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BROAD-PURPOSE ANTIMICROBIAL CHLORINE-ACTIVE POLYMERS: SUPPRESSION OF MULTIDRUG-RESISTANT MICROORGANISMS AND MICROBIAL PENETRATION RESISTANCE

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The aim of the work *was to evaluate the antimicrobial activity of polymeric materials with immobilized N-Chlorosulfonamide groups against multidrug-resistant hospital strains of common microorganisms and to determine the resistance to microbial penetration of these materials.*
Materials and methods: *the studied samples were copolymers of styrene with divinylbenzene in the form of staple fi bre and non-woven fabric with immobilized N-Chlorosulfonamide groups of various structures. Hospital strains of microorganisms have been isolated from clinical material; their antibiotic sensitivity has been determined by the Kirby-Bauer method. The agar diffusion method determines the antimicrobial activity of the polymers. Resistance to microbial penetration of samples of non-woven fabric has been determined by the membrane filtration method.*
Results: *polymer samples have been synthesized with immobilized N-Chlorosulfonamide groups in the Na- and H-forms, and with the N, N-dichlorosulfonamide group, with chlorine concentration range 3.7–12.5 %. All sam-ples demonstrated pronounced antimicrobial activity against both standard and hospital strains. Due to the higher specific surface area, staple fi bre is generally more e fficient. An increase in the zone of inhibition of the growth of microorganisms was observed with an increase in the concentration of immobilized chlorine. All the studied fabric samples are impermeable to S. aureus. The control samples containing the free sulfonamide group did not show antimicrobial properties.*
Conclusions: *synthesized chlorine-active polymers have a pronounced antimicrobial activity against multi-drug-resistant microorganisms, demonstrate high resistance to microbial penetration and therefore are promising for creating a wide range of medical products on their basis: dressings, protective masks, antimicrobial fi lters, etc.*
Keywords: *antimicrobial polymers, active chlorine, N-Chlorosulfonamides, immobilization, antibiotic resistance, microbial penetration resistance, dressings, face masks*

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1. Introduction

Creating new polymers and composites with microbiocidal properties is one of the topical areas of modern pharmaceutical chemistry. Such materials are widely used for the production of implants [1], catheters [2], unique clothing [3], medical equipment and furniture [4], dressings [5], filters [6], etc. In connection with the recent events related to the COVID-19 pandemic, the creation of personal protective equipment (respirators, medical masks, badges, etc.) with antimicrobial and antiviral effects, which are mostly polymer, has also become particularly relevant [7, 8]. The use of such products is an effective method of prevention [9, 10] and, in some cases, the treatment of infectious diseases of various aetiology [9–11]. The advantages of using microbiocidal polymers are the variety of their physical forms (from fibres with a highly developed surface to thin-layer coatings) [12, 13], multifunctionality [14], stability, the possibility of regeneration, etc.

Among the antimicrobial polymers, N-halamine-containing (usually N-Chloramine-)materials are of special interest [15, 16]. Compounds containing the "chlorine-active" fragment "N-Cl" are exceptionally widely used due to their potent and rapid bactericidal, fungicidal and virucidal activity [17, 18], relative stability, the impossibility of developing resistance to them, as well as ease of their synthesis, and availability [19]. There are several traditional ways to combine such compounds with a polymer carrier. In the simplest case, a suitable polymer is impregnated with the necessary N-Chloramine. Further, upon contact with a contaminated environment, the latter molecules are desorbed, causing an antimicrobial effect [20, 21]. Products from such materials are easy to manufacture and achieve an immediate effect, but, in most cases, they are disposable and sensitive to storage conditions (for example, if moisture gets in, the active component can simply be washed off from the carrier). Polymers are described in which N-Chloramines or their "non-charged" with chlorine precursors act as monomers [22, 23]. Such materials can contain high concentrations of active chlorine and pro-

