ISSN 1070-3632, Russian Journal of General Chemistry, 2010, Vol. 80, No. 7, pp. 1314–1319. © Pleiades Publishing, Ltd., 2010.
Original Russian Text © P.S. Vlasov, S.N. Chernyi, N.S. Domnina, 2010, published in Zhurnal Obshchei Khimii, 2010, Vol. 80, No. 7, pp. 1148–1153.

Functionalyzed Polyampholytes on the Basis of Copolymers of *N,N*-Diallyl-*N,N*-dimethylammonium Chloride and Maleic Acid

P. S. Vlasov, S. N. Chernyi, and N. S. Domnina

St. Petersburg State University, Universitetskii pr. 26, St. Petersburg, 198504 Russia
e-mail: Petr_vlasov@mail.ru

Received January 28, 2010

Abstract—Polyampholytes were prepared by radical copolymerization of *N,N*-diallyl-*N,N*-dimethylammonium chloride and maleic acid. The esterification and subsequent hydrazinolysis permitted to introduce hydrazide groups capable of formation of the coordination bond with copper ions. The formation of complex between the hydrazide groups of polyampholyte and Cu^{2+} ions was established by electronic spectroscopy. It was shown that the coordination of two hydrazide groups in the complex was hampered because of the polymeric nature of the ligand. It is shown that the created functionalized polyampholytes exhibit fungiostatic activity. The highest inhibiting effect on the growth of the pathogenic fungus *Fusarium oxysporum* is observed for complexes of the hydrazide-containing polyampholytes with Cu^{2+} ions.

DOI: 10.1134/S1070363210070169

Among the ionogenic polymers a great practical value have the polymers and copolymers of *N,N*-diallyl-*N,N*-dimethylammonium chloride used as technological additives in the processes of water purification, paper manufacturing, etc. [1, 2]. Besides, as many cationic polyelectrolytes they can exhibit fungiostatic activity and suppress the growth of bacteria and algae [3] being at the same time non-toxic for higher plants [4]. Copolymerization of *N,N*-diallyl-*N,N*-dimethylammonium chloride with ionogenic monomers such as unsaturated carboxylic acids and their salts leads to the formation of polyampholytes containing cationic as well as anionic groups in the polymer chain [5]. By varying the ratio of ionogenic groups in these copolymers, and also by the chemical modification their target properties may be improved and the area of their application may be broadened [6]. From this point of view the copolymers of *N,N*-diallyl-*N,N*-dimethylammonium chloride with maleic acid seem promising [7]. The presence of carboxy groups in the chain of such copolymers permits to introduce various functions, for instance, ligands capable of formation of coordination bonds with *d*-metals.

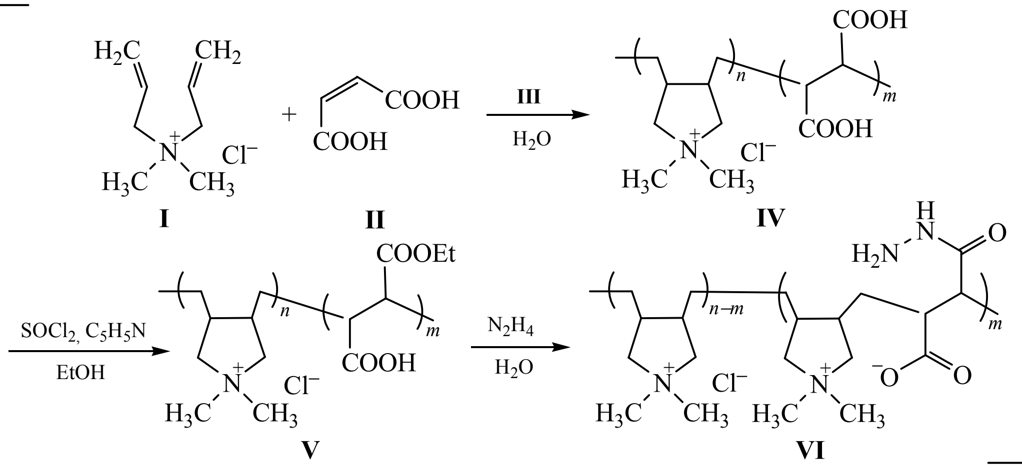
The aim of this work was the synthesis of copolymers on the basis of *N,N*-diallyl-*N,N*-dimethylammonium chloride **I** and maleic acid **II**, the introduction of hydrazide groups capable of complex formation with Cu^{2+} ions in the ampholytes obtained, and evaluation of fungiostatic activity of the created polymeric systems.

