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Novel guanidinium and phosphonium polysulfones: synthesis and antimicrobial activity

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ABSTRACT

The possibility of obtaining of new polymeric salts on the basis of guanidinium and phosphonium polysulfones was investigated. Guanidinium and phosphonium polysulfones were synthesized by free radical copolymerization technique. Polysulfonylpyrrolidinium and polysulfonylpyrrolidinylphosphonium hydroxides were obtained by ion chromatography. Chemical modification of hydroxides results in new optically and biologically active polymeric guanidinium and phosphonium salts for the first time. NMR, FTIR and element analysis were used to confirm the chemical structure and element composition. New polymeric guanidinium and phosphonium salts possess optical activity and are effective in controlling bacteria, spores and fungi.

Keywords: polymeric quanidinium and phosphonium salts, radical copolymerization, ion chromatography, antimicrobial activity.

INTRODUCTION

Many years the guanidine derivatives with antimicrobial activity have been investigated as medicals and antiseptics [1-4]. It was reported that among the guanidine derivates with antimicrobial activity the polymeric guanidine salts have gained importance [4, 5]. It is well known that functional polymers have the potential advantages of small molecules with the same functional groups. Their usefulness is related to both the functional groups and to their polymeric nature whose characteristic properties depend mainly on the extraordinarily large size of the molecules [6, 7]. These polymers are more effective and less toxic to humans as compared with low molecular weight compounds. Moreover, guanidine-based cationic polymers show excellent anti-bacterial [4, 5, 8-11], anti-fungal [4, 12] and anti-viral activities [13, 14]. That is why, during the last few decades much attention has been directed to guanidine-containing polymers.

Recently, the use of antiseptics and desinfectants has been questioned because of the possibility of hospital-acquired infections [15, 16]. Besides that, bacteria can be altered with respect to their susceptibility towards other antiseptics and antibiotics. Moreover some guanidine-containing compounds are stable to heat, which are the good additives for polymer materials.

Phosphoric polymer derivatives are of significant interest because they are used as extractants and complexing agents. Due to high selectivity phosphorylated polyamines can be used in the isolation of cations which are in hardly separable combinations. Complexation ability of aminopolyphosphonium compounds in reactions with different metals (copper, beryllium, uranium, lead) opens the possibility for their use in the removal of metal from organisms [17, 18]. Phosphorus containing compounds have attracted also considerable attention as antimicrobial compounds [7, 19, 20].

In the present study we prepared polymers with probable antimicrobial activity based on new water-soluble guanidinium and phosphonium polysulfones. Such studies are believed to be important in the development of new antimicrobials and anticeptics.

EXPERIMENTAL SECTION

Materials

2,2-Diallyl-1,1,3,3-tetraethylguanidiniumchloride (AGC) was synthesized from tetraethylurea as described in our publications [21]. Tetraethylurea (1 mol, 172 g) was dissolved in dry benzene (2.5 mol, 195 g). Phosgene was bubbled through the solution at 9–15°C with the intensive stirring until the reaction was completed (control by gas–liquid chromatography). Then the reactive mixture was heated slowly and was boiled until the gas stopped emanating. It was followed by the cooling of the reactive mixture and dry diallylamine (2.4 mol, 233 g) was added drop wise with the constant intensive stirring. Then the reactive mixture was stored for 2 hr at 50–60°C with stirring and sodium hydroxide (1.2 mol of 50% water solution) was added drop wise to the mixture. After that, the reactive mixture was filtered and the filtrate was evaporated under vacuum at 70°C. The obtained AGC was thoroughly rinsed with dry acetone to remove the residual formed NaCl. Finally, acetone was removed by distillation. The yield of AGC was 70% from the theory (201 g).

Tris(diethylamino)diallylaminophosphonium chloride (DAAP-Cl) was obtained as described in our publications [22]. To obtain tris(diethylamino)diallylaminophosphonium chloride the reaction flask was loaded with tris(diethylamino)phosphazohydride (1.5 mol, 392 g) and freshly distilled allylchloride (7.5 mol, 600 g) was added drop wise to the reaction mixture with stirring until the reaction temperature stopped rising. Then sodium hydroxide (6.4 mol of 50% aqueous solution) was added drop wise under stirring and the mixture was heated on a water bath at 38°C for 30-45 min then for 10-12 hr with stirring at a boiling temperature. After cooling and the middle layer was separated. DAAP-Cl was extracted with dichlormethane, the extract was evaporated under vacuum at 100°C. The yield of DAAP-Cl was 86.5% from the theory (490 g).