vide a powerful long-term microbiocidal effect. However, the technologies for their production are usually quite complex. The physical and mechanical properties of such polymers depend on the nature of the monomer used and are limited by it. The technologies for modifying an already finished polymer carrier with suitable properties by embedding chlorine-active functional groups into it are more relevant. The most common is the immobilization of fragments of cyclic amides or imides, for example, substituted hydantoins and imidazolidinones, which can be easily converted into N-Chloramine by interaction with chlorinating agents [24]. Such N-Chloramines are more stable due to the absence of an α-H-atom and, accordingly, the impossibility of decomposition by dehydrohalogenation mechanism [15]. Acyclic precursor groups of N-Chloramines, for example, acetamide, are also used often [15]. Recently, antimicrobial polymeric N-Chloramines based on chitosan, which initially contains a free amino group, have been actively studied [25]. In the above cases, the final step is the "charging" of immobilized functional groups with chlorine, most often by treatment with sodium hypochlorite. Many synthetic and natural polymers can be used as carriers: polyurethanes [26], cotton [27], silica gels [28], etc. The reactions of immobilization of precursors of the chlorine-active group are also diverse [15]; however, in most cases, the presence of specific reactive fragments in the structure of the polymer carrier is necessary, for example, a 6-hydroxy group in cellulose or an amide group in nylon. The introduction of antimicrobial N-Chloramine moieties in inert polymers such as polypropylene or polyethylene terephthalate are much more complex [29, 30]. The described approaches make it possible to obtain polymers of various physical forms and chemical structures with a powerful and long-lasting antimicrobial effect and, in many cases, capable of multiple regenerations. However, the grafting of complex organic fragments complicates the synthesis of such materials and increases the risk of allergic reactions in their medical use, such as dressings.

Another type of common and highly effective chlorine-active preparation with pronounced microbiocidal activity is N-Chlorosulfonamides. They have long and often been used in water treatment and for disinfection measures, for example, chloramines B and T [31]. Accordingly, the N-Chlorosulfonamide group is also a promising moiety for immobilization on a polymer carrier, especially since it is the most stable and does not contain organic fragments. The difficulty lies in the fact that the precursor sulfochloride group, from which the reaction with ammonia can subsequently obtain the target sulfamide group, can be embedded into the polymer only under very harsh conditions, for example, by treating the carrier with chlorosulfonic acid, sulfuryl chloride, or other very aggressive reagents. Most polymer-carriers do not withstand such conditions. Polymers of an aromatic nature, for example, polystyrene, as well as the products of its copolymerization with divinylbenzene, are suitable carriers for this purpose. Methods for the preparation and properties of some N-Chlorosulfonamides immobilized on such carriers have been described. Thus, Emerson et al. developed synthetic procedures and studied the chemical and antimicrobial properties of modified macroporous granular cation exchangers with immobilized N-Chlorosulfonamide groups [32, 33]. Similar granular materials, but using cation exchangers of other brands, have been actively studied by Bogoczek and colleagues [34–36]. These authors proved the antimicrobial activity of the synthesized polymers, described their oxidizing and other chemical properties, and proposed methods for their use in water treatment, for example, to remove iron ions, nitrites, cyanides, etc. However, all these polymers have a granular form with a relatively small surface area. Therefore, their use in pharmaceutical and medical purposes is limited, although they are promising for industrial goals due to their high-strength properties. Fibrous polymers of the styrene-divinylbenzene structure are described much less. Maddah and colleagues have developed a technology for producing chlorine-active polystyrene nanofibers, described its microbiocidal properties and demonstrated the possibility of its use, among other things, for the creation of protective clothing [37, 38]. At the same time, the electrospinning technology for obtaining such materials is quite expensive and inaccessible. Fibrous styrene-divinylbenzene polymers under the brand name FIBAN have been described and industrially produced using special radiation polymerization technology [39]. These materials withstand the harsh conditions of sulfochlorination without significant change in physical and mechanical properties, can exist in the form of a staple fibre with a developed surface or the form of the easily standardized non-woven fabric and are relatively affordable. We have developed methods for immobilizing N-Chlorosulfonamide groups of various structures on such carriers [40], studied the processes of active chlorine emission from them (and from their granular analogues) into aqueous media [40, 41], proved potent antimicrobial [42] and virucidal [43] properties, and conducted a number of *in vivo* studies of their effectiveness for treating open wounds [44]. Due to their fibrous form, such chlorine-active polymers are promising for use in medicine and pharmaceutics as components of antiseptic dressings, antibacterial protective masks and air filters, as well as for obtaining high-purity antiseptic and disinfection solutions *in situ*. Current work is a logical continuation of the direction we are developing.

This study aimed to establish the antimicrobial effectiveness of fibrous polymeric materials with immobilized N-Chlorosulfonamide functional groups in relation to hospital antibiotic-resistant strains of microorganisms and to determine their microbial penetration resistance for further evaluation of the prospects for their use as components of antiseptic medical and pharmaceutical products.

