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REVIEWS

Amphiphilic Copolymers of Different Structure Based on Poly(ethylene glycol): Synthesis, Physico-Chemical Properties, and Cytotoxicity

M. Yu. Zaremskii[a,*], N. S. Melik-Nubarov[a], I. D. Grozdova[a], E. E. Aliev[a], and S. A. Rumyantsev[a]

[a] *Department of Chemistry, Moscow State University, Moscow, 119992 Russia*

**e-mail: zaremski@mail.ru*

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Abstract—New procedures for the preparation of grafted and branched amphiphilic copolymers based on poly(ethylene glycol) have been suggested. Radical polymerization with TEMPO and sulfuric acid has afforded controlled synthesis of grafted copolymers of methyl methacrylate with poly(ethylene glycol) methacrylate. Radical copolymerization of allyl acetate with poly(ethylene glycol) acrylate in the presence of divinylbenzene has given branched copolymers. It has been shown that these copolymers can form micelles in aqueous medium; cytotoxicity of the copolymers and the ability to suppress the resistance of human cancer cells NCI/ADR-RES have been investigated.

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INTRODUCTION

Properties of amphiphilic copolymers strongly depend on their composition and morphology. For example, they determine size, shape, and stability of colloidal particles formed by amphiphilic copolymers in aqueous solutions. Accounting for these factors is especially important in the development of carriers for drug delivery, since size and shape of the polymer objects strongly affect their interaction with target cells and these of reticuloendothelial system (such as macrophages and dendritic cells). The control over stability of polymer containers determines their clearance rate and, hence, influences on bioavailability of the drug in the polymer particles [1].

The PEG-based copolymers are special among amphiphilic ones, due to their self-assembly affording wide range of structures in an aqueous solution [2]. The possibility to enhance the sensitivity of cancer cells to chemotherapy is among the most important features of the PEG copolymers, which allows their application in pharmacology. Above all, this applies to Pluronics (block copolymers of ethylene oxide and propylene oxide). Most of the Pluronics suppress the resistance of cells to drugs at concentration of one–two orders of magnitude below the cytotoxic concentration.

It is important to understand the physico-chemical features of Pluronics which determine their effect of the drug resistance of cancer cells. It is known that polyethoxylated α-tocopherol succinate, PEG block copolymers with polydimethylsiloxane or polycaprolactam, and copolymers of polyglycerol with poly(propylene oxide) exhibit the action similar to this of the Pluronics [3–6]. The mentioned polymers possess low mas of the hydrophobic block and act as low-molecular surfactants. Unfortunately, the range of macromolecular structures which are efficient in inhibiting the drug resistance of cancer cells is limited, on one hand, by block copolymers and, on the other hand, by the copolymers bearing ether or ester oxygen-containing units in the hydrophobic block backbone.

In this study, we attempted to resolve the following issues. Can the polymers bearing ester bonds in the pendant chains suppress the cells resistance? How does the copolymer architecture (block, grafted, or branched) affect its interaction with cancer cells? To do so, we prepared new copolymers and elaborated novel approaches to the synthesis of PEG-based copolymers prone to self-assembly in aqueous solutions.

EXPERIMENTAL

Methyl methacrylate (Aldrich) was purified of the inhibitor, hydroquinone monomethyl ether, via distillation under reduced pressure in an inert gas stream (T_b = 41°C at 80 mm Hg, n_D^{20} = 1.414). 2,2,6,6-Tetramethylpiperidine-1-oxyl (Sigma) was used as received. Allyl acetate (Show Denko K.K.) was purified by distillation (n_D^{20} = 1.404). Poly(ethylene glycol) acrylate monomethyl ether (**PEGA**) (Aldrich) with

M_n = 480 was used as received. Divinylbenzene (**DVB**) (Aldrich) was purified via passing through an alumina column. The radical initiator AIBN (Reakhim) was purified via recrystallization from ethanol. Poly(ethylene glycol methacrylate monoethyl ether (**PEGMA**) with M_n = 1900 was prepared as described elsewhere [7]. It contained 95% of terminal vinyl groups, as per the ^{1}H NMR spectroscopy data.

Grafted copolymers of MMA with PEGMA were prepared via dissolution of the desired amounts of AIBN, PEGMA, and TEMPO in MMA, followed by triple degassing of the solution under vacuum (5×10^{-3} mm Hg). The reagents concentrations were as follows: $[AIBN]_0 = [TEMPO]_0 = 0.05$ mol/L, $[H_2SO_4] = 0.035$ mol/L. The synthesis was performed 1 at 80°C in sealed ampoules. The copolymers were precipitated from a 10% solution in dichloroethane into an isopropanol : hexane (1 : 1) mixture and dried in vacuum.