Sulfur dioxide was dried by passing through concentrated sulfuric acid and freshly-sintered CaCl2.

Benzoic acid and optically active acids (OAA) – D-tartaric acid, α-aminopropionic acid (L-alanine), α-aminoisovaleric acid (L-valine) were obtained from Aldrich and used without further purification. Benzylpenicillin was obtained from BIOSYNTHESIS (Russia). The characteristics of applied initiator (2,2'-azobisisobutyronitrile (AIBN)) and solvents (DMSO, methanol, tetrahydrofurane) conformed to the reference data after purification by conventional methods.

Copolymerization

The monomers were mixed in suitable molar proportions and copolymerization of AGC and DAAP-Cl with SO_2 was conducted in bulk and in organic solvent in the presence of initiator AIBN ($3 \cdot 10^{-2}$ mol L^{-1}). The copolymerization experiments of AGC and DAAP-Cl with SO_2 were carried out in a glass reactor according to the following technique. A desired quantity of SO_2 was introduced into a liquid nitrogen-cooled reactor via condensation, then the necessary quantity of monomer, initiator and solvent were added. The reactor was sealed and the reaction was carried out at the chosen temperature. Copolymers were precipitated in tetrahydrofurane and purified by three-fold reprecipitation from methanol. The purified copolymers were dried under vacuum at 50 °C to constant weight. The copolymer composition was calculated from the elemental analysis.

Synthesis of polysulfonylpyrrolidinium and polysulfonylpyrrolidinylphosphonium hydroxides

Polysulfonylpyrrolidinium and polysulfonylpyrrolidinylphosphonium hydroxides (PSH) were obtained from chlorides by ion chromatography with using of AV-17-8 anionite. The anionite was rinsed with water, then with sodium hydroxide (7 and 10 % aqueous solution, sequentially) to remove the residual Cl^- ions (control by reaction with $AgNO_3$). Then the anionite was thoroughly rinsed with water again to achieve the neutral reaction medium. After that 20 % aqueous solution of polysulfonylpyrrolidinium or polysulfonylpyrrolidinylphosphonium chloride was passed through the anionite and the aqueous solution of hydroxide was obtained. Then the hydroxides obtained were separated by precipitation in acetone and rinsed with acetone twice. The purified hydroxides were dried under vacuum at 50°C to constant weight.

Synthesis of polymeric quanidinium and phosphonium salts

Synthesis of polymeric guanidinium and phosphonium salts was conducted by the action of active acids on hydroxides. 10% Aqueous solution of acid was added dropwise to the 10% aqueous solution of PSH at a ratio of hydroxide : acid = 1.0 : 1.1 and the reactive mixture was stored for 1 hour at 50°C. Then the salts were separated by

precipitation in acetone and rinsed with acetone twice. The purified salts were dried under vacuum at 50°C to constant weight. The yield of salts obtained was 50-57 % from the theory.

Measurements

NMR^{13}C spectra were registered on a "Bruker AM-300" spectrometer operating at 75,47 MHz using a broad-band proton suppression and in a JMOD mode. D_2O was used as solvent; 2,2-dimethyl-2-silapentan-5-sulphoacid (DSS) was used as internal standard.

IR spectra was recorded on a spectrophotometer Specord M-80 and spectra were measured in KBr pellets.

Polarimetric measurements ($[\alpha]_D^{20}$) were taken on Perkin Elmer 241 MC polarimeter at 589 nm in 0,5 % aqueous solutions. For D-tartaric acid $[\alpha]_D^{20}$ +10.8, for L-alanine +3.7, for L-valine +4.2.

Evaluation of antimicrobial activity

Acute toxicity of polymeric quanidinium and phosphonium salts was measured in mongrel white male mice weighing 18-20 g, using intraperitoneal doses. The mice were injected with these copolymers. The dose was up to 1000 mg · kg^{-1}. Each group consisted of six animals. The animals were observed for 48 h. LD_{50} values were calculated by Prozorovskiy's method [23].