2. Research planning

The experiment was planned based on several previous studies. Given that the primary goal of this area of our work is the creation of microbiocidal medical

products, the effectiveness of which strongly depends on their surface area (dressings, protective masks, antimicrobial filters, etc.), only fibrous chlorine-active polymers [40, 45] have been investigated, and repeatedly described by us [41] similar polymers in granular form have not been studied. It was essential to establish a correlation between the intensity of the antimicrobial action of the polymer and the concentration of immobilized chlorine in it; therefore, samples with a wide range of chlorine content have been synthesized. It was also necessary to establish the dependence of the antimicrobial activity of the polymer on the structure of the

The n-Chlorosulfonamide group strongly affects the active chlorine release kinetics [42, 46]. For this, samples with three types of functional groups have been synthesized: N-Chlorosulfonamide in the Na- and H-forms, and N, N-dichlorosulfonamide.

We proved the antimicrobial activity of some synthesized polymers against standard microorganisms strains [42, 44, 47]. The modified method of agar plates has been developed [42, 47], which makes it possible to reliably determine it, taking into account the specifics of the materials under study, which was also used in this work. It seemed most important to study the effectiveness of such polymers against resistant hospital strains, for which clinical material was taken from 33 patients of the Central Military Clinical Hospital of the Ministry of Defense of Ukraine, wounded during the conflict in the East of Ukraine. Separation and identification of individual hospital microorganisms have been carried out, their antibiotic sensitivity has been determined, and their resistance against the studied chlorine-active polymers has been evaluated, which was one of the goals of this work. The most common pathogens of nosocomial infections have been selected for the study: *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans,* and *Enterococcus hirae*. *Staphylococcus aureus* causes many complications – from inflammation of the skin and soft tissues to lethal pneumonia – and is one of the most rapidly spreading "superbugs" [48, 49]. *Pseudomonas aeruginosa* is associated mainly with the development of chronically infected wounds, significantly complicates their therapy due to the ability to actively form barrier biofilms, and is also characterized by increased antibiotic resistance [49, 50]. *Enterococcus hirae* has previously been observed relatively rarely. However, to date, it causes up to 3 % of enterococcal infections, is also responsible for septic shock, and therefore even proposed as a new test germ within the framework of the procedures for the European standardization of chemical and antiseptic agents for evaluation and validation of disinfectant products [51]. *Candida albicans* is the common cause of opportunistic mycoses worldwide and one of the significant contributors to wound infections [52]. Thus, the neutralization of these microorganisms is the essential element in the treatment of open wounds, so the effect of the synthesized polymers as potential components of antiseptic dressings and medical masks on them should have been studied in the first place.

A critical property of polymers used in the manufacture of personal protective equipment is their ability to resist the penetration of microorganisms through them. In the simplest case, it depends only on the physicomechanical properties of the polymer (specific surface area, electrostatic properties, pore diameter, etc.). However, for the synthesized polymers with immobilized.

N-Chlorosulfonamide groups, it is also necessary to consider the completeness and kinetics of the release of active chlorine into the contaminated medium passed through the sample. In this work, this aspect has been studied for the first time.

3. Materials and methods

Polymer samples with immobilized N-Chlorosulfonamide groups have been synthesized from a fibrous cation exchanger FIBAN K-1 in the form of a staple fibre ("cotton wool") and a non-woven fabric ("fabric") according to the procedure described in [41, 42, 44]. In addition, the concentration of immobilized chlorine has been determined according to a special method of iodometric titration developed and described by us in [41, 42].

To study the effectiveness of the antimicrobial action of polymeric chlorine-active materials, standard strains of microorganisms were used: for bactericidal activity – *Staphylococcus aureus* ATCC 6583 (*S.* a*ureus*), Pseudomonas aeruginosa ATCC 9027 (*P. aeruginosa*), for yeasticidal activity – *Candida albicans* ATCC 6583 (*C. Albicans*). Hospital strains have also been studied: *Staphylococcus aureus* (*S. aureus H*), *Pseudomonas aeruginosa* (*P. aeruginosa H*), and *Enterococcus hirae*

(*E.hirae H*), isolated from the patients. Hospital microorganisms were identified on the basis of their tinctorial microscopic signs and biochemical properties, and the full species identification was carried out according to biochemical parameters using a test system ID 32C (BioMerieux, France). Primary inoculation was carried out on several nutrient media depending on the type of clinical material: 5 % blood, chocolate, mannitol-salt agar, Endo's medium, thioglycol medium, cetrimide agar, tryptone soya agar (TSA), tryptone soya broth (TSB), Sabouraud agar.