The PMMA–*block*–PEG block copolymers were synthesized as described in [8]. PEG with a terminal bromoisobutyrate group (**PEGB**) was prepared as described elsewhere [9]. 52.5 mg (10 mmol/L) of PEGB was dissolved in an ampule in 100 μL of anisole and degassed via purging with argon during 15 min. 2.5 mg (10 mmol/L) CuCl and 21 mg (20 mmol/L) of dinonylbipyridyl (**dNbpy**) were weighed in a separate vessel. 400 μL of degassed MMA was added to the mixture. The final molar ratio of the components was as follows: PEGB : CuCl : dNbpy : MMA = 1 : 1 : 2 : 179. After that, 160 μL of the CuCl solution was transferred to the ampoule with the initiator, the ampoule was sealed, and the polymerization was performed at 95°C during 12 h. The product was dissolved in chloroform, the liquid fraction was separated and evaporated until disappearance of anisole smell. The polymer was further purified of the catalyst traces via chromatography on an Al_2O_3 column.

Branched copolymers were prepared via polymerization in a mixture of 0.36 mL of PEGA, 8.6 mL of allyl acetate, 0.18 or 0.09 mL (2 and 1 wt %, respectively) of divinylbenzene, and 15 mg of AIBN. The mixture was degassed as described above, and the reaction was performed at 80°C during 5 h. The products were precipitated with fivefold excess of hexane, and the precipitate was dried in a vacuum oven.

The polymerization kinetics was investigated by means of isothermal calorimetry using a DAK-1-1A differential automated calorimeter in the mode of direct registration of heat evolution. The value of methyl methacrylate heat of polymerization (57.7 kJ/mol) was used to calculate the conversion, whereas in the case of copolymerization of PEGA with allyl acetate heat of polymerization for both monomers was taken equal to 85.3 kJ/mol.

Molecular mass parameters of the copolymers were analyzed by means of GPC using a GPC-120 chromatograph (PolymerLabs) equipped with two PLgel 5 μL MIXEDB columns ($M = 5 \times 10^2$–1×10^7). The analysis was performed in DMF containing 0.1 wt % of LiBr at 50°C and flowrate 1 mL/min. The molecular mass was calculated using the calibration for narrow fractions of PMMA. GPC analysis of the branched copolymers was performed using an Agilent 1280 Infinity II chromatograph equipped with refractometric and LALLS detectors, eluting with THF (0.3 mL/min) at 40°C. Narrow fractions of PS were used as the calibration references.

The NMR analysis was performed using 2% solution of the analyzed substance in 0.6 mL of $CDCl_3$. The ^{1}H NMR spectra were recorded using a Bruker DRX500 spectrometer.

Dynamic light scattering experiments were performed using a PhotoCor scattered laser light goniometer equipped with a helium-neon laser (λ = 633 nm, 15 mW) as the light source. The copolymers solutions were dedusted via filtration through polyester filters 2 with pores diameter 0.45 μm prior to placing in the measurement cell. The correlator data were collected during 15 min using FlexCor software. Mean particles diameter and their size distribution were calculated using DynaLS software.

The copolymers effect on cells was tested using the NCI/ADR-RES human cancer line (earlier designated as MCF-7/ADR) using the procedure elaborated in [10]. To do so, 1 mg of a copolymer was dissolved in 20 μL of acetone and dispersed in 1 mL of a serumless culture medium; two series of sequential 3 dilutions of the copolymer from 1000 to 15 μg/mL were prepared. One of the series was prepared using a solution of 5 μg/mL of doxorubicin in the serumless 3 medium, the second series was prepared using the same medium without doxorubicin. The cells were incubated with 100 μL of the obtained solutions during 1.5 h under standard conditions. Each dilution was analyzed in three wells with cells. The cells incubated with 100 μL of the polymer-free medium were used as reference. Upon the incubation, the solution was removed, and the cells were incubated during 3 days under standard conditions in 0.2 mL of the culture medium with 10% of serum. After that, the solutions were removed, and the amount of living cells in the wells was determined using the MTT test [11]. Absorbance of the formazan solution in the wells at 550 nm (D550) was determined using a Multiscan photometer (Titertek, USA). The effect of the copolymer on the cells viability was assessed from the ratio of the D550 value for the wells incubated with the polymer and mean value of D550 in the reference wells taken as 100%.