Microbiological tests were performed by serial dilution of preparations in meat-peptone broth followed by inoculation of meat-peptone agar. Test cultures were *Staphylococcus aureus* strain 906, *Staphylococcus saprophyticus*, ATCC 15305, *Staphylococcus epidermis*, 33, *Micrococcus luteus*, ATCC 4698, *Escherichia coli* strain 25922, *Candida albicans*, 264/624; *Bacillus subtilis* ATCC 6633, *Bacillus antracoides*, 1312. Bacteria were grown for 20 h or 7 days. Microbial loads were 2.5 · 10^5 cells in 1 ml of preparation-containing liquid growth medium.

RESULTS AND DISCUSSION

Our attempt to directly enter the active acids into polysulfonylpyrrolidinium and polysulfonyl pyrrolidinyl phosphonium chlorides did not lead to the desired results. That is why our work was aimed at obtaining of optically and biologically active polymeric salts via the intermediate compound - hydroxide. Polysulfonylpyrrolidinium and polysulfonylpyrrolidinylphosphonium hydroxides were obtained by ion chromatography with using of AV-17-8 anionite (Figure 1).

Synthesis of optically active polymeric salts was conducted by the action of optically active acids on hydroxides (Figure 2). The composition of the salts obtained from the elemental analysis coincides with calculated data (Table 1).

