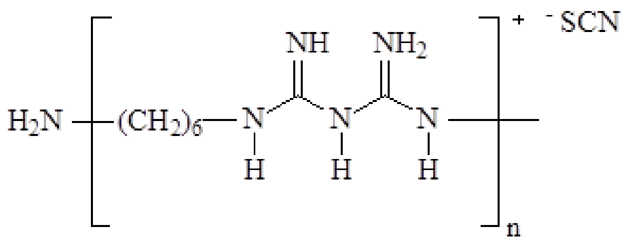
The antimicrobial activity of the test samples was assessed on the basis of the antimicrobial efficacy criterion described in Table 1.

The effectiveness of the antibacterial action	A
Poor	A<1
Satisfactory	1≤A≤2
Good	2≤A<3
Very good	A≥3

Table 1. Criterion for assessing the effectiveness of the antimicrobial activity

Wave number (cm^{-1})	Chemical bond or group	Type of chemical compound	Type of vibration
3318	N-H	Amine (biguanide)	Stretch
2934	C-H (alif.)	Alifatic hydrocarbons (polyhexamethylene..)	Stretch
2060	SCN	Nitrile	Stretch
1630	C=N	Biguanide	Stretch
1551	N-H	Biguanide	Deformation
1381	C-O	Carboxylate anion	Stretch (symetric)
1153	C-O	Carboxylate anion	Stretch (asymetric)
719	C-H (arom.)	Monosubstituted aromatic ring	Deformation

Table 2. Analysis of FTIR bands corresponding to individual vibrations in the tested samples

a)


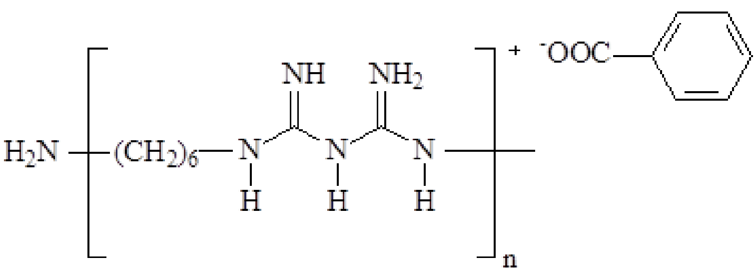
b)


Fig. 1. Structural formula of: a) PHMB-SCN; b) PHMB-BS

3. Results and discussion

3.1. Characterization of the structure of the ionic liquid (PHMB-SCN and PHMB-BS)

During the synthesis described in the methodology, two polymeric ionic liquids were obtained. The structure of the resulting liquids is shown in Figure 1. The first ionic liquid consisted of a cation derived from PHMB and a thiocyanate anion (Fig.1a), while the structure of the second ionic liquid consisted of the cation from PHMB and the benzoate anion (Fig. 1b).

The structure of the resulting ionic liquid was also confirmed on the basis of the FTIR spectrum (Table 2.). 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 wavenumber of 1551cm^{-1} presented in biguanide was observed. The characteristic absorption bands at the frequency of 1630 cm^{-1} attributed to the C=N stretching vibration of biguanide was also observed. The PHMB-SCN spectrum showed an intense absorption band at 1630 cm^{-1} characteristic of the triple bond present in nitriles, which confirmed the presence of the $^{-}$SCN ion. The absorption bands at 1381 cm^{-1} and 1153 cm^{-1} indicated the presence of a carboxylate anion. The carboxylate anion absorbs in a different region than other carbonyl compounds (1600-1870 cm^{-1}). 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 are also strongly mechanically coupled, therefore the FTIR spectrum showed a band of asymmetric carbon-oxygen stretching vibrations at the frequency of 1381 cm^{-1} and a weaker band of symmetrical stretching vibrations at 1153 cm^{-1}.

3.2. Solubility of the ionic liquids

The solubility of the obtained ionic liquids was also tested. On the basis of the dissolution test (10 mg of the ionic liquid was placed in 2 ml of the selected solvent and after 2 hours the solubility was determined), it was found that, unlike PHMB, both obtained ionic liquids were insoluble in acetone, toluene and hexane, while they were dissolved in ethanol, dimethyl sulfoxide, N-methylpyrrolidone, dimethylformamide, dimethylacetamide and tetrahydrofuran. The thiocyanate anion caused the lack of solubility of the obtained ionic liquid in water, while in the case of the PHMB-BS ionic liquid, limited water solubility (up to 1%) was observed.