For the cultivation of test strains and all experiments, the same nutrient media were used, the growth properties and sterility of which had been checked before the start of the research: TSA ("HiMedia", India) – to determine the number of bacteria; Sabouraud agar ("HiMedia", India) – to determine the number of fungi.

Preparation and preservation of test strains of microorganisms for research have been carried out according to EN 12353:2006 [53].

Determination of the sensitivity of microorganisms to antibiotics has been carried out by the Kirby-Bauer method [54]. The corresponding nutrient medium in the amount of 20 mL was poured into sterile Petri dishes. Before inoculation, the surface of the medium was dried at room temperature, keeping it in a slightly open dish for 30–40 min. For inoculation, an 18–20-hour agar culture of strain was used. When using an agar culture, it was first washed off with a small (4–5 mL) amount of physiological sodium chloride solution. Next, optical density on a KFK-3-01 photo colourimeter at a wavelength of

620 nm in a cuvette 10 mm thick determined the required number of microorganisms. The resulting inoculum was diluted 10 times with isotonic sodium chloride solution, and 1–2 mL of it was applied with a pipette to the surface of the medium, evenly distributed over the entire surface of the medium by rotating the dish. The excess of inoculum was removed. The dish was dried for 10–15 min, and sterile cotton pads impregnated with the corresponding antibiotic were applied with sterile tweezers. Then the dish was cultivated at 37±1 °C for 18–20 hours. After that, the diameter of the growth inhibition zone was measured with an accuracy of 1 mm, including the diameter of the disks. The results were evaluated using the standard tables, which indicate the limits of the values of the diameters of the growth inhibition zones of microorganisms for resistant, moderately resistant and sensitive strains [55]. The obtained values of the inhibition zones were compared with those indicated in the table, and the studied strains were assigned to one of these three sensitivity categories.

The antimicrobial effect of chlorine-active polymers in the form of "cotton wool" was studied by the agar diffusion method (well method), based on the ability of the microbicide (in this case, active chlorine released from the polymer upon contact with the amino groups of microorganisms or the agar itself) to diffuse into the nutrient medium over all directions. The melted and cooled TSA was contaminated with the test microorganism suspension. The microbial load was 1×10^7 CFU/mL. The contaminated medium (20 mL) was poured into sterile Petri dishes and left to solidify. Then, wells 8 mm in diameter were formed in the thickness of the nutrient medium via a sterile punch. The test samples of "cotton wool" in 0.1 cm^3 (about 0.035 g) were placed in separate wells. The cultures were incubated in a thermostat for 24–48 hours at 37±1 °C. After incubation, the diameters of growth inhibition zones around the wells were measured.

The antimicrobial activity of the "fabric" polymer samples was determined by the modified method of agar plates [41]. Suspension of the test microorganism was added to melted and cooled to 45 °C TSA at the concentration of 1×10^7 CFU/mL, and 20 mL of this mixture was poured into sterile Petri dishes. Round "fabric" test samples of 1 cm in diameter (which corresponded by mass to the studied samples of "cotton wool") were placed on the surface of the semi-hardened agar, slightly immersing them to increase the contact surface of the material with the nutrient medium. As shown earlier, if "fabric" samples are placed on the already hardened surface of the agar, effective diffusion of active chlorine into its volume is not achieved, which leads to a false negative result. Further actions were similar to those in the study of "cotton wool" samples. The antimicrobial activity of the material was assessed by determining the diameter of inhibition zones, measured from the edge of the sample to the growth limit of the microorganism.