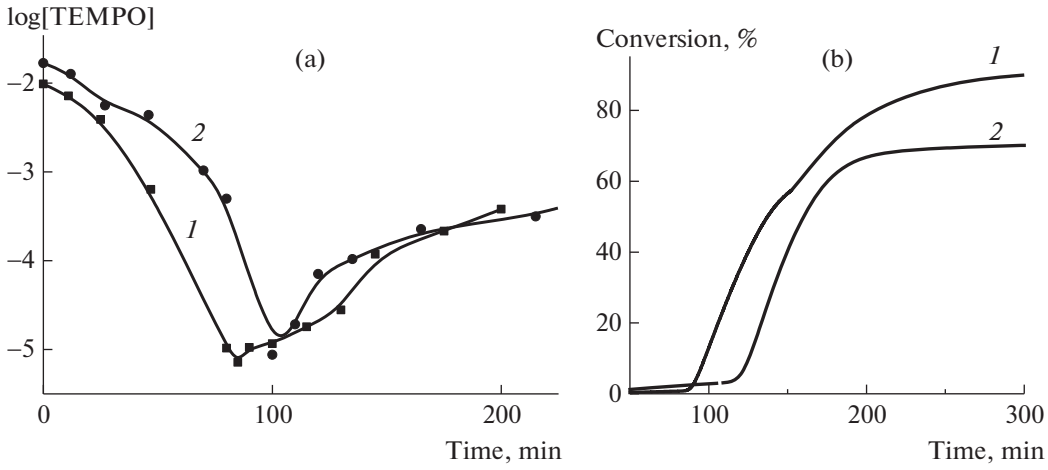
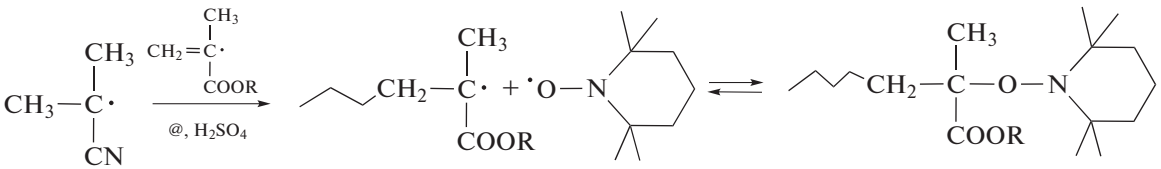


Fig. 1. Evolution of TEMPO concentration (a) and the copolymer yield (b) during polymerization of MMA with(*1*) 10 and (*2*) 30 wt % of PEGMA. [AIBN] = [TEMPO] = 0.05 mol/L, $[H_2SO_4]$ = 0.035 mol/L, T = 80°C.

RESULTS AND DISCUSSION

Synthesis of Graft Copolymers

The reversible inhibition approach [12, 13] was chosen for controlled synthesis of graft amphiphilic copolymers of MMA and PEG. Its distinct feature is that the obtained products contain no toxic admixtures [14]. It is known that controlled synthesis of MMA copolymers using this method is almost impossible due to two reasons [15, 16]. First, practically any nitroxyls disproportionate with the MMA propagating radicals. As a result, "dead" macromolecules are formed instead of "living" chains. Second, background concentration of nitroxyls, which are accumulated in the system due to the Ingold–Fischer persistent radical effect, is so high that the polymerization is inhibited at early to intermediate conversion. We have recently elaborated a simple approach to the controlled synthesis of amphiphilic copolymers of MMA with PEGMA via their simultaneous polymerization under the action of the AIBN–TEMPO–sulfuric acid system [15]:



where R = CH_3, $(CH_2CH_2O)_nCH_3$

Optimal conditions to minimize the probability of the propagating radicals disproportionation with TEMPO and reduction of the background concentration of the nitroxyl using sulfuric acid were elaborated, which allowed the targeted synthesis. Convenience of the chosen pair of monomers was due to their close reactivity in radical polymerization, hence the copolymer composition was not changed during the synthesis and coincided with that of the monomers mixture [17].

It was found that the addition of 0.035 mol/L of H_2SO_4 to the $[AIBN]_0 = [TEMPO]_0 = 0.05$ mol/L equimolar mixture resulted in the polymerization with the background concentration of free TEMPO 10^{-4}–10^{-5} mol/L upon the induction period, its absolute value being independent of the PEGMA content (Fig. 1). On one hand, that value was two to three orders of magnitude lower than that in the absence of the acid [15, 16], which ensured polymerization until deep conversion; on the other hand, that concentration was sufficient to maintain the reversible inhibition regime.

The latter fact was supported by regular shift of the copolymers MMD curve with conversion (Fig. 2) and low $Đ$ value of the polymer (Table 1). The only exception was the sample obtained in the presence of 30 wt % of PEGGMA at conversion 53%, which exhibited fairly high $Đ$ = 2.5, likely due to partial crosslinking of the copolymer under the action of admixture of PEGMA bearing two double bonds.

The so synthesized two series of amphiphilic graft copolymers contained 300 to 600 MMA units in the backbone and 2 to 11 pendant PEG chains (Table 2).