As is known, the radical homo- and copolymerization of *N,N*-diallyl-*N,N*-dimethylammonium chloride **I** leads to the formation of five-membered pyrrolidinium cycles regardless of the reaction conditions [8]. Lancaster et al. [8] and Vorob'eva et al. [9] have described copolymerization of *N,N*-diallyl-*N,N*-dimethylammonium chloride and maleic acid in water medium using ammonium persulfate as the initiator as well as in organic solvents in the presence of azobis(isobutyronitrile). It was found that in all cases the copolymers enriched with the units of the monomer **I** were formed, and the tendency to the alternation of units is observed.

In this work the synthesis of the copolymers was carried out in water medium using the water-soluble azoinitiator, 4,4'-azobis(4-cyanovaleric) acid **III** at the total concentration of monomers 4.8 mol l^{-1}. Preliminary experiments showed that such conditions lead to the formation of copolymers with the highest intrinsic viscosity [η] whose value characterize the dimensions of macromolecules in solution. Using the azo-initiator instead of ammonium persulfate permits to avoid side reactions characteristic of polymerization of the monomer **I** in water leading to decrease in the molecular mass of the polymers formed [10]. The composition of copolymers was calculated from the content of carboxylic groups evaluated by potentiometric titration. For the suppression of polyelectrolyte effects the titration was carried out in the 1 M NaCl solution [11]. Characteristics of the obtained copolymers are listed in Table 1.

The comparison of a series of samples (Table 1) shows that at the increase in the content of maleic acid **II** in the starting mixture a decrease in the intrinsic viscosity is observed indicating the low activity of the monomer **II** in the copolymerization. Highest [η] values were obtained for the samples containing the lowest amount of units of the maleic acid. Hence, by the copolymerization of monomers **I** and **II** copolymers were obtained differing in the composition and therefore in the balance of charges, and also in the intrinsic viscosity.

Carboxy groups in the composition of copolymers are weak ligands, but they can be transformed into hydrazide groups which are known to form strong complexes with Cu^{2+} ions [12, 13]. In the Scheme the stages of the synthesis of functionalized polyampholites are presented. The modification of the copolymers obtained was carried out through the intermediate stage of estrification of carboxy groups followed by hydrazinolysis.



The esterification was carried out by treating the alcoholic solution of metal carboxylate with thionyl chloride. This approach is widely used for the synthesis of methyl, ethyl, and benzyl esters of the amino acids [14–16]. The esterification of copolymers **IVa–IVe** was carried out by treating their solution in 96% ethanol with the excess of thionyl chloride in the presence of pyridine.

The reaction conditions were chosen in order to obtain on the average one carboxy and one ester group per one unit of the maleic acid. The conversion of the carboxy groups was calculated from the ^1H NMR spectra on the basis of signals at 4.24 ppm belonging to $COOCH_2CH_3$ group. The ^1H NMR spectrum of the polymer **Va** is presented in Fig. 1. The degree of esterification of carboxy groups in the copolymers **V** was ~50% (Table 2).