The resistance of "fabric" materials to the penetration of microorganisms was determined via a membrane filtration unit with a pressure of 35 kPa, which is used for drug research following the State Pharmacopoeia of Ukraine. It is the alternative to the method EN 14126:2003 «Protective clothing. Performance requirements and tests methods for protective clothing against infective agents». First, test strain *S. aureus* was cultured on TSA for 18–24 hours at 36±1 °C. Then 2–3 colonies were separated with a microbiological loop, resuspended in 5.0 ml TSB, and cultured for 18–24 hours at 36±1 °C. The obtained broth was diluted with phosphate buffer in a ratio of 1:10. The resulting suspension of microorganisms contained 1×10^4 CFU/mL. This suspension in a volume of 5.0 ml was filtered on the specified unit through chlorine-active "fabric" samples of the appropriate size, which were placed on the stage of the filtering unit above the "Millipore" microorganism filter with a diameter of 47 mm and a pore size of 0.45 μm. After filtering the suspension of microorganisms, the filter was placed on TSA and incubated at 36±1 °C for 24 hours. Then the presence or absence of growth of the microorganism was recorded. The presence of growth indicated the penetration of microorganisms through the polymer sample, and the absence of growth indicated its microbial impermeability.

All microbiological experiments were performed in triplicate. Samples of the initial polymer carrier containing the sulfonamide group $-SO_2-NH_2$ not "charged" with chlorine served as the control.

4. Results

To study the antimicrobial activity, 6 samples of polymeric materials with immobilized N-Chlorosulfonamide groups have been synthesized, and 2 control samples with a sulfonamide group "uncharged" with chlorine have also been prepared. The most important characteristics of these materials are given in Table 1; their appearance is shown in Fig. 1. The chemical structure and physical and mechanical properties of all samples correspond to those described earlier [41].

Table 1

The main characteristics of the studied chlorine-active polymers

Sample No.	Physical form	The structure of the functional group	Immobilized chlorine concentration
1	«cotton wool»	$-SO_2-NClNa$	6.8 %
2	«fabric»	$-SO_2-NClNa$	6.8 %
3	«fabric»	$-SO_2-NHCl$	3.7 %
4	«cotton wool»	$-SO_2-NHCl$	3.7 %
5	«cotton wool»	$-SO_2-NCl_2$	12.5 %
6	«fabric»	$-SO_2-NCl_2$	9 %
7	«cotton wool»	$-SO_2-NH_2$	–
8	«fabric»	$-SO_2-NH_2$	–

The results of determining the sensitivity of hospital strains of microorganisms isolated from patients to different antibiotics are shown in Table 2.

The results of the study of the antimicrobial activity of "cotton wool" samples (average of three reps) are

shown in Table 3. In addition, zones of growth inhibition of the studied strains of *S. aureus* under the action of these polymers are given in Fig. 2.

a *b*

Fig. 1. General appearance of the studied samples: *a* – "cotton wool"; *b* – "fabric"

Table 2

Sensitivity of hospital strains of microorganisms to antibiotics

Antibiotics	The number of strains sensitive to drugs (number / %)		
	E.hirae H (13 pieces)	*S.aureus H* (7 pieces)	*P. aeruginosa H* (5 pieces)
Amoxicillin	2/11 15.4/84.6*	5/2 71.4/28.6	0/5 0/100
Amoxicillin/ clavulanate	2/11 15.4/84.6	3/5 42.9/57.1	0/5 0/100
Ciprofloxacin	5/8 38.46/61.54	3/4 42.9/57.1	0/5 0/100
Norfloxacin	5/8 38.46/61.54	¾ 42.9/57.1	0/5 0/100
Ofloxacin	5/8 38.46/61.54	3/4 42.9/57.1	0/5 0/100
Lomefloxacin	5/8 38.46/61.54	3/4 42.9/57.1	0/5 0/100
Moxifloxacin	6/7 46.15/53.85	2/5 28.6/71.4	0/5 0/100
Ceftriaxone	6/7 46.15/53.85	5/2 71.4/28.6	0/5 0/100
Cephalexin	0/13 0/100	2/5 28.6/71.4	0/5 0/100
Nitrofurantoin	4/9 30.76/69.24	1/4 20.0/80.0	0/5 0/100
Furazolidone	0/13 0/100	1/4 20.0/80.0	0/5 0/100
Cefuroxime sodium	5/8 38.46/61.54	2/5 28.6/71.4	0/5 0/100
Cefpodoxime	5/8 38.46/61.54	2/5 28.6/71.4	0/5 0/100
Cefixime	5/8 38.46/61.54	2/5 28.6/71.4	0/5 0/100