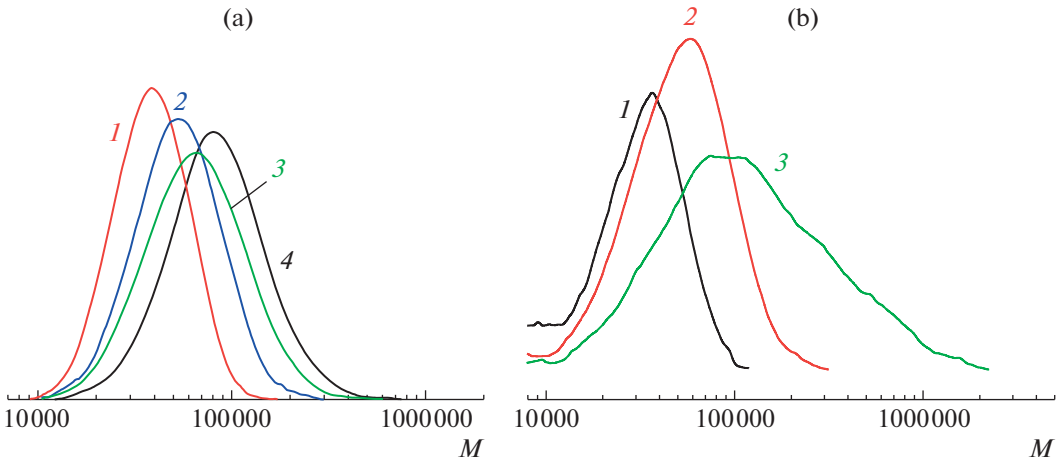
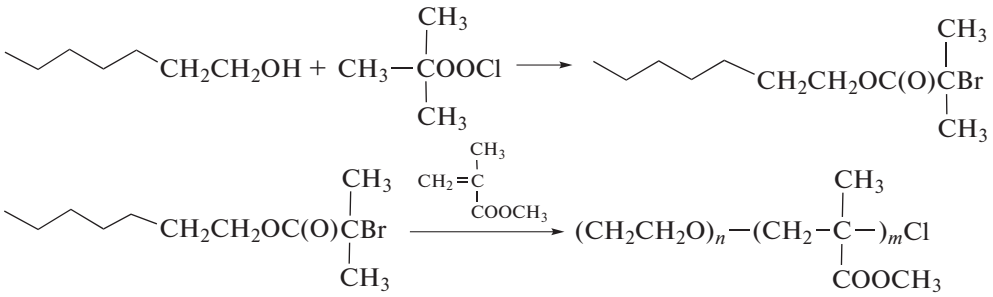


Fig. 2. Evolution of molecular mass distribution curves with conversion: a– 22 (*1*), 40 (*2*), 59 (*3*), and 88% (*4*); b– 11 (*1*), 22 (*2*), and 53 (*3*) for the MMA- PEGMA copolymers obtained at 10 (a) and 30 wt % of PEGMA (b).

Synthesis of Block Copolymers

Two samples of the PEG–*block*–MMA block copolymer were obtained via the standard method [17, 18]:



and characterized by means of GPC and NMR spectroscopy (Table 3).

Synthesis of Branched Copolymers

The approach described in [19–21] was used for the first time to prepare the PEG-based branched amphiphilic copolymers. The method consisted in copolymerization of vinyl monomers with divinyl ones in the presence of the chain transfer method. The "crosslinker" and the transfer agent were taken in large and comparable amounts to obtain highly branched polymer and avoid the product crosslinking. The branched polymers, although being irregular, exhibit the properties similar to these of dendrimers, but their synthesis is simpler and better accessible [22].

Table 1. Yield and molecular mass parameters of the P-(MMA-*co*-PEGMA) copolymers. $[AIBN]_0 = [TEMPO]_0 = 0.05$, $[H_2SO_4] = 0.035$ mol/L, $T = 80°C$

PEGMA, wt %	Time, min	Conversion, %	$M_n \times 10^{-3}$	$Đ$
10	105	22	33.7	1.2
30	125	40	44.6	1.3
	150	59	52.4	1.4
	250	88	67.7	1.4
	115	11	28.5	1.2
	122	22	41.7	1.4
	250	53	71.4	2.5

Originality of the approach consisted in combining the monomer and the chain transfer agent in the same molecule: the allyl monomer. In the absence of the crosslinker, radical copolymerization of allyl acetate and PEGA revealed the features of degradation chain transfer and afforded the oligomers due to the chain transfer to allyl acetate. In the presence of divinylbenzene, copolymerization of allyl acetate and PEGA gave amphiphilic branched polymers.

The copolymer yield was noticeably decreased with the increase in the allyl acetate content and decrease in the PEGA fraction in the monomer mixture f_{PEGA} (Table 4).

Table 2. Synthesized copolymers and their parameters

Sample	Monomer	Average composition	Synthesis notes	R_h, nm
G-1	MMA	$PMMA_{300}$–*graft*–$PEGMA_{1.8}$	10 wt % of PEGMA	130
G-2		$PMMA_{400}$– *graft*–$PEGMA_{2.4}$		110
G-3		$PMMA_{470}$– *graft*–$PEGMA_{2.8}$		112
G-4		$PMMA_{610}$– *graft*–$PEGMA_{3.5}$		110
G-5	MMA	$PMMA_{290}$– *graft*–$PEGMA_{6.5}$	30 wt % of PEGMA	32
G-6		$PMMA_{500}$– *graft*–$PEGMA_{11.3}$		29
B-1	MMA	$PMMA_{220}$–*block*–PEG_{115}	18 wt % of PEG	24
B-2		$PMMA_{58}$–*block*–PEG_{115}	46 wt % of PEG	17
G–allyl acetate	Allyl acetate	$PAA_{10.5}$–*graft*–PEG_3	–	69
Br-1	Allyl acetate	PAA_{36}–*branch*–$PEG_{7.5}$	1% of DVB	73
Br-2		PAA_{443}–*branch*–PEG_{92}	2% of DVB	86