The hydrazinolysis of the obtained polymers was carried out for 8 h at 100°C. According to ^1H NMR spectral data the conversion of ester to hydrazide groups was 82–85%. The evaluation of hydrazide groups content in the copolymers **Va, Vb** was carried out spectrophotometrically using sodium 2,4,6-trinitro-

Table 1. Copolymerization of *N,N*-diallyl-*N,N*-dimethylammonium chloride **I** and maleic acid **II** in water, [**I** + **II**] = 4.8 mol l^{-1}, [**III**] = 0.144 mol l^{-1}, 60°C, 16 h. M_{II} and m_{II} molar fractions in the solution of maleic acid and the copolymer respectively

Comp. no.	Reaction mixture		Copolymer			
	[**II**], mol l^{-1}	M_{II}	C_{COOH}, mmol g^{-1}	m_{II}	[η], dl g^{-1}	Yield, %
IVa	0.48	0.10	1.57	0.12	0.69	57
IVb	0.96	0.20	2.94	0.22	0.51	50
IVc	1.44	0.30	3.84	0.28	0.23	34
IVd	2.40	0.50	5.26	0.37	0.15	30
IVe	3.36	0.70	5.94	0.42	0.05	14
IVf	4.32	0.90	7.30	0.50	0.03	6

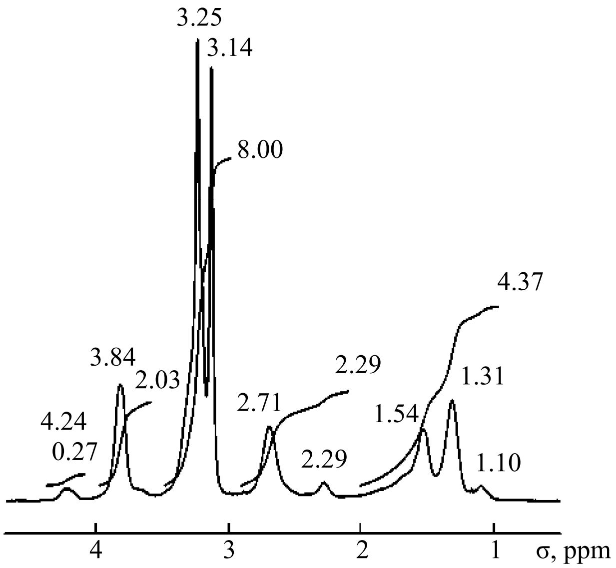


Fig. 1. ^{1}H NMR spectrum of polymer **Va**.

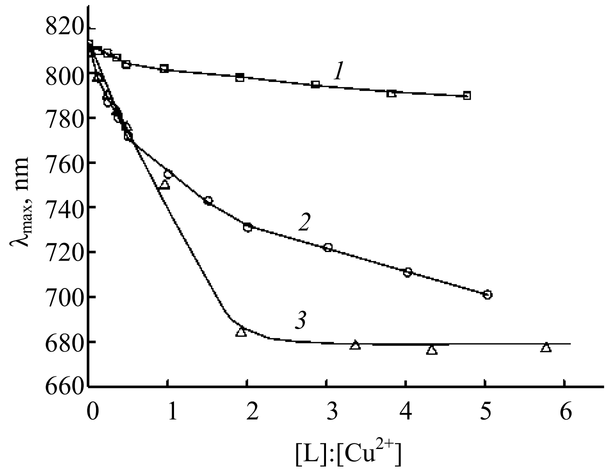


Fig. 2. Dependence of maximum point of the absorption band of Cu^{2+} at the complex formation on the ligand:metal ratio: (*1*) polymer **IVc**, (*2*) polymer **VIc**, and (*3*) isobutyrohydrazide.

benzenesulfonate [17, 18]. Note that the presence of residual ester groups should not affect the coordination of copper, but it may cause the formation of diacylhydrazide crosslinks. In connection with that the isolation and purification of hydrazide-containing copolymers was carried out under mild conditions. The samples dried at room temperature contained about 20% of water. Hence, the modification resulted in the formation of polyampholytes with the varied ratio of functional groups.

Complexes of Cu^{2+} ions with hydrazide-containing copolymers were prepared by mixing water solutions of copper(II) chloride and hydrazide-containing copolymers (in the basic form) and were isolated by liophylization of the mixture.

IR spectroscopy commonly used for establishing the formation of coordination bond in this case occurred to be uninformative because of overlapping of the characteristic bonds of C=O vibrations of carboxylate and hydrazide groups at 1562 and 1560 cm^{-1} respectively (amide **I**).