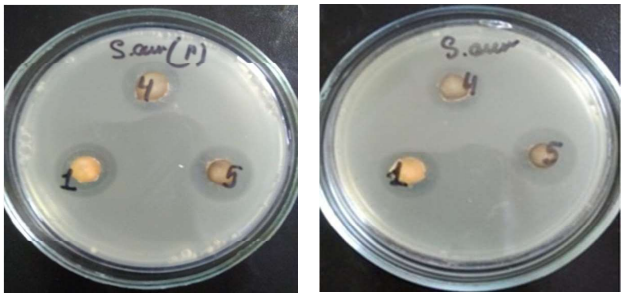
Note: % sensitive / % insensitive

The results of the study of the antimicrobial activity of "fabric" samples of N-Chlorosulfonamides immobilized on the polymer (average of three reps) are shown in Table 4. Zones of growth inhibition of the studied strains of *S. aureus* under the action of individual samples of these polymers are given in Fig. 3.

Table 3

Growth inhibition zones around the "cotton wool" samples

Sample No.	Microorganisms' growth inhibition zones(mm)					
	S. aureus	*P. aeruginosa*	*C. albicans*	*S. aureus (H)*	*P. aeruginosa (H)*	*E. hirae (H)*
1	10.0	7.0	10.0	6.0	8.0	14.0
4	8.0	5.0	10.0	6.0	5.0	10.0
5	12.0	10.0	20.0	10.0	12.0	10.0
7	0	0	0	0	0	0



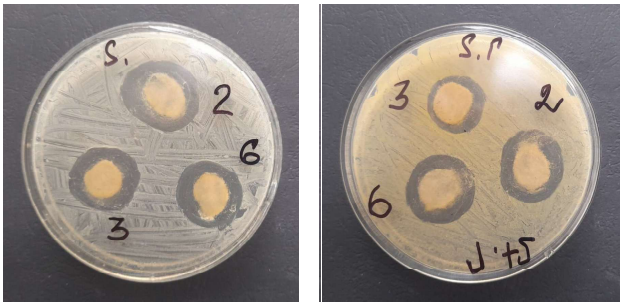
a *b*

Fig. 2. Growth inhibition zones under the action of "cotton wool" samples: *a – S. aureus H* 6583; *b – S. aureus* ATCC

Table4

Growth inhibition zones around the "fabric" samples

Sample No.	Microorganisms' growth inhibition zones (mm)					
	S. aureus	*P. aeruginosa*	*C. albicans*	*S. aureus (H)*	*P. aeruginosa (H)*	*E. hirae (H)*
2	12.0	8.0	9.0	10.0	8.0	12.0
3	6.0	3.0	10.0	5.0	3.0	8.0
6	11.0	10.0	12.0	10.0	9.0	10.0
8	0	0	0	0	0	0



a *b*

Fig. 3. Growth inhibition zones under the action of "fabric" samples: *a – S. aureus* ATCC 6583; *b – S. aureus H*

Resistance to microbial penetration has been studied only for "fabric" samples of chlorine-active polymers. After filtering the microbial suspension through samples No. 2, No. 3 and No. 6, no growth of microorganisms on the filter was observed, which indicates the impermeability of these polymers for *S. aureus*. Control sample No. 8 under the same conditions turned out to be permeable to this microorganism.

5. Discussion

Analysis of antibiograms of microorganisms isolated from clinical material showed that in more than 60 % of cases, they were insensitive to antibiotics of the

cephalosporin and penicillin series and nitrofurans and can be classified as multidrug-resistant opportunistic microorganisms. The presence of such microbes in the organism significantly complicates the treatment of the corresponding diseases and can lead to serious complications in their course.

Microbiological studies have shown that significant zones of growth inhibition formed around all the samples of chlorine-active polymers, proving their high antimicrobial activity against both standard strains of microorganisms and resistant hospital ones. There were no growth inhibition zones around control samples No. 7 and No. 8, despite the presence of immobilized sulfamide- and a certain amount of sulfogroups, which indicates that the microbicidal effect of such materials is due namely to the presence of immobilized chlorine, and not, for example, because of change of the nutrient medium, change of the charge of the cell membrane of a microorganism upon contact with the polymer, or the adsorption of microbe on the material. It has been proven that the suppression of the growth of microorganisms under the action of synthesized polymers in both physical forms is achieved directly under the sample and at a considerable distance from its edge. This means that such polymers not only have their antimicrobial activity (i.e., the ability to inactivate microorganisms upon direct contact) but also ensure the gradual release of microbicidal active chlorine into the environment, and, as we have shown earlier, this process starts precisely in the presence in this environment of amino compounds, including those of microbiological origin.