Table 3. Molecular mass parameters of the PEG–*block*–PMMA block copolymers obtained via radical atom transfer polymerization in anisole at 95°C

Sample	[M]/[I]	$M_n \times 10^{-3}$/ P_n (GPC)	P_n (NMR)	$Đ$ (GPC)
PEGB	–	4.8/108	109	1.09
B-1	220	16.2/114	220	1.11
B-2	53	8.5/45	58	1.12

Since the comonomers reactivity was unknown, the values of the copolymerization constants were first determined. To do so, the copolymers composition was found by means of ^{1}H NMR spectroscopy using the ratio of integral intensities of the signals at 3.36 ppm (terminal methoxy groups in PEGA) and 2.0–2.2 ppm (protons of the methyl group in allyl acetate) (Table 4). The copolymerization constants were calculated by means of the Fineman–Ross and the least squares methods (Table 5). The reactivity of PEGA was found almost two orders of magnitude higher than that of allyl acetate. Hence, the copolymers were enriched in the macromonomer, and it was necessary to decrease the PEGA molar fraction in the reaction mixture to 1–2% to obtain the samples with comparable mass fractions of both components.

Branched copolymers of PEGA with allyl acetate were obtained at the PEGA molar fraction 1% and divinylbenzene molar fraction 1 or 2% in the starting mixture:

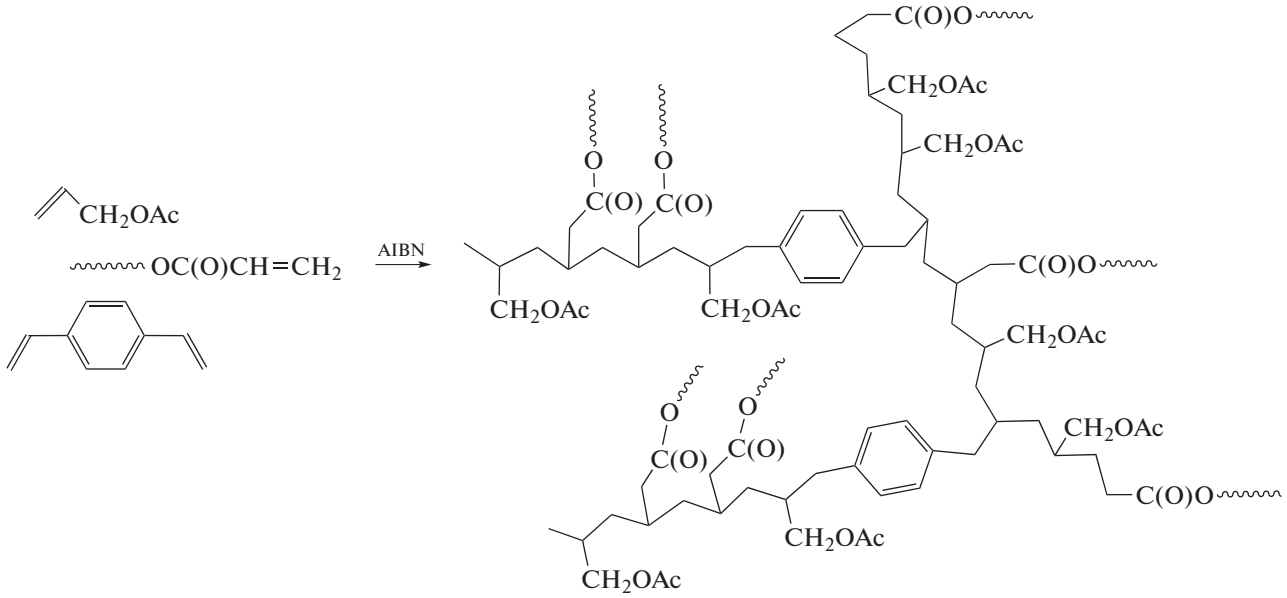


Table 4. Yield and composition of the allyl acetate copolymer with PEGA ([AIBN] = 10 mmol/L, $T = 80°C$)

f_{PEGA}, mol %	Copolymer yield, wt %	F_{PEGA}, mol %
50	95	–
30	56	78
20	–	74
15	28	–
10	22	48
7	–	38
5	12	30
4	9.1	–
2	5.1	15
1	3.3	13
0.5	2.4	–

Table 5. Copolymerization constants for PEGA and allyl acetate

Method	r_{PEGA}	$r_{allyl\ acetate}$
least squares method	8.5 ± 1.7	0.11 ± 0.01
Fineman–Ross method	7.5 ± 0.9	0.09 ± 0.01

Table 6. Molecular mass parameters of copolymers obtained at different DVB concentrations ([AIBN] = 10^{-2} mol/L, $T = 80°C$)

DVB, wt %	Refractometry detector		Light scattering detector	
	$M_n \times 10^{-3}$	$Đ$	$M_n \times 10^{-3}$	$Đ$
0	2.3	1.7	2.1	2.0
1	3.4	1.9	7.2	2.9
2	6.2	1.3	88.6	1.3

The copolymers yield was 3 and 4%, respectively. Using the ^{1}H NMR spectroscopy method, it was found that mass fraction of PEG in the copolymer was of 53–59% irrespectively of the divinylbenzene content.