Table 2. Conditions of esterification of copolymers **IV** and characteristics of the obtained polymers **V**

Comp. no.	$[C_5H_5N]$:[COOH]	$[SOCl_2]$: [COOH]	COOH conversion, %	Yield, %
Va'	5	17	56	82
Va	5	10	48	80
Vb	1.5	3.6	53	85
Vc'	2	4	41	87
Vc	2	7	50	58
Vd	1.5	3.6	53	84

The stoichiochemistry of complexes formed was evaluated from the electronic spectra studying the absorption of complexes in water solutions at different ligand/metal (L/Cu^{2+}) molar ratio.

Studying the dependence of location of the maximum point in the absorption spectra for copper ions on the concentration of polymers showed that only very weak interaction of starting copolymers **IV** with Cu^{2+} ions (Fig.2, curve *1*) takes place. The isobutyrohydrazide chosen as a low-molecular model forms a complex where copper ion coordinates two hydrazide groups (Fig. 2, curve *3*). For the hydrazide-containing polymer (Fig. 2, curve *2*) the maximum point of the absorption band coincides with that of the low-molecular model up to the 1:1 molar ratio. Polymer nature of the ligand results in the fact that formation of two-coordinate complex (Fig. 3) is possible only at high excess of the ligand.

As is known, the positive charge of polycations on the basis of *N,N*-diallyl-*N,N*-dimethylammonium chloride **I** provides their high affinity to biological membranes [19]. It permits an assumption that the created polymer system containing complexes of polyfunctionalyzed polyampholytes **VI** with copper ions can be adsorbed on the surface of cells of plants and fungi providing the directed transportation of Cu^{2+} ions.

Ascomycete *Fusarium oxysporum*, the pathogenic organism causing the rotting of many agricultural products [20] was used as an object for testing the fungiostatic activity of polymers. The results obtained are listed in Table 3.

The starting copolymers **IVa, IVc** and functionalized polyampholytes **Va, Vc** and **VIa, VIc** prepared from them differing in the ratio of functional groups and the balance of charges in the polyelectrolyte chain (Table 1) were chosen for these studies. It turned out that the samples with the intrinsic viscosity below 0.15 dl g^{-1} do not exhibit the fungiostatic activity.

The tests have shown that starting copolymers **IVa, IVc** containing carboxy groups with the quaternary ammonium ones practically do not inhibit the growth of fungi. The partial esterification of carboxy groups leads to the appearance of activity as is seen for the sample **Va**. The presence of hydrazide groups in the composition of copolymers **VIa, VIc** leads to the significant increase in the activity as compared to the starting polymers **IVa, IVc** as well as to the esterified **Va, Vc** polymers. The model experiment with the low molecular compound, isobutyrohydrazide, showed practically complete absence of inhibition of the mycelium growth (3% on the 5th day) what permits an assumption on the polymer nature of fungiostatic effect of the hydrazide-containing polymers.

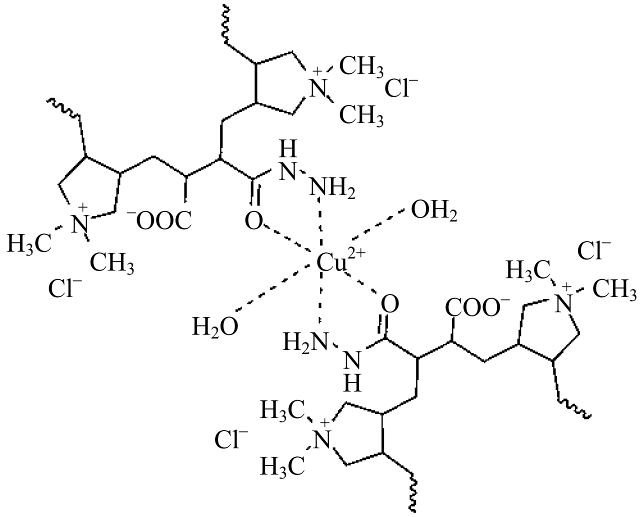


Fig. 3. Fragment of polymer complex of the composition L:Cu^{2+} = 2:1 (L is hydrazide-containing polyampholyte **VI**).

