

The peculiarities of polymerization of styrene in the presence of high-molecular alkoxyamines synthesized *in situ* on the basis of nitroneses of different structures

E.V. Kolyakina, A.B. Alyeva, D.F. Grishin

National Research Lobachevsky State University of Nizhny Novgorod, Gagarin Prospekt 23/2, 603950 Nizhny Novgorod, Russia

E-mail: kelena@ichem.unn.ru (E.V. Kolyakina)

High molecular weight alkoxyamines of various structure and molecular weight, used for the synthesis of polystyrene, were obtained by radical polymerization in the presence of C-phenyl-N-tert-butylnitronese and 2- (benzylideneamino) -2-methyl-1-phenylpropanol-1-N-oxide. It was shown that polymerization in the presence of alkoxyamines based on C-phenyl-N-tert-butylnitronese proceeds in a controlled manner. In this case, a constant rate of the process and linear growth of the molecular mass with conversion are observed, and the synthesis of polymers with low polydispersity ($M_w/M_n \sim 1.26$) is possible. Synthesis of polymers involving alkoxyamines based on 2- (benzylideneamino) -2-methyl-1-phenylpropanol-1-N-oxide is realized at higher rates, but in this case polystyrene is characterized by higher values of polydispersity coefficients. An increase in the polymerization temperature and the molecular weight of the starting alkoxyamines results in a decrease in the control over the molecular weight characteristics of the synthesized polymers.

Keywords: radical polymerization, polystyrene, macroinitiators, nitroxides, alkoxyamines.

1. Introduction

Polymerization in reversible inhibition is one of the three main directions of controlled radical polymerization (CRP) [1]. Nitroxide-Mediated Polymerization (NMP) [2-4] is most often used as reversible inhibiting agents, which are most effective in the polymerization of styrene and its homologues.

The use of precursors that generate nitroxide radicals directly in the process of polymerization (*in situ*) made it possible, on the one hand, to carry out the polymerization process in more comfortable temperature conditions (less than 100 °C), and on the other hand, to expand somewhat the range of monomers whose controlled polymerization can be carried out by this method [5, 6]. Nitrones commonly used in the chemistry of free radicals as spin traps are often used as sources of stable nitroxide radicals formed *in situ* [7-15].

Thus, in a series of works by Russian authors [6-12], a one-step approach to the formation of high molecular weight nitroxide radicals *in situ* in the medium of monomers during polymerization was applied. It is shown that this approach provides directional control of the molecular weight (M_w) characteristics of a number of vinyl and (meth)acrylic monomers, including methyl methacrylate, butyl methacrylate and butyl acrylate, styrene (ST), vinyl chloride, acrylonitrile and N-vinylpyrrolidone. This one-step approach allows the synthe-

sis of homo- and copolymers with specified molecular-weight characteristics over a wide temperature range of 50-120 °C with relatively high rates. At the same time, a certain "pay" for this gain in polymerization rate is a slight increase in the polydispersity coefficients of synthesized polymers compared with the use of nitroxide radicals introduced into the system from outside (*ex situ*).

A number of research groups aimed at obtaining polymers with improved molecular weight characteristics under conditions of radical initiation used a two-step approach to polymerization in the presence of nitroneses [13-16]. Synthesis of alkoxyamine (AA) based on nitroneses was initially performed at low temperatures of 60-80 °C for a short time directly in the medium of the monomer or in a solvent. As a result, the formation of spatially hindered AA occurred in the system. Further controlled synthesis of polymers was carried out at a higher temperature of 100-130 °C. In particular, Jerome R. and co-workers [5, 13, 14], using the polymerization of ST and butyl-acrylate in the presence of a number of nitroneses, have shown that the proposed approach allows the synthesis of polymers with polydispersity ($M_w/M_n = 1.4-1.6$) and also to obtain block copolymers of various structures. Barner-Kowollik C. et al. [15, 16] introduced the term Enhanced Spin Capturing Polymerization to characterize a two-stage approach to the controlled

synthesis of post- and block-copolymers using high molecular weight AA. Synthesis of high-molecular alkoxyamines ($M_w/M_n \sim 1.7$) was carried out by them in the low-temperature regime (60–80 °C) directly in the medium of monomers, in particular, ST and methyl methacrylate, on the basis of which, subsequently, at a higher temperature (100 °C and higher), post-polymers of styrene and various block copolymers were synthesized.

It should be noted that in recent years nitrones have been widely used not only for directly polymerization processes, but also for the implementation of so-called polymer-analogous transformations [17–19]. Undoubtedly, nitrones are very promising compounds with multifunctional capabilities. They can be positioned both as radical spin traps, and as polar 1,3-dipoles, and as effective regulators of polymerization. And the features and effectiveness of their application directly depends on the structure of the radicals bound with the nitron group. In this connection, studies of the influence of the structure of nitrones and high molecular weight AAs, which can be formed on their basis, on the CRP features, including with the aim of reducing the contribution of side reactions to the process of controlled polymer synthesis, are of undoubted interest.

2. Experiment

2.1. Reagents

The ST (Aldrich) was purified from the inhibitor by a 10% aqueous solution of sodium hydroxide, then washed with alkali water until neutral reaction and dried over calcined calcium chloride. Purified by vacuum distillation, collecting a fraction with a boiling point of 48 °C at 20 mm Hg. Art. for ST [20]. 2,2'-azo-bis-isobutyronitrile (AIBN) (Aldrich) was recrystallized from ethanol according to a standard procedure [21]. C-phenyl-N-tert-butyl nitron (PBN) was synthesized and purified by standard procedure by recrystallization from petroleum ether, $T_m = 74\text{--}75^\circ\text{C}$ [22]. 2- (benzylideneamino) -2-methyl-1-phenylpropanol-1-N-oxide (BMPO) was prepared by the procedure of [23]. Nitrones are characterized by IR and NMR spectroscopy. Methylene chloride, benzene, isopropyl alcohol and acetone (Reactive Component, Russia) were dried over calcined calcium chloride and distilled at atmospheric pressure. THF (Component Reagent, Russia) was initially dried over an alkali, distilled over metallic sodium. The boiling points of all the solvents corresponded to the literature data [24].

2.2. Experimental Procedure

Synthesis of polystyrene involving nitrones. Samples for polymerization were prepared as follows: AIBN (71.3 mg, 1 mol%) and nitron (1.5 mol%: 115.5 mg PBN or 174.9 mg BMPO) were dissolved in 5 ml of the pre-distilled monomer. The prepared solution was dispensed into 0.8 ml am-

poules. Under reduced pressure, the ampoule was degassed three times by repeating the freeze-thaw cycles to a residual pressure of $2 \cdot 10^{-2}$ mm Hg. Art. and soldered. Control of the kinetics of polymerization at temperatures of 70, 90 and 110 °C was carried out by gravimetric method. After a certain time, the ampoule was removed and freeze in liquid nitrogen to interrupt the polymerization. The resulting polymer was precipitated into isopropyl alcohol. In order to purify polymers from monomer, initiator and nitron residues, the samples were reprecipitated from methylene chloride solution, then, under reduced pressure and 50 °C., dried to constant weight.

The degree of conversion was calculated by formula:

$$P = \frac{m(\text{polymer})}{m(\text{monomer})} \times 100\%.$$

Preparation of high-molecular alkoxyamines. AA **1** and **4** were prepared in a solution of ST with benzene, for this, AIBN (196.8 mg, 2 mol %) and nitron (4 mol %: 424.8 mg PBN or 643.2 mg BMPO) were dissolved in 6 ml ST: benzene (1: 1) and loaded into an ampoule. AA **2**, **3**, **5**, **6** were prepared in a weight of ST, AIBN (42.8 mg, 1 mol %), and nitron (1.5 mol %: 69.3 mg PBN or 104.9 mg BMPO) were dissolved in 3 ml of pre-distilled ST, the resulting solution was loaded in the ampoule. The preparation of ampoules and the synthesis of high molecular weight AA were carried out in accordance with the above procedure. The main conditions for the synthesis of high molecular weight AA and their molecular weight characteristics are given in Table 1. Samples (**1**, **2**, **4**, **5**) withstood less than 10 AIBN half-lives ($t_{1/2}(\text{AIBN}) = 0.5 \text{ h}$ at 90 °C) were subjected to a three-fold reprecipitation in order to purify the resulting polymers from the initiator and nitron residues, and also low-molecular interaction products of the initiator and nitron. Samples (**3**, **6**) withstanding more than 10 AIBN half-life periods were precipitated from the methylene chloride solution into isopropyl alcohol after the termination of the polymerization, then, under reduced pressure and 50 °C, dried to constant weight. The resulting products were used as macroinitiators for the polymerization of styrene.

Homopolymerization of styrene using high-molecular alkoxyamines. The macroinitiator (0.1 mol %) was dissolved in 0.8 ml of styrene. Ampoules were prepared in accordance with the above procedure.