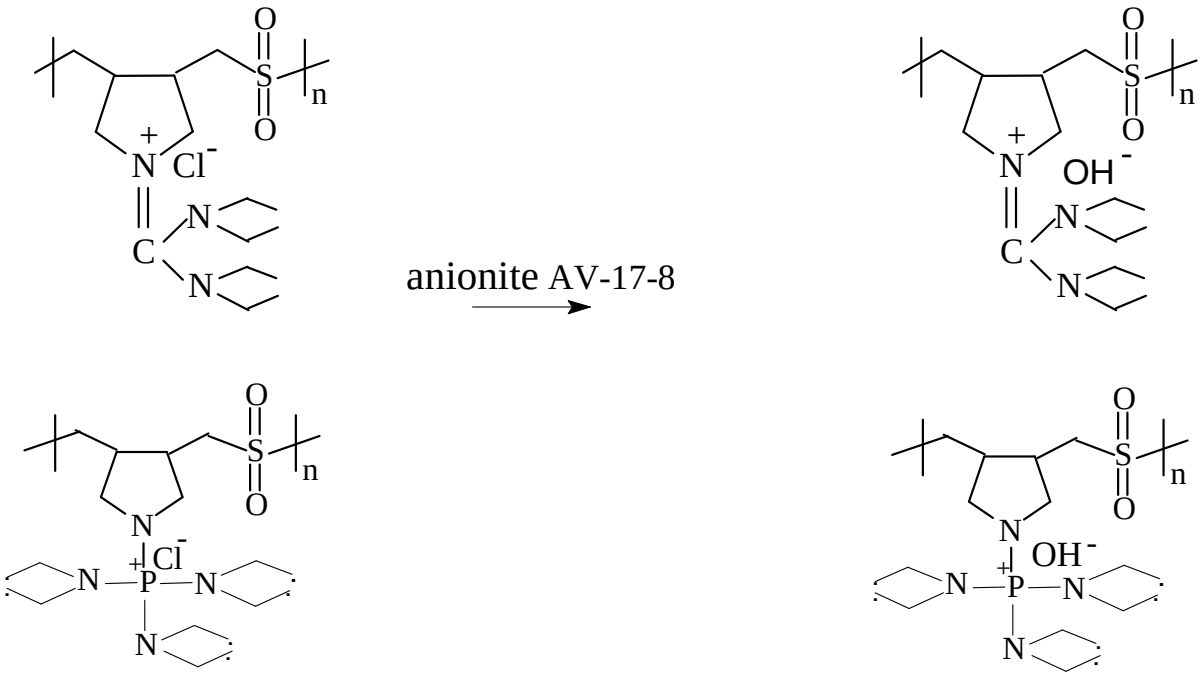
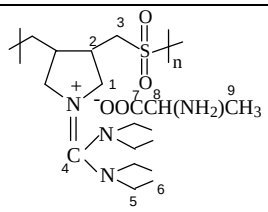
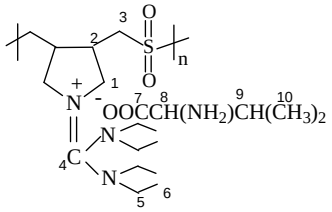
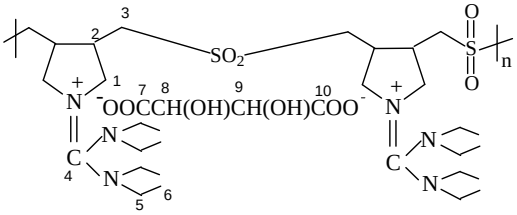
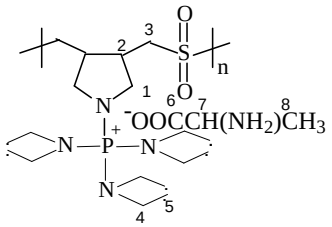
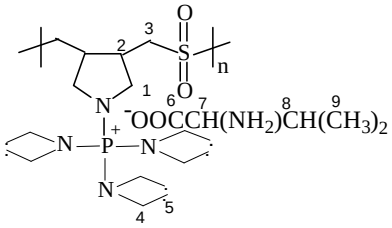
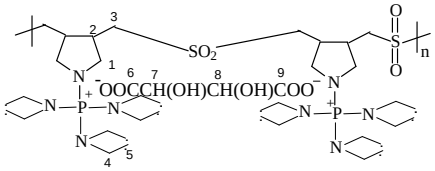
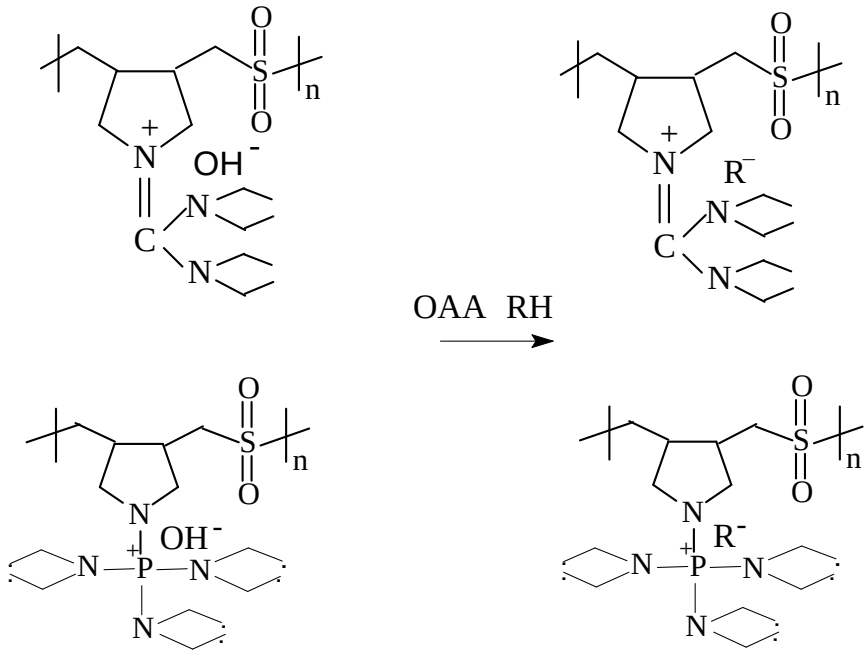


Figure 1. Synthesis of polysulfonylpyrrolidinium and polysulfonylpyrrolidinylphosphonium hydroxides.

Table 1. Optically active polymer salts (OAPS) on the basis of polysulfonylpyrrolidinium and polysulfonylpyrrolidinylphosphonium hydroxides.