Studies of fungiostatic activity of copper chloride showed that the degree of inhibition of the mycelium growth reaches significant values at the concentration 0.002 mol l^{-1} and becomes as large as 72% and 48% on the 5th and 10th days of cultivation respectively, while at the concentration 0.0002 mol l^{-1} this effect is absent. It agrees with the reported data [21].

For the samples **VIa, VIb** in presence of Cu^{2+} complete suppression of the mycelium growth is observed on the 5th day of cultivation. Note that the polymer **VIa** containing larger amount of units of the monomer **I** as compared to the polymer **VIb** exhibits higher activity as revealed on the 10th day of the experiment. It is connected evidently with the fact that fungiostatic effect of polymer system is determined both by the length of chains consisting of the units of monomer **I** carrying positive charge and by the

Table 3. Fungiostatic activity of polyampholytes; concentration of polymers 0.5 g l^{-1}, $[CuCl_2]$ 0.002 mol l^{-1}

Comp. no.	Ratio of functional groups in polymers, mol	Inhibition of growth of the *F. oxysporum* mycelium, % to control	
	COOH:COOEt:CONHNH$_2$	5th day	10th day
IVa	12:0:0	2	0
Va	6:6:0	29	22
VIa	6:1:5	56	33
VIa (Cu^{2+})	6:1:5	100	100
IVc	28:0:0	3	0
Vc	14:14:0	10	3
VIc	14:2:12	53	36
VIc (Cu^{2+})	14:2:12	100	83

presence of coordinated Cu^{2+} ions in the polyampholyte chain.

Hence, functionalized polyampholytes **VIa, VIc** on the basis of *N,N*-diallyl-*N,N*-dimethylammonium chloride and maleic acid containing hydrazide groups form coordination complexes with Cu^{2+} ions and exhibit high fungiostatic activity suppressing the growth of the *F.oxysporum* mycelium.

EXPERIMENTAL

N,N-Diallyl-*N,N*-dimethylammonium chloride (Aldrich) and maleic acid were purified by crystallization from acetone. Initiator **III**, 4,4'-azo-bis-(4-cyanovaleric) acid was purified by crystallization from ethanol. All the solvents used were freshly distilled. Commercial reagents like hydrazine hydrate, thionyl chloride, copper(II) chloride dihydrate were used without the additional purification. Sodium 2,4,6-trinitrobenzenesulfonate was prepared according to [22]. Membranes from the regenerated cellulose D9527-100FT (Sigma-Aldrich) were used for the dialysis. Viscosity of polymer solutions was measured by the capillary Ubbelode viscometer (Cannon Instruments Co) in 1M NaCl solution at 25°C. Flow time of pure solvent was 116.2 s. Intrinsic viscosity was calculated from the average value as described in [23]. ^1H NMR spectra were taken on a Bruker DPX-300 spectrometer (300.13 MHz) in D_2O. UV spectra were obtained on a UV-1700 Shimadzu device in 1 cm quartz cells.

Synthesis of copolymer IVa. A solution containing monomer **I** in the concentration 4.32 mol l^{-1} and the monomer **II** in the concentration 0.48 mol l^{-1}, 10 ml, was placed in an ampule containing 0.404 g of the initiator **III**, and the mixture obtained was degassed. The ampule was sealed under argon and maintained at 60°C for 16 h. The reaction was performed until the high degree of conversion and was terminated by precipitation with 200 ml of acetone. The admixtures of monomers were removed from the product by extraction with hot 2-propanol and the subsequent reprecipitation from methanol to acetone. The product was dried in a desiccator over phosphorus pentoxide for no less than 3 days at 56°C (1 mm Hg). Copolymer **IVa**, 6.11 g (75%) was obtained. Molar fraction of units of the monomer **II** 0.12. ^1H NMR spectrum (D_2O), δ, ppm: 1.0–2.0 br.m (4H, CH_2 of the link), 2.29 br.m (0.3H, CH of *trans*-units of **I**), 2.45–2.90 br.m (2H, CH of *cis*-units of **I**, CHC=O), 3.0–3.5 br.m (8H, CH_3, CH_2N pseudoaxial). The other samples of copolymers **IVb–IVd** with different ratio of monomer units were obtained by varying molar ratio of monomers (Table 1).