The obtained samples were reprecipitated from methylene chloride to isopropyl alcohol. The samples were dried under reduced pressure at 50 °C. The degree of conversion was calculated by formula:

$$P = \frac{m(\text{polymer}) - m(\text{AA})}{m(\text{monomer})} \times 100\%.$$

Synthesis conditions of high-molecular alkoxyamines at 90 °C

AA	Synthesis conditions					$M_n \times 10^{-3}$	$M_w \times 10^{-3}$	M_w/M_n
	System	AIBN concentration, mol. %	Nitron	Nitron concentration, mol. %	Time, h			
1	$V(C_6H_6)/V(ST)$ 1:1	2	PBN	4	2.0	2.6	3.0	1.15
2	ST	1		1.5	0.7	6.4	9.5	1.48
3	ST	1		1.5	62.4	10.8	15.7	1.45
4	$V(C_6H_6)/V(ST)$ 1:1	2	BMPO	4	2.0	2.5	3.0	1.20
5	ST	1		1.5	0.7	6.3	10.0	1.59
6	ST	1		1.5	40.0	7.9	15.2	1.92

Analysis of the molecular-weight characteristics of polymers. The molecular weight characteristics of the polymers were determined by Gel permeation chromatography GPC using a liquid chromatograph KNAUER equipped with a cascade of Phenomenex columns (300 mmr \times 7.8 mm, phenogel, 10 μ m) with pore diameters of 105 and 103Å and two detectors (refractometric and UV). The eluent was THF, the column temperature was 25.0 \pm 0.1 °C. For the calibration, narrow-dispersed PS standards were used [25].

3. Results and discussion

As mentioned above, the synthesis of polymers in the presence of nitroxide radicals formed *in situ* is of major importance among the methods of conducting an CRP on the mechanism of reversible inhibition. As a source of stable radicals a wide range of organic compounds is used, including nitroneses, nitrosocompounds, oxytriazenes, amines and other compounds [5, 6]. Interest in this direction of polymerization is determined by a number of important aspects: the ability to synthesize polymers based on monomers of different structures with specified molecular weight characteristics, the modification of the structure of the polymer molecule, and commercial availability and relatively low cost of the foregoing CRP regulators precursors. In

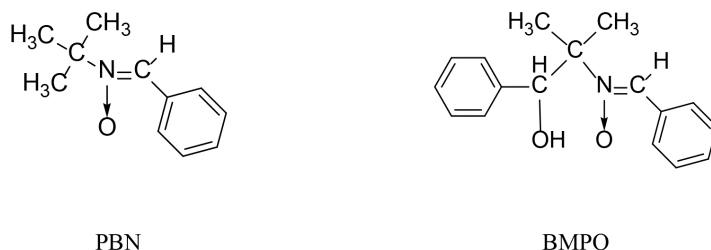
the literature, there are single data on the correlation of the structure and effectiveness of sources of nitroxide radicals in the processes of controlled synthesis of macromolecules [5, 6, 13].

In this work, we conducted a comparative analysis of the features of polymerization of ST in the presence of nitroneses of different structures (Scheme 1) and also high-molecular AA of various M_w synthesized on their basis (Table 1).

3.1 Radical polymerization of styrene in the presence of alkoxyamines formed *in situ*, based on nitroneses

It was found that PBN and BMPOs as potential sources of stable nitroxide radicals are able to effectively regulate the kinetic parameters of ST polymerization, as well as the molecular weight characteristics of synthesized polymers, due to the formation of alkoxyamines *in situ*.

Comparing the regularities of polymerization of ST in the presence of PBN and BMPOs, which are structural analogues, it is necessary to emphasize that in the presence of BMPOs, the polymerization rate of the ST at 90 °C is much higher than the polymerization rate in the presence of PBN (Fig. 1). The kinetic curves 2 and 3, shown in Fig. 1, have two sections. The first section (up to 5 hours) corresponds to the initial process of formation of alk-



Scheme 1

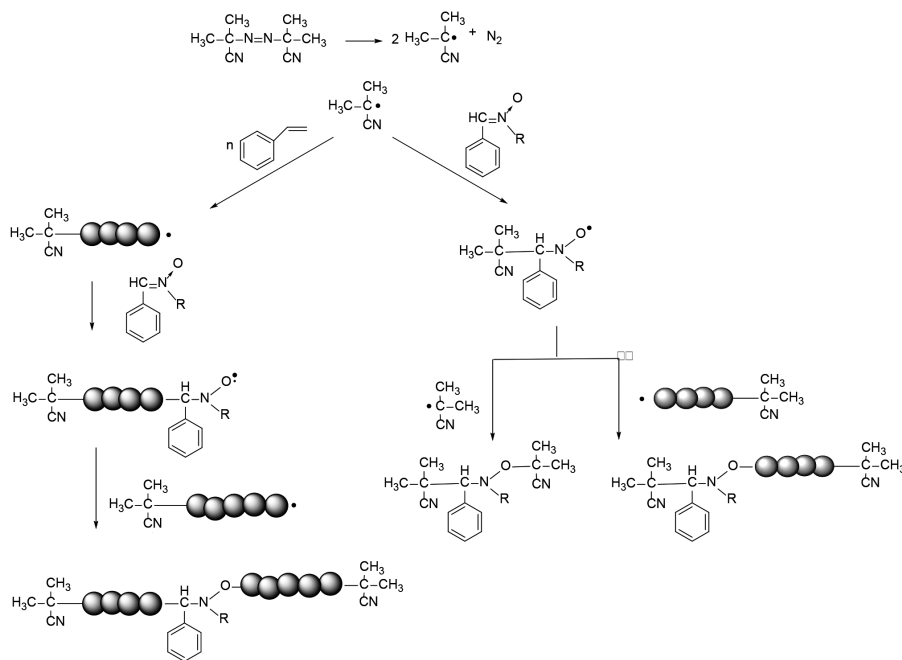
oxyamine *in situ*. At this stage, the AIBN decomposes with the generation of radicals, the initiation of polymerization, and the formation of high-molecular alkoxyamines (Scheme 2). The second section of the kinetic curve, both in the case of FBNs and BMPOs, differs sharply in rate from the initial stage. In this case, the polymerization proceeds at a lower rate than the first stage. This is due to the fact that after 5 hours of polymerization there is practically no radical initiator of AIBN in the system (its half-life at 90 °C is 0.5 h), and the process is realized before deep conversion due to the reversible breakage of the growing radical on high-molecular nitroxide, i.e. due to the implementation of the NMP mechanism [2].

Molecular weight characteristics of the PS synthesized in the presence of nitroxides generating AA during the polymerization are shown in Fig. 2a and b. Some features of the *in situ* polymerization of ST in the presence of PBN and its structural analogue of BMPOs should be noted. The dependences of M_w on conversion (Figure 2) are uncharacteristic for NMP polymerization. In particular, at the initial stage of formation of high molecular weight AA, growth of M_w with conversion is not observed (sections of curves up to 40%), which may be due to the gradual decay of the AIBN in the system. The further process is accompanied by a linear increase in M_w with an increase in conversion. However, the regularities of the increase in M_w in the presence of PBN and BMPO are different. In the case of polym-

erization in the presence of PBN, a significant increase in the M_w and a decrease in the polydispersity coefficient are observed with an increase in the monomer conversion to a minimum value of 1.45 (Fig. 2a). The control of the polymerization process in the presence of BMPO is less pronounced, while the values of the polydispersity coefficients after 40% conversion of the monomer to the polymer decrease somewhat compared with the initial polydispersity from 1.67 to 1.57, but at deep degrees of conversion increase to 1.90 (Fig. 2b).

The molecular weight distribution (MWD) curves of polymers synthesized by the *in situ* method are shown in Fig. 3a and b. In the case of ST polymerization in the presence of PBN, narrow unimodal curves are observed, which gradually shift to the region of higher M_w values. Samples synthesized in the presence of BMPOs are characterized by a broader MWD of macromolecules. Moreover, in the polymerization in the presence of BMPOs, the low-molecular-weight shoulder is not shifted to the region of higher M_w values (curves 2,3 of Fig. 3b), and at high degrees of conversion of the monomer to the polymer macromolecules with higher M_w values are formed, which leads to a sufficiently significant increase in polydispersity synthesized samples.

Such influence of BMPOs and nitroxides formed on its basis is probably due to the effect of the interaction of the hydroxyl group and the nitroxide radical. Indeed, in [26], the possibility of interaction of



Scheme 2

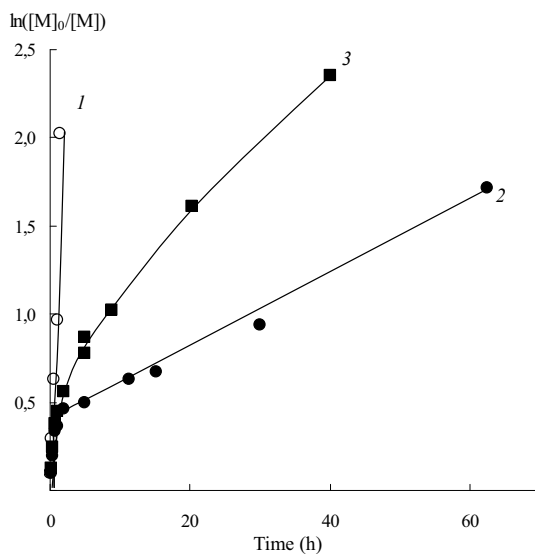
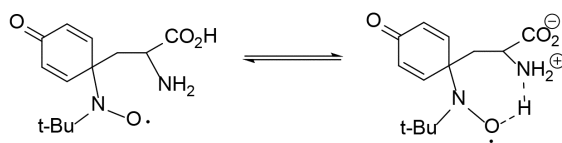


Fig. 1. Semi-logarithmic kinetic dependences of polymerization of ST at 90°C in the presence of 1.0 mol.% of AIBN and nitrones (1.5 mol.%): 1 - without nitrone; 2 - PBN; 3 - BMPO.