Zones of inhibition of the growth of microorganisms around polymer samples are not the same. In general, “cotton wool” samples demonstrated slightly more pronounced antimicrobial activity than “fabric” ones, primarily due to their larger surface area. Samples No. 3 and No. 4 with immobilized

N-Chlorosulfonamide groups in H-form were expectedly less active than the corresponding samples No. 1 and No. 2 with the same groups in Na-form. This is due to the lower concentration of chlorine in them and the structural features of these functional groups. We assume that in the case of Na-forms, the dissociation of the N-Na bond is possible, which facilitates the emission of active chlorine from the formed anion; in the case of the H-form, such a process is less likely. In addition, microorganisms can be inactivated upon direct contact with the polymer. In the case of the more hydrophilic -N(Na)-Cl group, this interaction seems to be more effective than for the -N(H)-Cl group. The largest growth inhibition zones were observed around dichloro-substituted samples No. 5 and No. 6, which, taking into account the previously established lower rate of chlorine emission from them [46, 47], is better explained by the higher concentration of immobilized chlorine. However, it should be noted that the direct correlation “chlorine concentration – inhibition zone diameter” was not observed in all cases.

Of the microorganisms, *P. aeruginosa* was found to be the least sensitive to the action of studied polymers; nevertheless, the activity of all samples against it was pronounced. The increased resistance of this microorganism to active chlorine was described earlier [56]. There were no significant differences in the effectiveness of polymer samples on standard and hospital strains. Data regarding the antimicrobial activity of materials against standard microorganisms are generally consistent with those previously obtained. It has been repeatedly confirmed that the activity of chlorine preparations in the presence of an organic load, which in our case is the nutrient medium itself, is significantly reduced due to the consumption of a significant amount of active chlorine for interacting directly with this medium. Therefore, the wide zones of growth inhibition obtained even under the conditions of the described experiments indicate the extremely high antimicrobial activity of the synthesized polymers and, accordingly, the expediency of their use for the creation of medical devices with increased resistance to microbial contamination and antiseptic/disinfection properties. Suspension microbiological tests, which will be the subject of our separate research, will likely demonstrate this more clearly. The interaction of chlorine with agar, the layer thickness of which is difficult to control in these experiments, can also explain some of the “fallouts” from the described dependences, for example, the greater sample activity No. 2 against *S. aureus* (*H*).

Experiments to determine resistance to microbial penetration have shown that all studied «fabric» samples of chlorine-active polymers are impermeable to

S. aureus, as opposed to an «uncharged» control sample. The conditions of the experiment do not allow us to determine what is more responsible for this effect: mechanical retention of the microorganism upon direct contact with the fibre for a time sufficient for its inactivation or rapid emission of active chlorine in a concentration sufficient for inactivation. However, considering the permeability of the control sample, it can be stated that it is the presence of immobilized chlorine that plays the key role and not the physical and mechanical properties of the carrier, which do not change significantly during functionalization. This property of the synthesized polymers confirms their prospects for manufacturing regenerated antimicrobial filter materials and medical devices based on them.

Study limitations. The limitations of this study include the impossibility of accurately determining the mechanism of suppression of microorganisms upon contact with the polymer, the difficulty in calculating the effective concentration of immobilized active chlorine, as well as the interaction of the released active chlorine with the components of microbiological nutrient media, which can lead to an underestimation of the growth inhibition zones around the sample.

Prospects for further research. In the course of further research, we will study the microbial permeability of these polymers when contaminated air is

passed through them to determine the possibility of their use to protect respiratory organs from aerogenic infectious diseases. In addition, the absence of acute toxicity upon inhalation of high-purity vapours of active chlorine, which can theoretically be released into the treated air in this case, has been recently proved by us in the model experiment [57]. Also, in vivo research on the application of such materials for the treatment of wounds of various origins and conditions will be continued.

6. Conclusion

Synthesized fibrous polymers with immobilized N-Chlorosulfonamide groups of various structures exhibit pronounced antimicrobial activity against both standard and hospital strains of microorganisms and demonstrate high resistance to microbial penetration. Such properties of these materials confirm their prospects for creating a wide range of medical products: wound dressings, personal protective equipment, antimicrobial wipes, special clothes, water and air filters, etc.

Conflict of interest

The authors declare that they have no conflict of interest concerning this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

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