Type of bacteria	Sample	A	Assessment
E. coli	PHMB-SCN	1.73	Satisfactory
	PHMB-BS	1.49	Satisfactory
S. aureus	PHMB-SCN	2.41	Good
	PHMB-BS	3.00	Very good effect

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

	Zone 1	Zone 2	Zone 3	Three-wire head
T (℃)	150	160	170	180

Table 4. Extruder zone temperatures

	Zone 1	Zone 2	Zone 3	Flat-slot head
T (℃)	170	170	165	160
Screw rotation (1/min)	100			
Film width (mm)	160			
Film thickness (mm)	0.08-0.085			

Table 5. Extruder zone temperatures and the processing parameters

3.2.1. Application of a new ionic liquid (PHMB-SCN or PHMB-BS) on a cotton fabric

Alcoholic solution of ionic liquid (PHMB-SCN or PHMB-BS) in the amount of 1.5%. was introduced on cotton fabric by the dipping method. Then, the cotton, after immersion in an alcoholic ionic liquid solution, was allowed to dry and its antibacterial properties were examined (Table 3).

The obtained results from microbiological tests prove that the applied ionic liquids differently affect the microbiological activity of the obtained fabrics against *E. coli* and *S. aureus*. In the case of *E. coli*, both PHMB-SCN and PHMB-BS showed a satisfactory inhibitory effect on the growth of microorganisms. The value of antibacterial activity against *E.coli* was 1.73 and 1.49 for PHMB-SCN and PHMB-BS, respectively. Whereas, better antibacterial properties of the synthesized ionic liquids were observed against *S. aureus* bacteria. The antibacterial activity of these samples was above 2.4.

3.2.2. Preparation of PE based film with the addition of an ionic liquid (PHMB-SCN or PHMB-BS)

PE/PHMB-SCN and PE/PHMB-BS granulate was obtained by means of a simple mixing virgin PE with ionic liquids in an intensive planetary mixer heated up to 80°C and then subjected to further processing in a co-rotating twin-screw extruder (type BTSK-20 Bühler, Germany) to get a regranulated PE composition. Extruder zone temperatures were as follows (Table 4).

Due to the suspicion of uneven distribution of the ionic liquids in the polymer matrix, the pre-granulated composition was extruded again. The concentration of ionic liquids in PE was obtained at the level of 10%. From the concentrate, by diluting (mixing) the concentrate with the PE, the compositions with a 1,5% concentration of ionic liquids were obtained. The PE composition prepared in this way was subjected to ribbon extrusion in a single-screw extruder (type Brabender PLV 151, Germany) with a screw with a compression ratio of 3:1 and a 170 x 0.5 mm flat-slot head. Table 5 shows the temperature range of the extruder zones and the processing parameters for the production of the PE film with the addition of PHMB-SCN or PHMB-BS.

In case of the PE/PHMB-SCN the change in the color of the granulates was observed during processing. The color change was due to the formation of a colored iron thiocyanate resulting from the fusion of the thiocyanate anion with the iron cation from the processing equipment. The presence of iron was confirmed by Inductively Coupled Plasma Optical Emission Spectrometry - ICP-OES 5110 Agilent spectrometer. The iron content in PHMB-SCN is only 1.9 mg/kg, which is a marginal value for both the film (136.4 mg/kg) and the granulate (389.4 mg/kg) values. This phenomenon was not observed in iron-free devices. During processing with the addition of PHMB-BS, such a phenomenon did not occur. It was found that the ionic liquid containing the thiocyanate anion cannot be used in polymer processing due to the significant discoloration of the granules and the film. Therefore, products based on PHMB - BS were further tested.

3.3. Thermal Analysis of PHMB-BS

During heating the sample in a controlled atmosphere at constant temperature increment, changes in the mass of the test substance are observed as a result of chemical reactions (decomposition, oxidation, or reduction) and physical changes (evaporation, sublimation, desorption). The first stage of the PHMB-BS thermal decomposition started at a temperature from 111°C to 175°C. The 9.82% weight loss in this temperature range was probably due to the loss of water absorbed on the surface of the material. On the other hand, the loss of 26.46% of the mass in the temperature from 175°C to 300°C indicated the initial decomposition of PHMB-BS. Probably in this range the lowest energy bonds breakdown occurred. Further decomposition of PHMB and the organic anion leading to fragmentation occurred