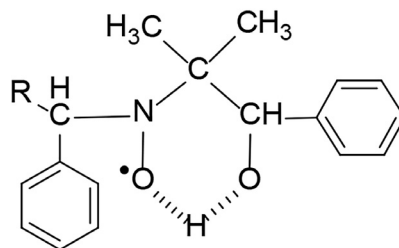
the nitroxide fragment with the hydrogen atom of the amino group through the formation of a seven-membered cyclic complex (Scheme 3) is noted.

In our case, analogous coordination of the hydrogen atom of the hydroxyl group is possible with the formation of an energetically favorable six-membered ring (Scheme 4):



Scheme 3

It should be noted that such interaction of the hydroxyl group with the nitroxide fragment can lead to intramolecular transfer of the hydrogen atom and, as a result, the gradual death of nitroxide radicals and the formation of a “dead” polymer. This fact causes a lower efficiency of high molecular weight alkoxyamine based on BMPO as a regulator of molecular weight characteristics in comparison with analogs based on PBN.



Scheme 4

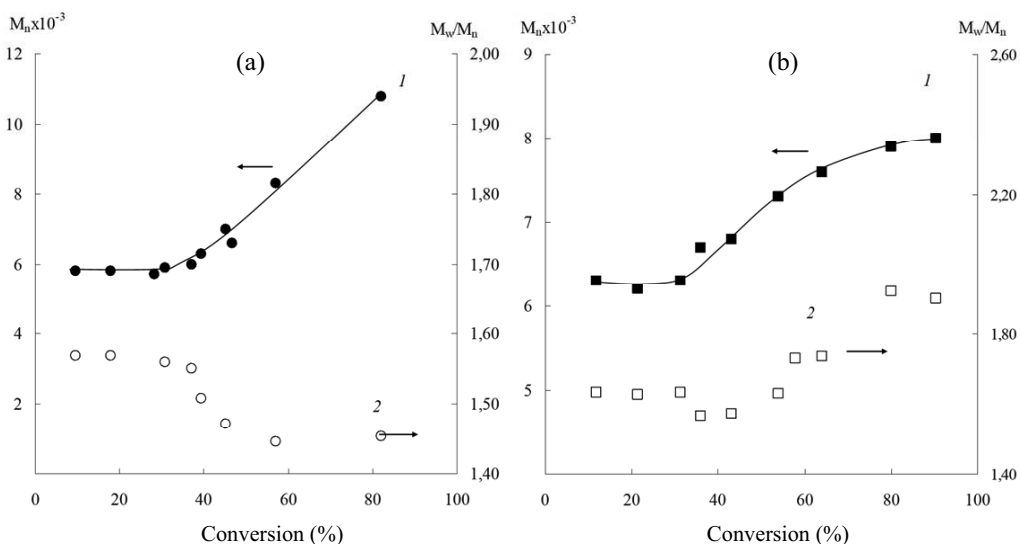


Fig. 2. Dependence of M_n (1) and polydispersity coefficient (2) of PS samples obtained in the presence of 1.0 mol.% of AIBN and 1.5 mol.% of nitrone at 90°C, on the conversion: a - FBN, b - BMPO.

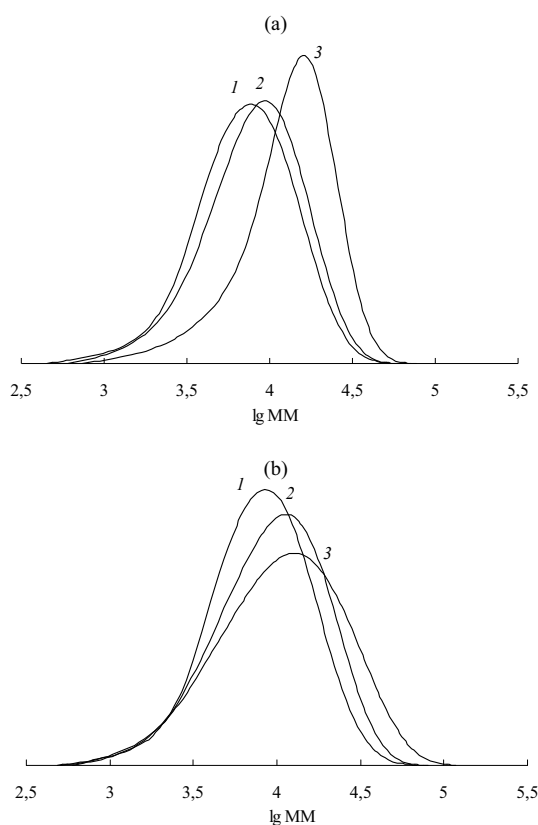
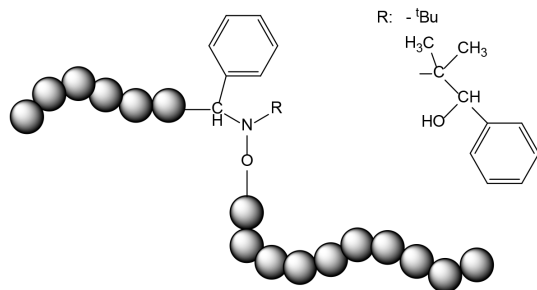


Fig. 3. MWD curves of the PS synthesized in the presence of AIBN (1 mol.%) and nitron (1.5 mol.%). a - PBN, conversion, %: 1-10; 2-50; 3-82; b - BMPO, conversion, %: 1-12; 2-58; 3-91.

3.2 Characteristics of the polymerization of styrene in the presence of high-molecular alkoxyamines

To carry out a controlled synthesis of PS, high molecular weight AAs with a certain M_w were used, the structures of which are presented in Scheme 5.



Scheme 5

Synthesis of AA was carried out in the presence of PBN and BMPOs, and the synthesis conditions were selected in such a way that the M_w regulators were as comparable as possible. The main characteristics of alkoxyamines are given in Table 1.

Obtained data (Table 2) indicate that not only the initial molecular weight characteristics of AA but also the structure of the nitron, a source of high-molecular AA, influence on the features of polymerization of ST. It was found that AA based on PBN (1, 2, 3) are most effective in the polymerization of CT at 90 °C and above. At 70 °C, these alkoxyamines are also capable of initiating a polymerization process, but with the exception of AA having a M_w of 15,700 Da, the conversion of the monomer within 60 hours is only 20%. In the case of the highest-molecular-weight AA, the rate of polymerization at 70 °C increases somewhat. We note that in all cases polymers with sufficiently low values of polydispersity coefficients are obtained, which increase somewhat when passing from low molecular weight AA to high molecular weight.

Synthesis of PS in the presence of AA based on BMPO differs somewhat from polymerization in the presence of PBN-containing alkoxyamines. At 70 °C, the polymerization of STs with the participation of alkoxyamines (4 and 5) with M_w 3000 and 10,000 Da is realized before deeper conversion than in the case of PBN-containing AA. It was found that the rate of polymerization decreases upon transition from a low molecular weight to a higher molecular weight AA. In the case of BMPO-containing AA, the change in their MW is also significantly reflected in the molecular weight characteristics of the synthesized polymers. Thus, with the growth of M_w alkoxyamines, a significant increase in the polydispersity of the samples occurs (Table 2).

The obtained data indicate that the polymerization of ST in the presence of AA both on the basis of PBN and BMPOs proceeds at a much higher rate than its autopolymerization (Figs 4a and b). Moreover, the nature of the initial nitron has a direct effect on the degree of control of the synthesis of the PS. Thus, in the polymerization of ST in the presence of AA synthesized on the basis of PBN (1, 2, 3), the process proceeds without autoacceleration at a constant rate (Fig. 4a). At the same time, the rate of polymerization is practically independent of the MW of the alkoxyamines used.

Absolutely different results were obtained in the polymerization of ST in the presence of AA 4 and 5 on the basis of BMPO (Fig. 4b). It was found that the polymerization rate of ST in the presence of low molecular weight AA is higher than the polymerization rate of ST in the presence of AA 6 with $M_w = 15200$ Da. We emphasize that the overall process speed in the presence of AA 4 and 5 is higher than the polymerization rate of the monomer with AA based on PBN. Thus, the polymerisation process in

Table 2

Molecular mass characteristics of PS synthesized in the presence of 0.1 mol. % AA at various

Nº	AA	$M_w \times 10^{-3}$	$T, ^\circ$	Time, h	Conversion, %	$M_n \times 10^{-3}$	$M_w \times 10^{-3}$	M_w/M_n
1	1	3.0	70	60	20	32	46	1.44
2			90	60	92	81	116	1.43
3			110	12	87	86	124	1.44
4	2	9.5	70	60	22	26	45	1.73
5			90	60	90	95	143	1.51
6			110	12	94	88	128	1.45
7	3	15.7	70	60	45	49	88	1.79
8			90	60	90	95	141	1.48
9			110	12	89	89	127	1.43
10	4	3.0	70	60	59	112	167	1.49
11			90	22	98	102	225	2.20
12			110	12	82	89	212	2.39
13	5	10.0	70	60	58	60	141	2.35
14			90	22	94	44	158	3.59
15			110	12	84	39	168	4.31
16	6	15.2	70	60	32	38	335	8.81
17			90	60	82	70	499	7.13
18			110	12	85	49	300	6.12

the presence of AA based BMPO is realized to deep degrees of polymerization in 20 hours, and in the case of polymerization involving PBN-containing alkoxyamines, deep conversions are achieved only in 60 hours (Fig. 4a and b).