The branched copolymers were characterized by means of GPC using the instrument equipped by refractive index and light scattering detectors. In the case of linear copolymers, the determined M_n value should be independent of the detector type, while the second (absolute) value should be higher than the second (relative) one for branched copolymers. That difference should be increased with the increase in the branching degree. Indeed, the M_n values determined for the linear copolymer using both detectors coincided within the measurement accuracy limits (Table 6). The increase in the crosslinker content in the polymerization mixture led to faster growth of the absolute molecular mass of the copolymer in comparison with the relative one. It is interesting to notice that the polydispersity was simultaneously decreased (Fig. 3; Table 6).

Colloidal Properties of the Copolymers in Aqueous Solutions

It is known that the copolymers containing PEG fragments are prone to self-assembly in aqueous solutions with the formation of wide range of structures, depending on the composition [2]. We investigated the aggregation of the prepared amphiphilic copolymers in aqueous solutions by means of dynamic light scattering. Analysis of the correlation functions using the Tikhonov regularization method revealed the presence of a single peak in most of the samples, mean hydrodynamic radius of which was calculated under approximation of spherical shape of the particles.

The block copolymers of PMMA with PEG, B-1 and B-2, containing 18 and 46 wt % of PEG formed the particles with R_h 24 and 17 nm, respectively.

Mean hydrodynamic radius R_h of the polymer particles formed by the graft copolymers of PMMA with 10 wt % of PEG was of 110–130 nm (cf. Table 2). Size of the particles formed by the graft copolymers with 30% of PEG was close to 30 nm. That result was expected, since the increase in the PEG content facilitated the shielding of the MMA skeleton from the contact with water. In both cases, the particles size was nearly independent of the increasing degree of the macromolecules polymerization.

Mean hydrodynamic radius of the formed associates was slightly increased with the increase in the polymer concentration. That fact revealed that water was a good solvent for the obtained particles, i.e. their surface was hydrophilic.

Hence, it was shown that the structure of the macromolecules strongly affected the behavior of the MMA–PEG copolymers in aqueous solutions. Micelles of the block copolymers were significantly smaller than those of the grafted analogs (Fig. 4).

Graft copolymers of allyl acetate and PEGA also formed the micelles with mean hydrodynamic radius of 70 nm (cf. Table 2). That size was twice larger in comparison with the micelles formed by the graft copolymers of PEG with 70% of hydrophobic MMA. The difference in the micelles size could be due to different hydrophobicity of MMA and allyl acetate and, hence, the difference in the intrachain hydrophobic interactions.

The increase in the crosslinker content and, hence, the molecular mass of the graft copolymer led to the increase in mean hydrodynamic radius of the copoly-

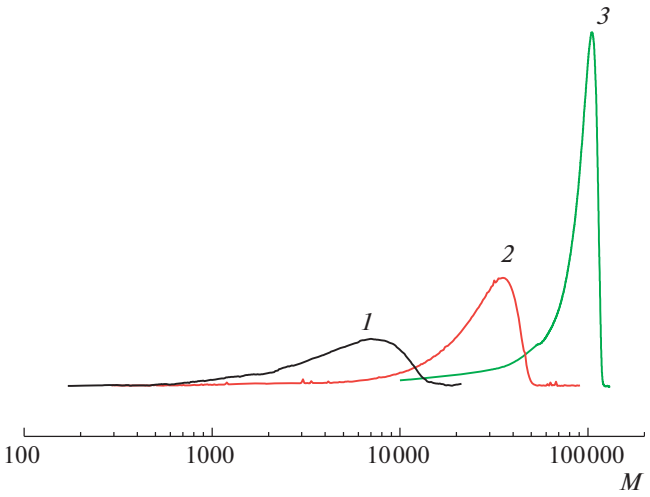


Fig. 3. Molecular mass distribution curves (LALLS detector) of the copolymers of PEGA with allyl acetate obtained in the absence of the crosslinker (*1*) and in the presence of 1 (*2*) and 2% (*3*) of the crosslinker.