Structure	OAA	Founded, % / Calculated, %				IR-spectrum	$[\alpha]_D^{20}$
		C	H	N	S		
 (I)	L-α-alanine	53,35 / 53,50	8,85 / 8,91	13,38 / 13,91	6,99 / 7,90	1124, 1300 – SO_2 1618 – NH_2 1580 – COO^-	not determined
 (II)	L-α- valine	53,85 / 55,56	8,85 / 9,26	11,38 / 12,96	6,49 / 7,41	1124, 1299 – SO_2 1614 – NH_2 1587 – COO^-	+2.8
 (III)	D-tartaric acid	50,80 / 52,30	7,90 / 8,20	9,91 / 10,77	8,08 / 9,20	1116, 1296 – SO_2 1584 – COO^- 1034, 1072, 3422 – OH	+ 8.3
 (IV)	L-α-alanine	49,40 / 50,90	8,40 / 9,29	13,10 / 14,14	6,11 / 6,46	1125, 1304 – SO_2 1624 – NH_2 1590 – COO^-	+ 2.1
 (V)	L-α- valine	50,36 / 52,80	8,50 / 9,56	13,08 / 13,40	5,44 / 6,12	1124, 1299 – SO_2 1612 – NH_2 1587 – COO^-	+ 0.5
 (VI)	D-tartaric acid	50,36 / 51,56	8,47 / 9,02	11,11 / 12,03	6,53 / 6,87	1125, 1298 – SO_2 1630 – COO^- 1088, 3400 – OH	+ 3.3



R : $^{-}OOCCH(NH_2)CH_3$, $^{-}OOCCH(NH_2)CH(CH_3)_2$, $^{-}OOCCH(OH)CH(OH)COO^{-}$

L-alanine L-valine D-tartaric acid

Figure 2. Synthesis of optically active polymer salts

In IR spectra of salts we observe the appearance of the high intensity bands between 1580 and 1612 sm^{-1} (depending on the acid used) assigned to COO-groups and the bands at 1620 sm^{-1} attributed to NH_2-group, respectively. When D-tartaric acid at the ratio hydroxide : acid of 1 : 1 is used, except for the band at 1612 sm^{-1} IR spectrum of salt contains peak at 1705 sm^{-1} attributed to COOH-group. When the hydroxide : acid ratio of 2 : 1 is used the disappearance of these bands is observed (Table 1). The new polymeric guanidinium and phosphonium salts based on OAA possess optical activity; values of $[\alpha]_D^{20}$ are presented in Table 1.

In NMR ^{13}C spectra of the new polymeric guanidinium and phosphonium salts there are no signals of initial PSH and any other signals, this fact suggests full substitution of OH- anion to corresponding OAA anion (Table 2).

Table 2. NMR^{13}C spectra of optically active polymer salts.

Structure	Chemical shift values and signals multiplets of the atoms (d, ppm)									
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
I	51,85 t	35,81 d	51,05 t	161,67 s	43,13 t	12,99 k	176,5 s	50,66 d	17,01 k	
II	51,49 t	35,50 d	50,88 t	163,40 s	43,40 t	13,10 k	175,4 s	60,8 d	30,3 t	18,5 k
III	51,34 t	35,64 d	51,03 t	162,80 s	43,80 t	13,28 k	177,20 s	71,9 d	177,20 s	
IV	52,60 t	36,71 d	51,98 t	41,27 t	13,94 k	172,30 s	50,4 d	16,99 k		
V	53,34 t	38,77 d	54,82 t	40,37 t	13,89 k	174,70 s	59,8 d	30,4 t	18,2 k	
VI	53,28 t	37,30 d	53,28 t	40,62 t	12,90 k	175,30 s	70,8 d	175,30 s		

Biologically active benzoic acid and benzylpenicillin were entered into polymer matrix of PSH on the basis of guanidinium salt by ion exchange method (Figure 3). The passing of the reaction was controled by the disappearance of the band at 1650 sm^{-1} and appearance of the band at 1597 $см^{-1}$ attributed to COOH и COO^- - group, respectively (Table 3). The structures of the salts were confirmed by elemental analysis, IR- and NMR ^{13}C-spectroscopy data (Table 4).