On the other hand, when using high molecular weight BMPO-containing AA **6** (MW = 15200 Da), the overall polymerization rate at 90 °C is even slightly lower than in the case of AA based on PBN. It should be noted that alkoxyamine with MW = 15200 Da is isolated at deep degrees of ST transformation during *in situ* polymerization and is characterized by the highest polydispersity coefficient in the number of AAs under study (Table 1). It is not excluded that the observed features of polymerization with its participation are due to the presence of a large number of "dead" chains.

We carried out a comprehensive analysis of the molecular-weight characteristics of the PS with the participation of AAs of different structures and MWs. It was found that in the case of ST polymerization in the presence of PBN-containing AA, the experimental values of MW synthesized samples (Table 3) correlate quite well with theoretically calculated MWs according to the equation:

$$M_{n,th} = \frac{[M]_0}{[AA]_0} \times M_{ST} \times conversion + M_{n,AA}$$

where $[M]_0$ is the initial monomer concentration, $[AA]_0$ is the concentration of alkoxyamine,

M_{ST} – MW monomer, $M_{n,AA}$ is the number-average MW of the parent alkoxyamine.

For PBN-containing AA irrespective of their MW, the initiation efficiency (f) throughout the entire polymerization process is high and within the error of the determination by MW chromatographic method is close to 1 (Table 3). Moreover, MW PSs increase with conversion linearly for all types of initial AA (Fig. 5a). In this case, the polydispersity of samples synthesized on the basis of PBN-containing AA decreases with an increase in the degree of monomer conversion to some minimum values ($M_w/M_n = 1.26$ for AA **1** и **2**, $M_w/M_n = 1.36$ for **3**), however, on deep conversions its growth is observed (Fig. 6a). It is found that the absolute values of the polydispersity coefficients increase with increasing MW of high-molecular AA based on PBN (Fig. 6a).

MWD curves of PS samples obtained in the presence of high molecular weight AA based on PBN are shown in Fig. 7. Regardless of the alkoxyamine used, all MWD curves are unimodal, the mode being successively shifted to the region of high MW with increasing conversion. This type of curves is typical for processes that occur by the mechanism of reversible inhibition.

The presence of a hydroxyl fragments capable of intramolecular transfer of a hydrogen atom in the structure of high-molecular nitroxides based on BMPO has a significant effect on the molecular weight characteristics of polymers. In the case

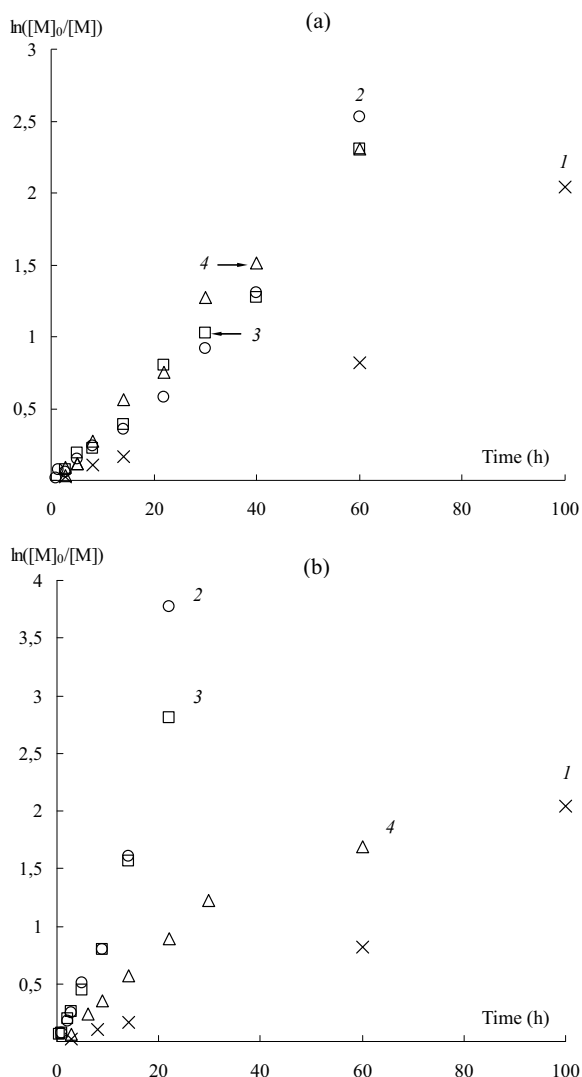


Fig. 4. Semi-logarithmic kinetic dependences of polymerization of ST at 90°C in the presence of 0.1 mol.% of alkoxyamines. 1 - thermal polymerization of ST.

a: AA synthesized in the presence of PBN. M_w AA, Da: 2 - 3000; 3 - 9500; 4 - 15700.

b: AA synthesized in the presence of BMPO. M_w AA, Da: 2 - 3000; 3 - 10,000; 4 - 15200.

of using a low molecular weight AA $M_w = 3000$ Da at 90 °C, linear growth of MW with an increase in the degree of monomer conversion is observed (Fig. 5b). However, practically throughout the entire polymerization process, the experimental values of M_w polymers are higher than theoretically calculated, that is, the efficiency of initiating this

alkoxyamine is significantly below 1 (Table 3). In general, the polydispersity coefficients of polymers synthesized in the presence of AA on the basis of BMPO ($M_w = 3000$ Da), initially decrease in the course of the process (Fig. 6b), but on deep conversions increase and reach values of 2.2. In this case, the MWD of PS samples synthesized on the basis of this AA on deep conversion remain unimodal (Fig. 8a).

An increase in the alkoxyamine M_w to 10,000 Da leads to a more significant deviation of the growth of MW with conversion, in contrast to the ideal controlled polymerization (Table 3, Fig. 5b). The experimental values of M_n are significantly lower than the theoretical expected M_w of PS, while at the deeper levels of the monomer conversion, the MW growth slows down (Fig. 5b). The “apparent” initiation efficiency (Table 3) when using this AA is greater than one, this indicates a number of side processes, in particular the lower experimental values of the M_w of PS can be due to the death of the nitroxide radical due to the intramolecular transfer of the hydrogen atom in the nitroxide fragment, and, as a consequence, the presence of a low-molecular “dead” polymer. When this AA is used, a PS with high polydispersity is obtained, whose values increase with increasing conversion (Fig. 6b). The MWD of PS samples synthesized in the presence of AA with $M_w = 10,000$ Da is wide enough and have a low-molecular-weight shoulder at deep conversion, indicating the presence of a “dead” polymer (Fig. 8b)

A few other regularities in terms of molecular weight characteristics were revealed when using BMPO-containing AA with $M_w = 15200$ Da and a sufficiently high polydispersity coefficient. The samples are characterized by a bimodal distribution (Fig. 8c) and high polydispersity coefficients at the level of 7-8 (Table 3), while it is difficult to interpret the results on M_w growth with conversion and initiation efficiency. It should be noted that the low-molecular fashion samples corresponding to the original AA hardly shifted to higher values of MW (Fig. 8c), and only somewhat reduced in intensity. The second high molecular mode although shifted to higher values of M_w , but deep degrees of conversion comparable to MW of PS produced by thermal initiation (Fig. 8c). These laws testify to the fact that with the increase of M_w alkosiaminov based on BMPO an abrupt loss of control of polymerization occurs, which is associated with the occurrence of side reactions leading to the conclusion of AA from the reaction.

Temperature has a significant effect on the polymerization of ST in the presence of a BMPO-containing AA. The initial screening of polymerization of ST in the presence of AA in various temperature regimes showed that the process involving BMPO-containing AA (4 and 5) in contrast to AA based on PBN (1 and 2) at 70 °C is realized until deep-

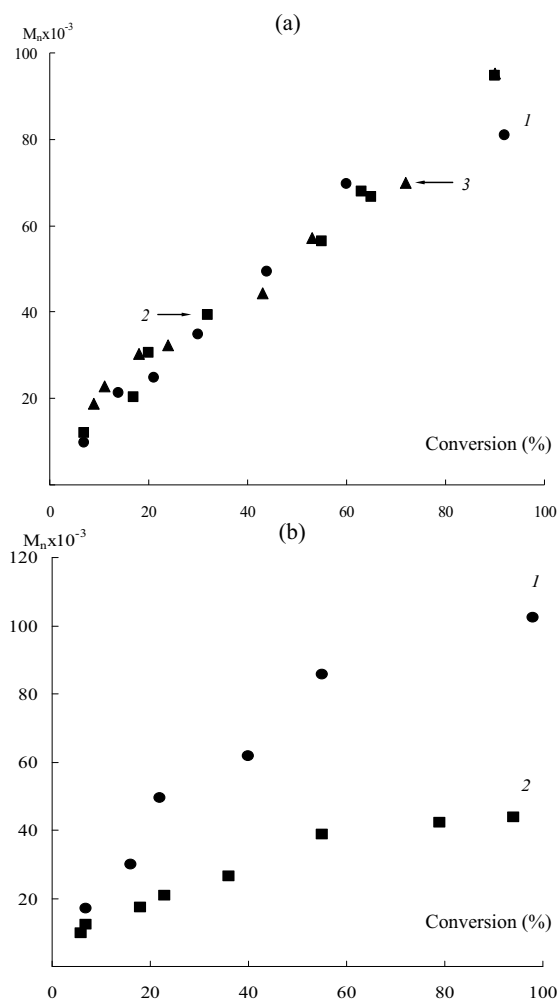


Fig. 5. Dependence of M_n PS samples obtained in the presence of 0.1 mole% alkoxyamines at 90°C, on conversion. a: AA synthesized in the presence of PBN. M_w AA, Da: 1 - 3000; 2 - 9500; 3 - 15700. b: AA synthesized in the presence of BMPO. M_w AA, Da: 1 - 3000; 2 - 10000.