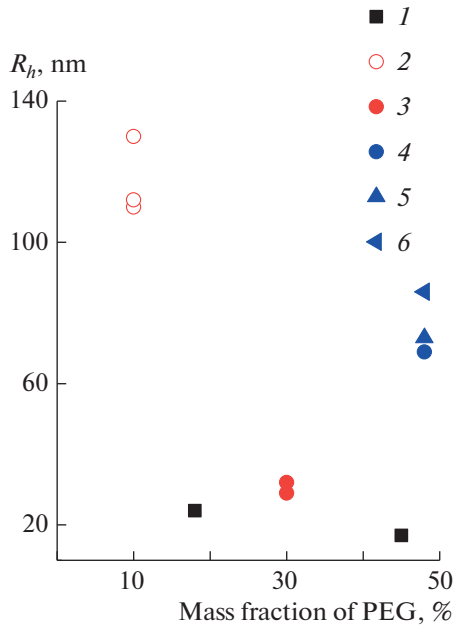


Fig. 4. Size of the micelles of block B-1 and B-2 (*1*), graft G-10-1–G-10-4 (*2*), G-30-1–G-30-2 (*3*), and G–allyl acetate (*4*), and branched Br-1 (*5*) and Br-2 (*6*) PEG-based copolymers (see details in the text and in Table 2).

mer micelles. Since the fraction of divinylbenzene in the copolymers did not exceed 2% the change in the hydrodynamic radius was unlikely due to the change in the copolymer hydrophobicity. Most probably, the increase in the copolymer crosslinking degree led to the increase in the macromolecules size and, hence, that of the associates.

Interactions of the Copolymers with Cells

The action of the obtained copolymers on the cells was investigated using the NCI/ADR-RES human ovary cancer cells exhibiting enhanced resistance to drugs, for example, to antitumor antibiotic doxorubicin. The copolymers effect on the cells viability was analyzed using a drug-free medium via incubation of the cells with solutions of polymers differing in the concentration. The ability of the copolymers to suppress the cells resistance to drugs was evaluated via incubation of the cells with the copolymers in the medium containing doxorubicin in the nontoxic concentration.

Analysis of the graft copolymers of PMMA with PEG (cf. Table 2) in the doxorubicin-free medium revealed that they were nontoxic towards the cells in concentration up to 1.5 mg/mL in the case of degree of conversion 22% (G-5, Fig. 5a) as well as 53% (G-6, Fig. 5b). However, their block analogs B-1 and B-2 exhibited toxicity at concentration above 0.1 mg/mL (Fig. 5c).

It is known that PEG-based copolymers possess a unique property of enhancing cells viability. That fact has been demonstrated using numerous examples of amphiphilic PEG-containing block copolymers such as Pluronics and the siloxane-based polymers. At the same time, the copolymers containing a branched polyglycerol hydrophilic block instead of PEG have not exhibited the said effect, despite the hydrophobic block similarity. That result has revealed that the enhancement of the cells viability was determined by the PEG block. It is prone to form hydrogen bonds with the polysaccharides, in particular with hyaluronic acid, one of the components of glycocalyx, surface layer of cells [23]. The relationship between this interaction and the cells viability has been unknown. Therefore, any data on the cells interaction with PEG is of interest.

In view of this, it is remarkable that the ability to increase the amount of living cells was the most prominent in the case of graft copolymer of allyl acetate and lightly branched (Br-1) copolymer of allyl acetate with PEG (Fig. 5d, curves *1* and *2*). The amount of the cells reached the maximum under the action of 0.5 mg/mL of those copolymers. The effect of highly branched copolymer (Br-2) (Fig. 5d, curve *3*) was less pronounced. Hence, the linear and lightly branched copolymers of PEG with allyl acetate were much more efficient in the enhancement of the cells viability in comparison with the highly crosslinked copolymer.

The MCF-7/ADR cells exhibit resistance to the drugs action, due to presence of the P-glycoprotein in their membrane, which can excrete various drugs from the cell. From extensive reports on the subject it is known that certain amphiphilic block copolymers containing a block of poly(propylene oxide), polydimethylsiloxane, or poly-ε-caprolactone can