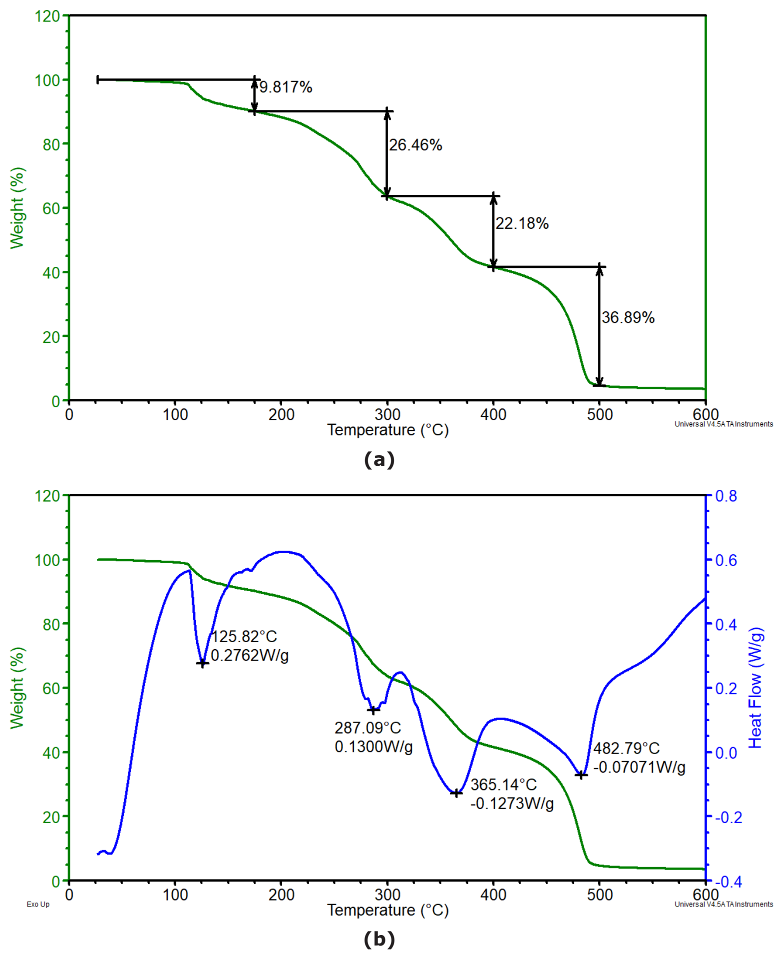


Fig. 2. TGA (a) and DTG (b) of PHMB-BS

Temperature range (°C)	Maximum decomposition temperature (°C)	Weight loss (%)
111-175	125	9.82
175-300	287	26.46
300-400	365	22.18
400-500	482	36.89

Table 6. Thermal stability of PHMB-BS

Type of bacteria	Sample	A	Assessment
E. coli	PE/PHMB-BS	3.9	Very good effect
S. aureus	PE/PHMB-BS	3.1	Very good effect

Table 7. The results of the antibacterial activity of the tested films against E. coli

in the temperature range 300-400°C with 22.18% weight loss (Figure 2, Table 6). In the last stage (temperature range 400-500°C), the carbonization process probably started, accompanied by 36.89% weight loss [20].

3.3.1. Antibacterial properties of PE/PHMB-BS film

Determination of antibacterial properties for the produced PE films with the addition of 1.5% ionic liquid was tested against *E.coli.* The result of these tests are presented in Table 7.

The produced PE films with the addition of ionic liquid (PHMB-BS) showed very good bactericidal properties. In the case of both samples, the antibacterial activity reached values above 3.0, which proves a very good inhibitory effect on the growth of microorganisms in these materials.

4. Conclusions

Ionic liquids offer new prospects for new environmentally friendly technologies. They can also be used in the modification of polymers and textiles. New ionic liquid based on a biguanide cation and a rhodate (thiocyanate) or benzoate anion with antimicrobial properties were synthesized. They were used as a functional additive to plastics. It was applied in the processing of polyethylene. The PE films produced with the addition of PHMB-BS showed good bioactive properties, while the films with the addition of PHMB-SCN changed colour and showed no antibacterial activity. The ionic liquid PHMB-SCN has a limited use in the processing of plastics. Both ionic liquids are perfect for functionalizing textiles to give them antimicrobial properties.