er conversions Table 2). Analysis of the kinetic regularities of polymerization of ST with the participation of BMPO-containing AA with M_w 3000 Da and 10,000 Da at 70 °C indicates that the processes in its presence proceed at a faster rate than the autopolymerization of the ST (Fig. 9). The linear dependence of $\ln([M]_0/[M])$ on time for the described systems shows that the reaction rate is first-order in monomer, and the concentration of growth radicals is constant throughout the process. As expected, the total polymerization rate at 70 °C is lower than

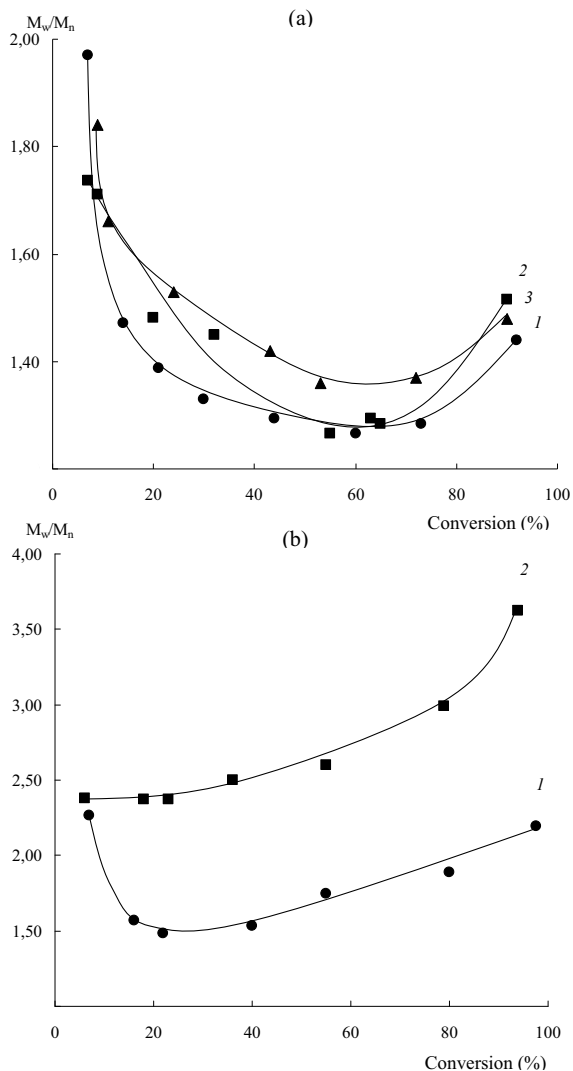


Fig. 6. Dependence of polydispersity coefficients of PS samples synthesized in the presence of 0.1 mol.% of alkoxyamines at 90 °C, on conversion. a: AA synthesized in the presence of PBN. M_w AA, Da: 1 - 3000; 2 - 9500; 3 - 15700. b: AA synthesized in the presence of BMPO. M_w AA, Da: 1 - 3000; 2 - 10000.

the polymerization rate of the ST in the presence of BMPO-containing AA at 90 °C. Thus, at 70 °C the degree of conversion reaches 70% in 80 hours, and at 90 °C the polymerization is realized to deep polymerization in 20 hours (Figures 9 and 4b).

It is shown that in ST polymerization in the presence of BMPO-containing AA, the process is accompanied by a linear growth of MW with an increase in the degree of conversion (Fig. 10), with

Table 3

Molecular mass characteristics of PS synthesized in the presence of 0.1 mol. % high-molecular AA at 90 ° C

Nº	AA	Time, h	Conversion, %	$M_n \times 10^{-3}$	M_w/M_n	$M_{n,th} \times 10^{-3}$	f^a
1	-	2.3	2	275	2.36	-	-
2	-	60.0	56	335	2.30	-	-
3	1	1.5	7	10	1.97	10	1.00
4		30.0	60	70	1.26	65	0.93
5		60.0	92	81	1.43	98	1.21
6	2	1.5	7	12	1.74	14	1.17
7		22.0	55	57	1.26	64	1.12
8		60.0	90	95	1.51	100	1.05
9	3	2.8	9	19	1.84	20	1.05
10		22.0	53	57	1.36	66	1.16
11		60.0	90	95	1.48	105	1.11
12	4	2.0	16	30	1.57	19	0.63
13		9.0	55	86	1.74	60	0.70
14		22.0	98	102	2.20	105	1.02
15	5	2.0	18	17	2.37	25	1.47
16		9.0	55	39	2.60	64	1.64
17		22.0	94	44	3.59	104	2.36
18	6	2.8	6	16	7.23	14	0.88
19		6.2	22	22	8.19	31	1.41
20		9.0	30	31	7.95	39	1.26
21		14.0	44	33	8.30	54	1.64
22		22.0	59	55	7.56	69	1.25
23		29.8	71	69	7.18	82	1.19
24		60.0	82	70	7.13	93	1.32

^a f – the efficiency of initiation, calculated by the formula:

$$f = M_{n,th} / M_n$$

MW growth of polystyrene with conversion more pronounced with a decrease in MW alkoxyamine. At the same time, the experimentally determined values of the MW of the PS synthesized in the presence of AA **4** are higher than theoretically expected (Table 4). In this case, the efficiency of AA **4** initiation at 70 ° C is less than 1, it increases with the degree of monomer conversion and on deep conversions is about 0.5. This suggests that the molecules of AA at a lower temperature decay non-simultaneously. From the dependences presented in Fig. 11, it follows that the polydispersity of samples synthesized in the presence of AA **4** decreases with an increase in the degree of monomer conversion to some minimum values (1.49), but on deep conversions its growth is observed.

A BMPO-containing AA **5** with a higher MW at 70 ° C is more effective in initiating than a low molecular weight analog (Table 4). However, at deep degrees of transformation, the growth of the M_w of

the PS slows down (Figure 10) due to the accumulation of a low molecular “dead” polymer, which affects the “visible” initiation efficiency values, which are greater than one at high degrees of monomer increment (Table 4). In this case, the polydispersity of the samples, as in the case of AA **4**, decreases at the beginning of the polymerization process (Figure 11), however, the absolute values of the polydispersity coefficients are quite high and increase with increasing MW of the high molecular weight alkoxyamine based on BMPOs (Table 4).

MWD curves of PS samples synthesized in the presence of high-molecular AA on the basis of BMPO at 70 ° C are shown in Fig. 12a and b. As follows from the presented data, when using BMPO-containing AA with $M_w = 3000$ Da, the MWD curves are unimodal, the mode is successively shifted to the region of large M_w values with increasing conversion. When using BMPO-containing AA with $M_w = 10000$ Da, MWD curves have a low-molecular-

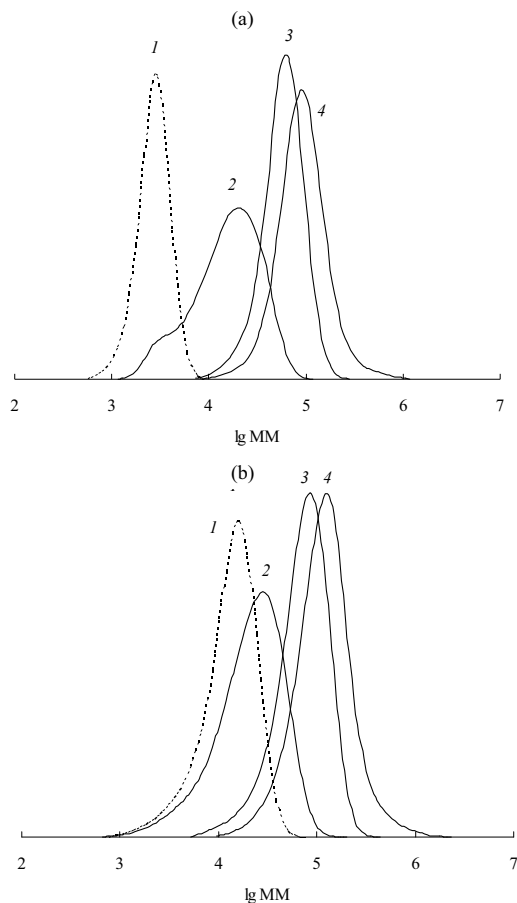


Fig. 7. MWD curves of a PS synthesized in the presence of a PBN-containing high-molecular alkoxyamine. $T=90^{\circ}\text{C}$. a: AA with $M_w = 3000$. 1 - Initial macroinitiator. Conversion, %: 2 - 7; 3 - 44; 4 - 92; b: AA with $M_w = 15700$. 1 - Initial macroinitiator. Conversion, %: 2 - 10; 3 - 51; 4 - 90.