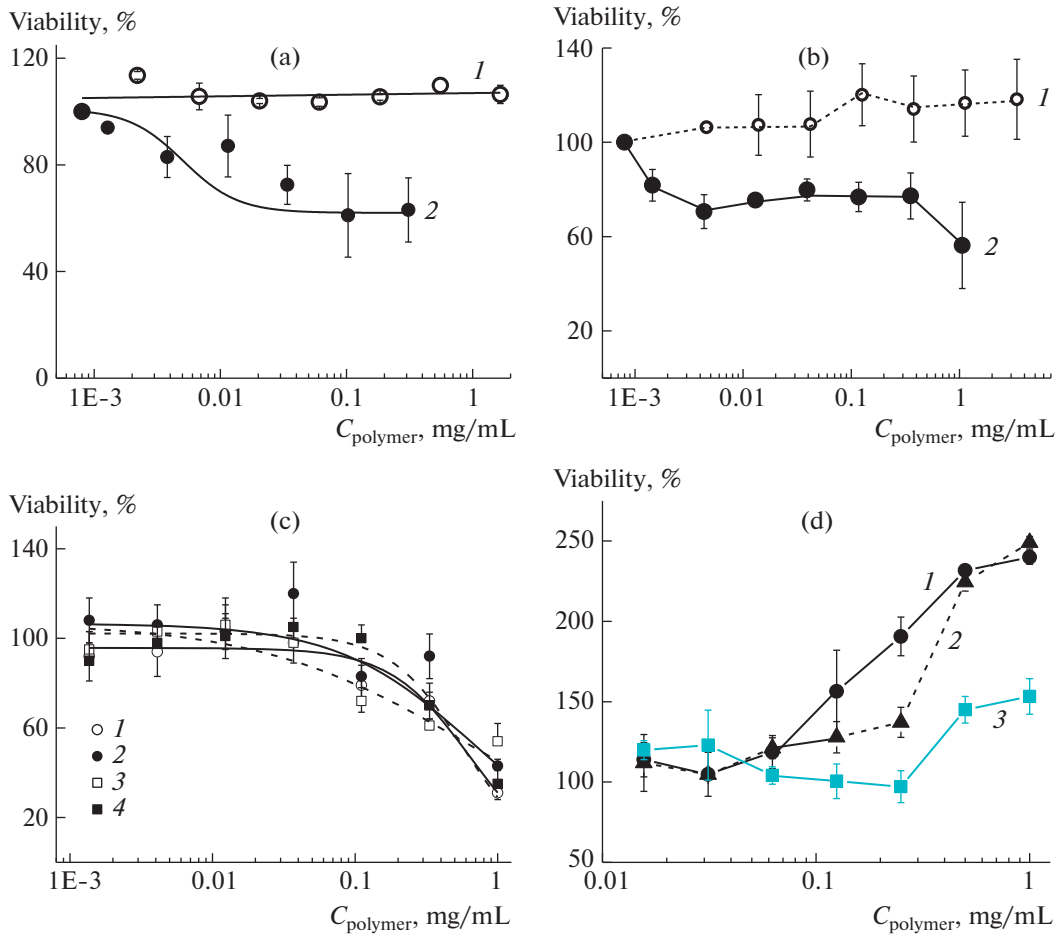


Fig. 5. Influence of PMMA–PEGMA graft copolymers G-30-1 (a) and G-30-2 (b) on the amount of living cells without a drug (*1*) and in the presence of 5 μg/mL (8.6 μM) of doxorubicin (*2*). c – Influence of PMMA–PEG block copolymers B-1 (*1*) and B-2 (*3*) without a drug and in the presence of 5 μg/mL (8.6 μM) of doxorubicin (*2* and *4*, respectively). d – Viability of the cells upon incubation with graft allyl acetate (*1*) and branched copolymers of allyl acetate and PEGA Br-1 (*2*) and Br-2 (*3*).

suppress the activity of this protein, thus favoring the drug accumulation in the cell.

We explored the possibility of the synthesized copolymers differing in the molecular architecture to affect the sensitivity of cancer cells to antitumor antibiotic doxorubicin. It was found that the graft copolymers in concentration of 0.003 (G-6, Fig. 5b) or 0.1 mg/mL (G-5, Fig. 5a) enhanced the doxorubicin cytotoxicity, which led to the decrease in the number of cells (curves *2*). On the contrary, linear block copolymers B-1 and B-2 did not suppress the resistance of the cell to the drug (Fig. 5c).

It could be expected that the said difference in the behavior of graft and block copolymers was due to the presence of negative charge at the former ones. Let us remind that the graft copolymers were obtained in the system containing sulfuric acid which can catalyze hydrolysis of the ester groups in MMA during the synthesis. Doxorubicin is a cationic molecule and can thus bind to polyanions [24].

Direct measurement of ζ-potential of the micelles formed by the graft polymers partially supported that suggestion: ζ-potential of the micelles was indeed weakly negative, whereas that of the block copolymers was almost zero. At the same time, ζ-potential of the micelles formed by the graft polymers was practically independent of conversion, i.e. the duration of the contact with sulfuric acid. Moreover, the value of the ζ-potential of those micelles (about −10 mV) was not sufficiently high to solidly state the possibility of efficient binding of the single-charge doxorubicin cation by such micelles.

Another possible reason for the difference between the considered series of the copolymers was higher cytotoxicity of the copolymers obtained via the atom transfer polymerization. It could not be ruled out that the said cytotoxicity not allowing demonstration of the suppression of the drug resistance could be due to the admixture of 4,4'-dinonyl-2,2'-bipyridyl in the block copolymers. We observed the presence of those

admixtures in the samples by means of ^{1}H NMR spectroscopy. Probably, removal of those admixture could allow the increase in the concentration of the block copolymers and observation of the block copolymers effect on the cells resistance to drugs.

The difference between the graft and block copolymers could also be due to their molecular architecture. The graft copolymers, being bulkier, could disturb the cancer cells membrane strongly in comparison with the linear block copolymers, thus suppressing the activity of P-glycoprotein.

CONCLUSION

It was shown for the first time that graft and branched polymers based on PEG and PMMA can suppress the resistance of cancer cells to the action of drugs. The graft architecture favored the enhancement of the copolymers efficiency.

The ability of amphiphilic linear block copolymers of poly(ethylene oxide) and poly(propylene oxide) to improve the cells viability was also typical of the copolymers of poly(ethylene oxide) with allyl acetate. However, in that case the increase in the copolymers branching led to weakening of their action on the cells viability.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

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SPELL: 1. ampoules, 2. dedusted, 3. serumless