References

1. Denyer S. P., Stewart G. Mechanisms of action of disinfectants. International Biodeterioration & Biodegradation. 1998; 41:261-268.
2. Mansour S.C., Pena O.M., Hancock R.E.W. Host defense peptides: front-line immunomodulators. Trends in Immunology. 2014;35:443-450. DOI:10.1016/j.it.2014.07.004
3. Ting D.S.J., Beuerman R.W., Dua H.S., Lakshminarayanan R., Mohammed I. Strategies in Translating the Therapeutic Potentials of Host Defense Peptides. Frontiers in Immunology. 2020;11:983. DOI: 10.3389/fimmu.2020.00983
4. Grigoras A.G. Natural and synthetic polymeric antimicrobials with quaternary ammonium moieties: a review. Environmental Chemistry Letters. 2021;19:3009–3022. DOI:10.1007/s10311-021-01215-w
5. Akter M., Sikder T., Rahman M., Ullah A.K.M.A., Hossain K.F.B., Banik S., Hosokawa T., Saito T., Kurasaki M.A. A systematic review on silver nanoparticles-induced cytotoxicity: Physicochemical properties and perspectives. Journal of Advanced Research. 2018;9:1-16. DOI:10.1016/j.jare.2017.10.008
6. Hung N.V., Bac N.V., Chung T., Luong T.D. Synthesis of oligoguanidine - Based on polycondensation and compare their Antimicrobial Activities with Chloramine B. International Journal of Engineering Research & Science. 2018;4:63-67.
7. Buchberger T., Himmelsbach M., Buchberger W. Trace analysis of biocidal oligoguanidines in environmental water samples. Journal of Chromatography A. 2013;1318:22-26. DOI: 10.1016/j.chroma.2013.10.005
8. Feiertag P., Albert M., Ecker-Eckhofen E.M., Hayn G., Hönig H., Oberwalder H.W., Saf R., Schmidt A., Schmidt O., Topchiev D. Structural Characterization of Biocidal Oligoguanidines. Macromolecular Rapid Communications. 2003;24:567-570. DOI: 10.1002/marc.200390085
9. Bueno C.Z., Moraes A.M. Influence of the incorporation of the antimicrobial agent polyhexamethylene biguanide on the properties of dense and porous chitosan-alginate membranes. Materials Science and Engineering C. 2018;93:671-678. DOI:10.1016/j.msec.2018.07.076
10. Gendaszewska D., Szuster L., Wyrębska Ł., Piotrowska M. Antimicrobial Activity of Monolayer and Multilayer Films Containing Polyhexamethylene Guanidine Sulphanilate. FIBRES & TEXTILES in Eastern Europe. 2018;26:73-78. DOI: 10.5604/01.3001.0011.5742
11. Osada, I., de Vries H., Scrosati B., Passerini S. Ionic-Liquid-Based Polymer Electrolytes for Battery Applications. Angewandth Chemie Intnational Edition 2015;55:500–513. DOI:10.1002/anie.201504971
12. Zhang S.-Y., Zhuang Q., Zhang M., Wang H., Gao Z., Sun J.-K., Yuan J. Poly(ionic liquid) composites. Chemical Society Reviews. 2020;49:1726-1755. DOI:10.1039/c8cs00938d
13. Wu H., Fang F., Zheng L., Ji W., Qi M., Hong M., Ren G. Ionic liquid form of donepezil: Preparation, characterization and formulation development. Journal of Molecular Liquids. 2020;300:112308. DOI:10.1016/j.molliq.2019.112308
14. Mateus N.M.M., Branco L.C., Lourenço N.M.T.,Afonso C.A.M. Synthesis and properties of tetra-alkyl-dimethylguanidinium salts as a potential new generation of ionic liquids. Green Chemistry. 2003;3:347-352.
15. Carrera G.V.S.M., Frade R.F.M., Aires-de-Sousa J., Afonso C.A.M., BrancoL.C. Synthesis and properties of new functionalized guanidinium based ionicliquids as non-toxic versatile organic materials. Tetrahedron. 2010;66:8785-8794.
16. Butschies M., Neidhardt M.M., Mansueto M., Laschat S., Tussetschläger S. Synthesis of guanidinium–sulfonimide ion pairs:towards novel ionic liquid crystals. The Journal of Organic Chemistry. 2013;19:1093–1101.
17. Masłowska-Lipowicz I., Wyrębska Ł., Lasoń-Rydel M., Ławińska K., Słubik A. New polymeric ionic liquids, their preparation and application. Patent Application P. 440793 2022, Poland.
18. Zang Y., Yang L., Dong Q., Li L. Fabrication of antibacterial fibrous films by electrospinning and their application for Japanese sea bass (Lateolabrax japonicus) preservation. Food Science and Technology. 2021;149:111870.
19. Ramasamy S., Muthusamy S., Nagarajan S., Nath A.V., Savarimuthu J.S., Jayaprakash J., Gurunadhan R.M. Fabrication of collagen with polyhexamethylene biguanide: A potential scaffold for infected wounds. Journal of Biomedical Materials Research Part B: Applied Biomaterials 2022;3:535-546.
20. De Paula G.F., Netto G.I., Mattoso L.H.C. Physical and Chemical Characterization of Poly(hexamethylene biguanide) Hydrochloride. Polymers. 2011;3:928-941. DOI:10.3390/polym3020928