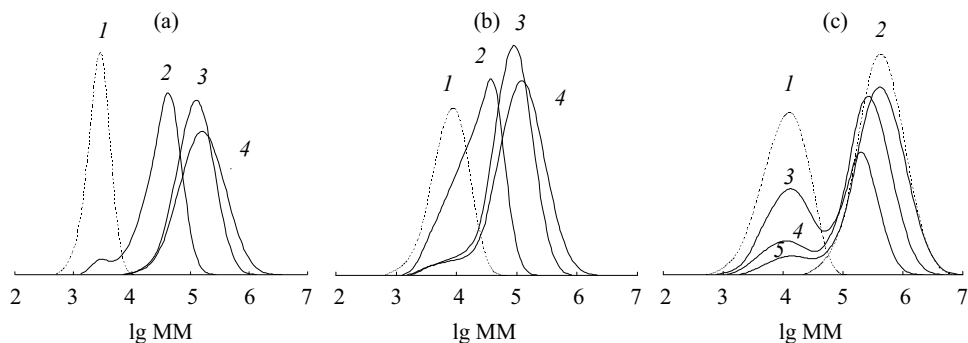


Fig. 8. MWD curves of a PS synthesized in the presence of a high-molecular BMPO-containing alkoxyamine. $T=90^{\circ}\text{C}$. a: AA with $M_w = 3000$. 1 - Initial macroinitiator. Conversion, %: 2 - 7; 3 - 55; 4 - 98. b: AA with $M_w = 10000$. 1 - Initial macroinitiator. Conversion, %: 2 - 7; 3 - 55; 4 - 94. c: AA with $M_w = 15200$. 1 - Initial macroinitiator. 2 - Thermal polymerization. Conversion, %: 3 - 3; 4 - 44; 5 - 82.

weight shoulder (Fig. 12b, curves 3, 4, 5). Moreover, both low-molecular and high-molecular mode shift to the region of large values of MW with an increase in the degree of monomer conversion, while the MWD of the PS synthesized in the presence of BMPO-containing AA 5 is incomparable with the MWD polymer obtained by thermal initiation. It should be noted that a clear displacement of the MWD curves of the PS synthesized at 70°C into the region of large M_w indicates a controlled character of the polymerization in which the fragmentation growth of the chains occurs by the mechanism of reversible initiation.

3.3 Post-polymerization of styrene in the presence of high-molecular alkoxyamines

As is shown above, high molecular weight AAs obtained by in situ on the basis of PBN and BMPO are able to initiate CRP. However, the use of AA with high MW values leads to a significant increase in the polydispersity of the samples. Reduction of control over molecular weight characteristics is associated with a number of side processes leading to the formation of "dead" macromolecules, both in the synthesis of high molecular weight AA in situ, and also during the polymerization itself. The loss of control is greatest in the case of high-molecular-weight AA 6 based on BMPO. With the purpose of more detailed explanation of the degree of contribution of side processes and the development of a procedure for the synthesis of polymers with even higher MW values, we carried out the post-polymerization of ST on AA, obtained in the second stage of the work. At the same time AA was purposefully used with different molecular-weight characteristics (Table 5).

It is found (Table 5) that in the case of using high molecular weight AA based on PBN (3-1, 3-1^a and 3-2), the polymerization of the ST proceeds with a further increase in MW polymers. The calculat-

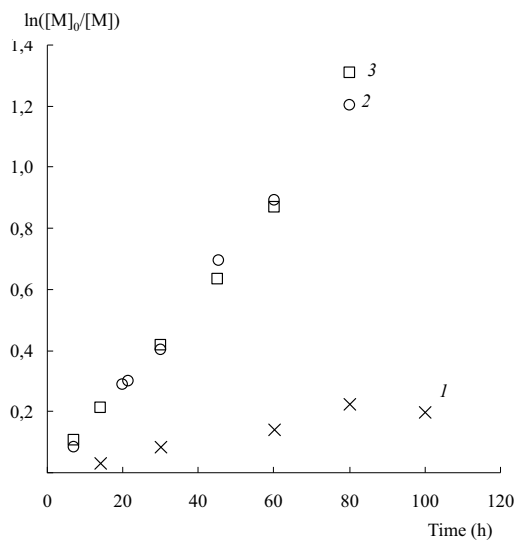


Fig.9. Semilogarithmic kinetic dependences of the polymerization of ST at 70°C in the presence of 0.1 mol.% of BMPO-containing high-molecular alkoxyamines. 1 - thermal polymerization of ST. M_w AA, Da: 2 - 3000; 3 - 10000.

ed values of MW polymers are rather close to those found experimentally, but in all cases the polydispersity coefficient increases. The MWD curves of post-polymers (Fig. 13a) are unimodal and clearly shifted to the region of high MWs at each subsequent stage of synthesis of macromolecules. Note that the use of different AAs in the second stage, isolated at different degrees of monomer conversion (3-1 and 3-2), is reflected in the post-polymerization process of

the ST. With an increase in the conversion and M_w alkoxyamines, some loss of control over the molecular weight characteristics of post-polymers occurs. Attention is drawn to the fact that in the case of the fourth stage of polymerization (Table 5, line 4) and the use of alkoxyamine at low concentration (Table 5, line 6), the “apparent” initiation efficiency is high, but the degree of control is lowered. This is due to the death of nitroxide radicals and the accumulation of a low molecular weight “dead” polymer in the process of multistage synthesis of PS, the presence of which leads to a “seemingly” overestimate of the initiation efficiency greater than one.

Similar data were obtained in the case of the use of low-molecular-weight BMPO-containing AA 4 in the initial stage. Post-polymerization on high-molecular alkoxyamines is accompanied by a gradual growth of MW polymers (Table 5, lines 8-12) and a shift in the MWD curves to the region of high molecular weights (Fig. 13b). However, as mentioned above, the use of BMPO-containing alkoxyamines results in the preparation of samples with greater polydispersity. At the same time, control over the polymerization process decreases with each stage of PS synthesis.

As can be seen from the data given in Table 5 and Fig. 13b, in the case of use AA based on BMPO ($M_w = 15200$ Da), the control over the polymerization process is completely lost, as evidenced by the lack of growth of the MW polymer (when comparing the high-molecular-weight regions for 6-1 and 6-1^a) and shifting the MWD curve with the conversion. The absence of a low-molecular mode in Fig. 13 in the case of the third stage of polymer synthesis is due to a low concentration of the original AA 6-1 and a sufficiently high error in the GPC analysis.

Table 4

Molecular mass characteristics of PS synthesized in the presence of 0.1 mol. % Of BMPO-containing high-molecular AA at 70 ° C

№	AA	Time, h	Conversion, %	$M_n \times 10^{-3}$	M_w/M_n	$M_{n,th} \times 10^{-3}$	f^a
1	-	30	8	508	2.15	-	-
2		100	18	518	2.17	-	-
3		14	7	30	2.83	10	0.33
4	4	30	33	84	1.58	37	0.44
5		80	70	139	1.58	75	0.54
6		14	19	27	2.55	26	0.96
7	5	30	34	41	2.31	42	1.02
8		80	73	63	2.49	82	1.30

^a f – the efficiency of initiation, calculated by the formula:

$$f = M_{n,th} / M_n$$

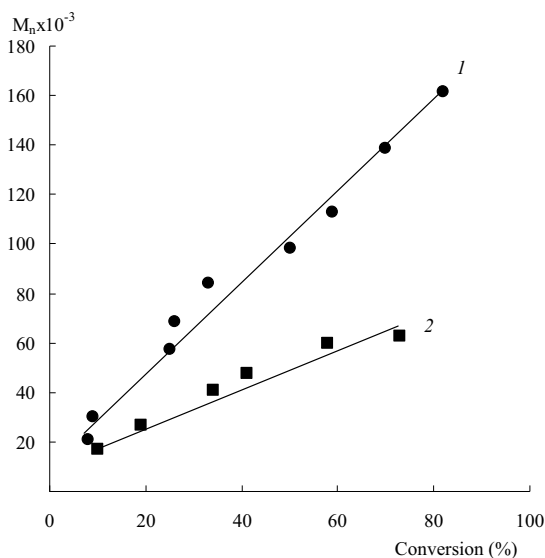


Fig. 10. Dependence of M_n PS samples obtained in the presence of 0.1 mol.% of BMPO-containing high-molecular AA at 70°C, on conversion. M_w AA, Da: 1 - 3000; 2 - 10000

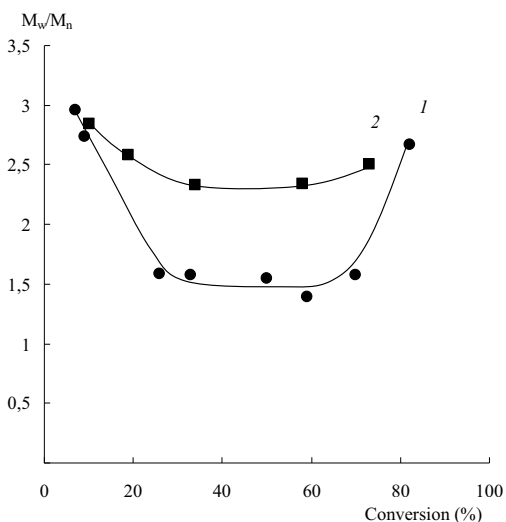


Fig. 11. Dependence of PS polydispersity coefficients obtained in the presence of 0.1 mol.% AA based on BMPO at 70°C, on conversion. M_w AA, Da: 1 - 3000; 2 - 10000.