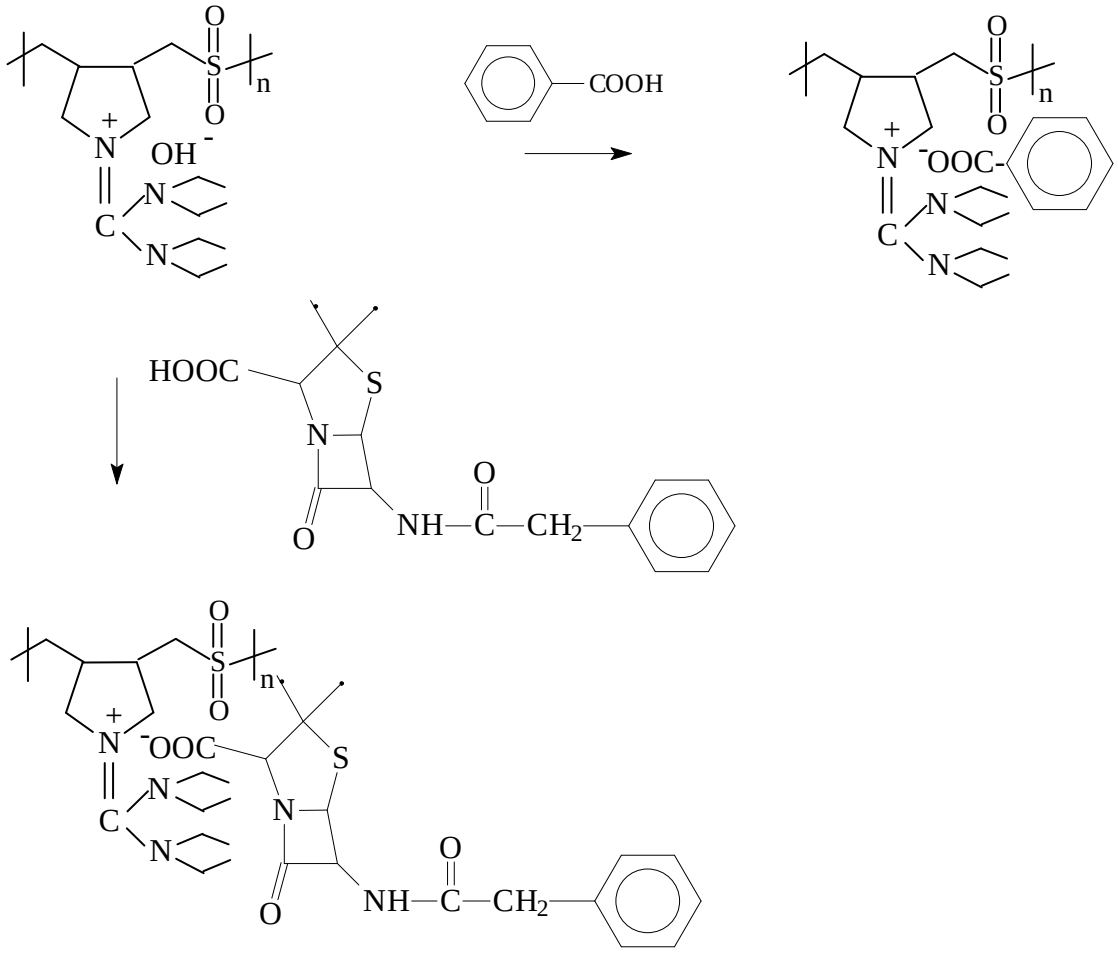
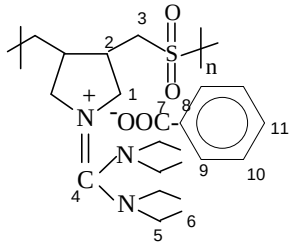
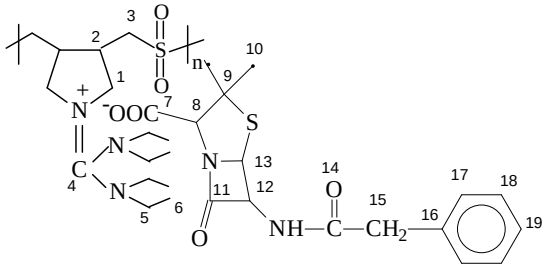


Figure 3. Synthesis of biologically active polymer salts.

Table 3. Polymer salts on the basis of polysulfonylpyrrolidinium hydroxide and biologically active benzoic acid and benzylpenicillin.

Structure		Founded, % / Calculated, %				IR-spectrum
		C	H	N	S	
 (VII)	Benzoic acid	59,85 60,31	8,85 8,22	9,38 9,61	6,49 7,32	1124, 1300 – SO_2 1600 – COO^-
 (VIII)	Benzylpenicillin	58,05 57,32	7,85 7,24	10,36 10,79	9,47 9,86	1125, 1302 – SO_2 1590 – NH 1680, 1690 – C=O 1608 – COO^-

Guanidine-containing polymers are mainly investigated as the antimicrobial agents. Due to its high water solubility, excellent biocide efficiency and nontoxicity they are widely used as anticeptics and fungicides. Antimicrobial agents are defined as those materials capable of killing pathogenic micro-organisms. The great limitations of low molecular weight compounds are based on their residual toxicity even when suitable amounts of the agent are added

[24]. The use of antimicrobial polymers prevents the limitation of low molecular weight analogues: reduces the residual toxicity and increases efficiency and selectivity of the agents. Moreover antimicrobial polymers are chemically stable and do not permeate through skin.