Esterification of copolymer IVa. To a solution of copolymer **IVa**, 2.67 g, (4.2 mmol of COOH) in 40 ml of 96% ethanol 1.7 ml of pyridine was added. After that 3.0 ml of thionyl chloride was added under the intense stirring and cooling with a mixture of ice and calcium chloride. After that the reaction mixture was heated for 2 h at 80°C. The product was precipitated with acetone (200 ml) and exposed to dialysis at first against 0.0001 M HCl, and then against the distilled water. After liophylization and drying over phosphorus pentoxide 2.14 g (80%) of the product **Va** was obtained. The degree of esterification of carboxy groups according to ^1H NMR spectral data was 48%, $[\eta]$ 0.74 dl g^{-1}. ^1H NMR spectrum (D_2O), δ, ppm: 1.0–2.0 br.m (4.4 H, CH_2 of the chain, $CH_2\mathit{CH_3}$), 2.15–2.90 br.m (2.3 H, CH of the units **I**, CHC=O), 3.0-3.5 br.m (8H, CH_3, CH_2N pseudoaxial), 3.6–4.0 br.m (2H, CH_2N pseudoequatorial), 4.24 br.m (0.26H, $\mathit{CH_2}CH_3$). The esterification of copolymers having another composition was carried out analogously (Table 2). The purification of samples **Vc'** and **Ve** was carried out by thrice repeated reprecipitation with acetone.

Hydrazinolysis of polymer Va. Polymer **Va**,1.46 g (1.1 mmol of COOEt) was dissolved in 30 ml of hydrazine hydrate and heated for 8 h at 100°C. After that the reaction mixture was exposed to dialysis against 0.1 M NaCl and then against water. Yield of the product **VIa** after liophylization was 1.31 g (70%) at the content of water 22%, $[\eta]$ 0.69 dl g^{-1}, content of $CONHNH_2$ groups 0.5 mmol g^{-1}. ^1H NMR spectrum (D_2O), δ, ppm: 1.0-2.0 br.m (4H, CH_2 of the chain, $CH_2\mathit{CH_3}$), 2.0–2.9 br.m (2.4H, CH_2 of the units **I**, CH=O), 3.0–3.5 br.m (8H, CH_3, CH_2N pseudoaxial), 3.6–4.0 br.m (2H, CH_2N pseudoequatorial), 4.24 br.m (0.04 H, $C\mathit{H_2}CH_3$).

Analogously from 1.23 g of polymer **Vc** $[\eta]$ 0.26 dl g^{-1} 0.99 g (63%) of polymer **VIc** with moisture content 21% was obtained, $[\eta]$ 0.33 dl g^{-1}, content of $CONHNH_2$ groups 1.2 mmol g^{-1}.

Synthesis of complex of the polymer VIa with Cu^{2+}. Hydrazine-containing polymer **VIa,** 0.20 g, was dissolved in 1 ml of water, and 1 ml of 0.1 M solution of copper(II) chloride was added. Dark green solution was stirred for 30 min. After liophylization 0.21 g of the product was obtained.

Fungiostatic activity. The investigation of fungiostatic activity of polymers was carried out in the Russian Institute of Plant Protection of Russian Academy of Agricultural Sciences by the agar block method and characterized by the degree of growth inhibition of the *Fusarium oxysporum* micelium against the control samples on the 5th and the 10th day of cultivation. The fungus was cultivated at 25°C on the Chapek medium with the addition of substance under study dissolved in the minimum amount of water.

AKNOWLEDGMENTS

Authors express their gratitude to the NMR laboratory of Chemical faculty of St. Petersburg State University for the registration of NMR spectra and to the Candidate of biological sciences E.V. Popova (VIZR, RASKhN) for the great help in the fungiostatic activity studies.