Thus, carrying out multistage synthesis of PS on isolated macroinitiators allows obtaining polymers with MW in the range from 3000 to 200000 Da. However, each subsequent stage leads to some degeneration of high-molecular AA and loss of con-

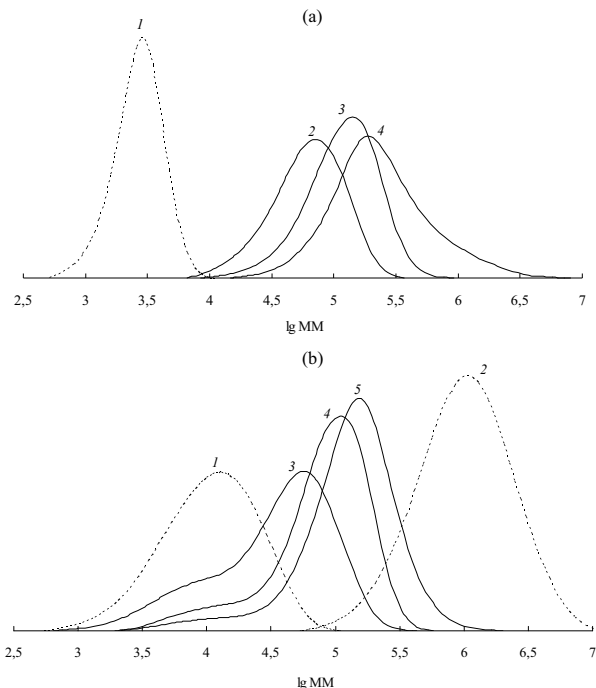


Fig. 12. MWD curves of a PS synthesized in the presence of a high-molecular alkoxyamine-containing BMPO. $T=70^\circ C$. a: AA with $M_w = 3000$. 1 - Initial macroinitiator. Conversion, %: 2 - 7; 3 - 33; 4 - 82. b: AA with $M_w = 10000$. 1 - Initial macroinitiator. 2 - thermal polymerization. Conversion, %: 3 - 10; 4 - 34; 5-73.

trol over the molecular-weight characteristics of the polymers. We emphasize that in this case the processes will proceed against the background of low concentrations of nitroxide radicals, and as a consequence, the effect of stable radicals responsible for polymerization control degenerates. As a result, the implementation of multi-stage processes without additional nitroxide radicals will be accompanied by the expected loss of control and an increase in the polydispersity of the resulting polymers.

4. Conclusion

The results of the conducted studies indicate that the *in situ* polymerization of ST in the presence of PBN and BMPO at a temperature of 90°C is realized in two stages. The first stage is characterized by high speed, while high-molecular AA is formed, the molecular-weight characteristics of which do not depend on the depth of monomer conversion. In the second stage, the polymerization rate decreases and the MW polymer grows with conversion, i. e. the process proceeds in a controlled manner.

The *in situ* polymerization method was used to synthesize high molecular weight AAs of dif-

Table 5

Post-polymerization of ST in the presence of high-molecular AA based on PBN and BMPO at 90 °C

Nº	AA	Conditions	Time, h	Conversion, %	$M_n \times 10^{-3}$	$M_w \times 10^{-3}$	M_w/M_n	$M_{n,th} \times 10^{-3}$	f
1	3	ST + 1 mol.% AIBN + 1.5 mol.% PBN	62	82	10.8	15.7	1.45	-	-
2	3-1	ST + 0.1 mol.% AA 3	30	84	82.0	119.0	1.45	98.3	1.20
3	3-1 ^a	ST:C ₆ H ₆ (50:50 vol.%) + 0.05 mol.% AA 3-1	60	42	153.5	242.9	1.58	125.7	0.82
4	3-1 ^b	ST:C ₆ H ₆ (50:50 vol.%) + 0.05 mol.% AA 3-1 ^a	60	95	196.6	384.4	1.96	252.4	1.28
5	3-2	CT + 0.1 mol.% AA 3	60	90	95.0	140.6	1.48	104.5	1.10
6	3-2 ^a	ST:C ₆ H ₆ (50:50 vol.%) + 0.01 mol.% AA 3-2	60	35	194.1	365.4	1.88	277.3	1.43
7	4	ST:C ₆ H ₆ (50:50 vol.%) + 2 mol.% AIBN + 4 mol.% BMPO	2	19	2.5	3.0	1.20	-	-
8	4-1	ST + 0.1 mol.% AA 4	5	40	61.6	94.6	1.54	44.2	0.71
9	4-1 ^a	ST:C ₆ H ₆ (50:50 vol.%) + 0.05 mol.% AA 4-1	60	32	138.4	324.9	2.35	94.9	0.68
10	4-1 ^b	ST:C ₆ H ₆ (50:50 vol.%) + 0.05 mol.% AA 4-1 ^a	60	94	169.5	423.3	2.50	237.3	1.39
11	4-2	ST + 0.1 mol.% AA 4	22	97	102.3	224.6	2.20	103.5	1.01
12	4-2 ^a	ST:C ₆ H ₆ (50:50 vol.%) + 0.05 mol.% AA 4-2	60	37	182.0	476.2	2.62	140.8	0.77
13	6	ST + 1 mol.% AIBN + 1.5 mol.% BMPO	40	91	7.9	15.2	1.92	-	-
14	6-1	ST + 0.1 mol.% AA 6	30	71	68.3 285.3 ^c	488.3 570.8 ^c	7.15 2.00 ^c	-	-
15	6-1 ^a	ST:C ₆ H ₆ (50:50 vol.%) + 0.05 mol.% AA 6-1	60	31	265.4	653.5	2.46	-	-

^a Polymer obtained in the third stage of polymerization^b The polymer obtained in the fourth stage of polymerization^c The data calculated on the basis of GPC analysis without taking into account the low molecular weight mode

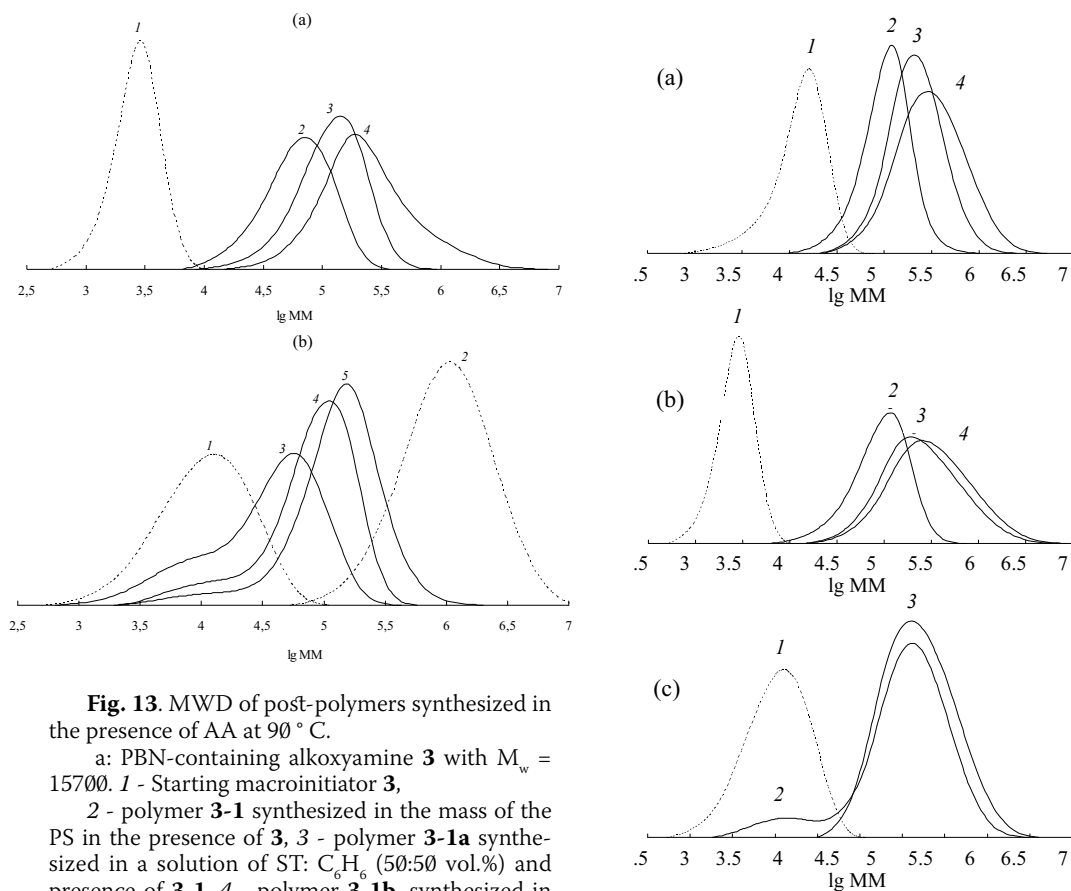


Fig. 13. MWD of post-polymers synthesized in the presence of AA at 90 °C.