Table 4. NMR^{13}C spectrum of the polymer salts VII and VIII.

Structure	Chemical shift values and signals multiplets of the atoms (d, ppm)														
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}
VII	51,8 t	35,8 d	51,1 t	161,7 s	43,1 t	13,0 k	176,5 s	130,6 d	130,1 d	128,4 d	133,7 d				
VIII*	52,8 t	33,8 d	51,6 t	161,0 s	43,4 t	13,0 k	179,8 s	57,0 m	32,0 d	20,4 k	175,3 s	50,7 d	40,7 d	171,8 s	23,3 t

**For VIII $C_{16\text{-}19}$ 135,6; 129,8; 128,9; 127,3 m*

All new polymeric salts are non-toxic and can be used for medical purposes (LD50 values were more than 1000 $mgkg^{-1}$). Studies of antimicrobial activity showed that the polymeric salts have a significant antimicrobial activity against both Gram positive and Gram negative microflora (Table 5). The antimicrobial activity decreases with increasing chain length of alkyl group of amino acid. The microbial screening results of new polymer salts show higher activity is due to the the presence of two guanidinium or phosphonium groups. The breadth of antimicrobial activity of new polymeric salts on the basis of guanidinium and phosphonium polysulfones makes their perspective for development of the new water-soluble antiseptics and antibacterial agents.

Table 5. Antimicrobial activity of copolymers of 2,2-diallyl-1,1,3,3-tetraethylguanidiniumchloride and tris(diethylamino) diallylaminophosphonium chloride with sulfur dioxide and new polymer salts (I-VIII).

№	Test cultures	Minimal bacteriostatic concentration (MBsC), µg/ml									
		AGC with SO_2	DAAP-Cl with SO_2	I	II	III	IV	V	VI	VII	VIII
1	*Escherichia coli*, ATCC 25922	500,0	62,5	125	250,0	125	125	125	62,5	31,2	15,6
2	*Staphylococcus aureus*, 906	7,8	7,8	15,6	31,2	7,8	15,6	31,2	7,8	7,8	3,9
3	*Micrococcus luteus*, ATCC 4698	7,8	>7,8< 15,6	31,2	31,2	7,8	15,6	<31,2> 15,6	7,8	7,8	7,8
4	*Staphylococcus saprophyticus*, ATCC 15305	15,6	<31,2> 15,6	15,6	<31,2> 15,6	15,6	31,2	62,5	15,6	7,8	7,8
5	*Staphylococcus epidermis*, 33	7,8	250,0	15,6	62,5	15,6	500,0	500,0	250,0	<31,2> 15,6	15,6
6	*Candida albicans*, 264/624	15,6	31,2	31,2	<31,2> 15,6	31,2	15,6	31,2	<31,2> 15,6	15,6	7,8
7	*Bacillus antracoides*, 131	–	<500 >250	500,0	–	–	–	500,0	250,0	–	125,0
8	*Bacillus subtilis*, ATCC 6633	>31,2< 62,5	<15,6> 7,8	31,2	62,5	62,5	15,6	31,2	15,6	15,6	15,6

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CONCLUSION

Polysulfonylpyrrolidinium and polysulfonylpyrrolidinylphosphonium hydroxides were obtained by ion chromatography, chemical modification of them results in new optically and biologically active polymeric guanidinium and phosphonium salts for the first time. The structure of the polymer salts obtained was investigated by IR and NMR ^{13}C. New polymeric polysulfonylpyrrolidinium and polysulfonylpyrrolidinylphosphonium salts with L-isomers of some α-aminoacides and D-tartaric acid are optically active. All polymeric polysulfonylpyrrolidinium and polysulfonylpyrrolidinylphosphonium salts are nontoxic and possess antimicrobial activity with respect to both Gram positive and Gram negative microorganisms.

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