REFERENCES

1. Butler, G.B., *Cyclopolymerization and Cyclocopolymerization,* New York: Marsel Deker Inc., 1992, p. 485.
2. Wandrey, Ch., Hernandez-Barajas, J., and Hunkeler, D., *Adv. Polym. Sci*., 1999, vol. 145, p. 123.
3. Ottenbrite, R.M. and Ryan, W.S*., Ind. Eng. Chem. Prod. Res, Dev*., 1980, vol. 19, no. 4, p. 528.
4. Gilyazetdinov, Sh.Ya., Balakhontsev, E.N., Leplyanin, G.V., Vorob'eva, I.A., Iskhakov, F.F., and Radtsenova, O.V., USSR Inventor's Certificate no. 744797; *Byull. Izobret*., 1992, no 24, p. 205.
5. Thomas, D.S., Vasilieva, Y.A., Armentrout, S.R., and McCormick Ch.L., *Macromolecules*, 2003, vol. 36, no. 26, p. 9710.
6. Aleksandrova, V.A., Kotlyarova, E.B., Odin, A.P., Domnina, N.S., Shevchenko, V.A., and Topchiev, D.A., *Radiatsionnaya biologiya*. *Radioekologiya*, 1995, vol. 35, no 5, p. 746.
7. Vorob'eva, A.I., Gaitsina Kh.A., Vasil'eva, E.V., and Prochukhan, Yu.A., *Vysokomol. Soedin., Ser, B*, 1999, vol. 41, no. 4, p. 726.
8. Lancaster, J.E., Baccei, L., and Panzer, H.P., *J. Polym. Sci., Polym. Lett. Ed*., 1976, vol. 14, no. 9, p. 549.
9. Vorob'eva, A.I., Sagitova, D.P., Kuznetsov, S.I., Kunakova, R.V., and Monakhov, Yu.B., *Vysokomol. Soedin., Ser. A*., 2008, vol. 50, no. 2, p. 230.
10. Dautzenberg, H., Goernitz, E., and Jaeger, W., *Macromol. Chem. Phys*., 1998, vol. 199, no. 8, p. 1561.
11. Koetz, J. and Kosmella, S., *Polyelectrolytes and Nanoparticles*, Berlin: Springer-Verlag, 2007, pp. 11, 14.
12. Grekov, A.P. and Suhorukova, S.A., *Polymery na osnove gidrazina* (Hydrazine-Based Polymers), Kiev: Naukova Dumka, 1976, p.183.
13. Nonaka, T., Momono, E., and Egawa, H., *Bull. Chem. Soc. Japan*, 1979, vol. 52, no. 4, p. 1147.
14. Brener, M. and Huber, W., *Helv. Chim. Acta*, 1953, vol. 36, no. 5, p. 1109.
15. Uhle, F.C. and Harris, L.S., *J. Am. Chem. Soc*., 1956, vol. 78, no. 2, p. 381.
16. Patel, R.P. and Price, S., *J. Org. Chem.,* 1965, vol. 30, no. 10, p. 3575.
17. Cayot, Ph. and Tainturier, G., *Anal. Biochem*., 1997, vol. 249, no. 2, p. 184.
18. Lu Zh-R., Kopechkova, P., Wu, Zh.-Ch., and Kopecek, J., *Bioconjugate Chem*., 1998, vol. 9, no. 6, p. 793.
19. Afinogenov, G.E. and Panarin, E.F., *Antimikrobnye polimery* (Antimicrobal Polymers), Sankt-Peterburg: Gippokrat, 1993, pp. 4, 12.
20. Bilay, V.I., *Fuzarin* (Fusarine), Kiev, Naukova Dumka, 1997.
21. Domsch, K.H. and Corden, M.E., *Arch. Microbiol*., 1973, vol. 88, no. 4, p. 345.
22. Pettitt, D.J. and Helmkamp, G.K., *J. Org. Chem*., 1964, vol. 29, no. 9, p. 2702.
23. Elias, H-G., *An Introduction to Polymer Science*, 1st ed, Weinheim: VCH, 1997, p. 201.
24. Vlasov, P.S., Kiselev, A.A., Domnina, N.S., Popova, E.V., and Tyuterev, S.L., *Zh. Prikl. Khim*., 2009, vol. 82, p. 1571.