a: PBN-containing alkoxyamine **3** with $M_w = 15700$. 1 - Starting macroinitiator **3**,

2 - polymer **3-1** synthesized in the mass of the PS in the presence of **3**, 3 - polymer **3-1a** synthesized in a solution of ST: C_6H_6 (50:50 vol.%) and presence of **3-1**, 4 - polymer **3-1b**, synthesized in a solution of ST: C_6H_6 (50:50 vol.%) and presence of **3-1a**.

b: BMPO-containing alkoxyamine **4** with $M_w = 2500$. 1 - Starting macroinitiator **4**,

2 - polymer **4-1** synthesized in the mass of the PS in the presence of **4**, 3 - polymer **4-1a** synthesized in a solution of ST: C_6H_6 (50:50 vol.%) and presence of **4-1**, 4 - polymer **4-1b**, synthesized in a solution of ST: C_6H_6 (50:50 vol.%) and presence of **4-1a**.

c: BMPO-containing alkoxyamine **6** with $M_w = 15200$. 1 - Starting macroinitiator **6**,

2 - polymer **6-1** synthesized in the mass of the PS in the presence of **6**, 3 - polymer **6-1a** synthesized in a solution of ST: C_6H_6 (50:50 vol.%) and presence of **6-1**. (The conditions for the synthesis of post-polymers and their molecular-mass characteristics are given in Table 5).

ferent structures and MW. Their initiating ability in ST polymerization was studied. It is shown that PBN-containing AA allows to more effectively regulate the molecular weight characteristics of the polymer in comparison with AA based on BMPO. The introduction of the hydroxyl group into the structure of the nitron leads to a decrease in the degree of control over the molecular weight characteristics of the PS. However, high-molecular AA on the basis of BMPO allow controlled synthesis of PS with sufficiently high rates at lower temperatures (70°C). These features of polymerization of ST in the presence of BMPO-containing alkoxyamines can be due to intramolecular trans-

fer of the hydrogen atom from the hydroxyl group to the nitroxide radical, which, on the one hand, leads to an increase in the polymerization rate with their participation, and on the other hand, to the loss of AA data and, as a consequence, to loss of control over the molecular-weight characteristics of polymers.

Acknowledgements

The work was financially supported by RFBR (№17-03-00498)

The authors are grateful to Ph.D. Kirilyuk I.A. for the 2- (benzylideneamino) -2-methyl-1-phenylpropanol-1-N-oxide provided for the experiments.

References

- [1] D. Braun, H. Cherdrón, M. Rehahn, H. Ritter, B. Voit, *Polymer Synthesis: Theory and Practice Fundamentals, Methods, Experiments*. 5th ed. Berlin–Heidelberg: Springer-Verlag, 2013.
- [2] *Nitroxide Mediated Polymerization: From Fundamentals to Applications in Materials Science*, Ed. by D. Gígmes. UK: The Royal Society of Chemistry, 2016.
- [3] M. Yu. Zaremski, Kinetic features of pseudo-living radical polymerization under conditions of reversible inhibition by nitroxide radicals, *Polym. Sci. Ser. C*. 57 (2015) 65–85.
- [4] J. Nicolas, Y. Guillauneuf, C. Lefay, D. Bertin, D. Gígmes, B. Charleux, Nitroxide-Mediated Polymerization, *Prog. Polym. Sci.* 38 (2013) 63–235.
- [5] V. Sciannamea, R. Jérôme, C. Detrembleur, In-Situ Nitroxide-Mediated Radical Polymerization (NMP) Processes: Their Understanding and Optimization, *Chem. Rev.* 108 (2008) 1104–1126.
- [6] E.V. Kolyakina, D.F. Grishin, Nitroxide radicals formed in situ as the regulators of polymer chain propagation, *Russ. Chem. Rev.* 78 (2009) 535–568.
- [7] M.V. Pavlovskaya, E.V. Kolyakina, V.V. Polyanskova, L.L. Semenycheva, D.F. Grishin, Polymerization of Styrene in the Presence of Nitroxide Radicals Generated Directly in the Course of the Polymer Synthesis (in situ), *Russ. J. Appl. Chem.* 75 (2002) 1868–1872.
- [8] M. Yu. Zaremski, A.P. Orlova, E.S. Garina, A.V. Olenin, M.B. Lachinov, V.B. Golubev Pseudoliving free-radical polymerization mediated by macromolecular nitroxides based on nitrones, *Polymer Science, Ser. A*. 45 (2003) 502–512.
- [9] D.F. Grishin, L.L. Semenycheva, S.A. Bulgakova, L.M. Mazanova, E.V. Kolyakina, Free-radical homo- and copolymerization of styrene and acrylonitrile in the presence of C-phenyl-N-tert-butyl nitroxide, *Polymer Science, Ser. A*. 45 (2003) 305–310.
- [10] D.F. Grishin, L.L. Semenycheva, K.V. Sokolov, E.V. Kolyakina, The controlled radical polymerization of vinyl chloride in the presence of C-phenyl-N-tert-butyl nitroxide, *Polymer Science, Ser. B*. 42 (2000) 189–190.
- [11] M. Yu. Zaremski, A.L. Reznichenko, Yu.V. Grinevich, E.S. Garina, M.B. Lachinov, V.B. Golubev, Pseudoliving free-radical copolymerization of vinyl acetate with styrene under conditions of nitroxide-mediated reversible inhibition, *Polymer Science, Ser. A*. 47 (2009) 536–545.
- [12] D.F. Grishin, E.V. Kolyakina, M.V. Pavlovskaya, M.A. Lazarev, A.A. Shchepalov in *Controlled/Living Radical Polymerization: Progress in RAFT, DT, NMP & OMRP*. Ed. by K. Matyjaszewski. ACS Symposium Series. American Chemical Society. Washington DC. 1024 (2009) 95–114.
- [13] V. Sciannamea, C. Guerrero-Sanchez, U. S. Schubert, J.-M. Catala, R. Jerome, C. Detrembleur, Ability of nitrones of various structures to control the radical polymerization of styrene mediated by in situ formed nitroxides *Polymer*. 46 (2005) 9632–9641.
- [14] V. Sciannamea, J.M. Catala, R. Jerome, C. Detrembleur, Controlled radical polymerization of styrene mediated by the C-Phenyl-N-tert-butyl nitroxide/AIBN pair: kinetics and electron spin resonance analysis *J. Polym. Sci., Part A: Polym. Chem.* 45 (2007) 1219–1235.
- [15] E.H.H. Wong, T. Junkers, C. Barner-Kowollik, Enhanced spin capturing polymerization: An efficient and versatile protocol for controlling molecular weight distributions, *J. Polym. Sci., Part A: Polym. Chem.* 46 (2008) 7273–7279.
- [16] L. Zang, E. H.H. Wong, C. Barner-Kowollik, T. Junkers. Control of methyl methacrylate radical polymerization via Enhanced Spin Capturing Polymerization (ESCP), *Polymer*. 51 (2010) 3821–3825.
- [17] E.H.H. Wong, T. Junkers, C. Barner-Kowollik, Nitrones in synthetic polymer chemistry, *Polym. Chem.* 2 (2011) 1008–1017.
- [18] J.P. Wu, C.W. Pan, K. E. Heiler, M.E. Ching, E.S. Tillman, Altering the effectiveness of radical traps in atom transfer radical coupling reactions of polymer chains, *Polymer*. 127 (2017) 66–76.
- [19] M. Du, C. Deng, X.Wu, H. Liu, H. Liu, Nitroxide Mediated Coupling of Hyperbranched Polymer Radicals, *Macromol. Chem. Phys.* 218 (2017) DOI:10.1002/macp.201700069
- [20] *Enziklopediya polimerov [Polymer Encyclopedia]*, ed. by V.A. Kabanov, Moscow: Sovetskaya Enziklopediya, 1972, V.1. (in Russian).
- [21] *Organic Syntheses*, third ed., New York–London: Interscience Publishers, 1946–1952. V. 26–32.
- [22] W.D. Emmons, The preparations and properties of oxaziranes, *J. Am. Chem. Soc.* 79 (1957) 5739–5744.
- [23] U. Paulsen, B. Lindeke, Autoxidation of N-Hydroxyphenylalkylamines. IV. The Formation of N-Benzylidene-phenylalkylamine-N-Oxides During Copper Catalyzed Autoxidation of Beta,N-Bis(hydroxy)phenylalkylamines, *Acta. Pharm. Suec.* 15 (1978) 264–273.
- [24] A. Weissberger, E. Proskauer, J. Riddick, E. Toops, *Organic Solvents*. New York: Interscience Publishers. Inc., 1955.
- [25] B.G. Belenkii, L.Z. Vilenchik, *Chromatography of Polymers*. M.: Khimiya, 1977
- [26] Y.Q. Steven, Y.R. Chen, L.J. Deterding, Y.C. Fann, Identification of protein-derived tyrosyl radical in the reaction of cytochrome c and hydrogen peroxide: characterization by ESR spin-trapping, HPLC and MS, *Biochem. J.* 363 (2002) 281–288.