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NOVEL PEROXIDE CONTAINING PEGYLATED POLYAMPHOLYTIC BLOCK COPOLYMERS

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Received: May 06, 2013 / Revised: July 07, 2013 / Accepted: December 20, 2013

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Abstract. The synthesis of block-copolymers of controlled chain length and content of end peroxide groups *via* polymerization of dimethylaminoethyl methacrylate initiated by Ce⁴⁺-PEG system using a functional peroxide-containing chain transfer agent was developed. Rheological and colloidal-chemical characteristics of novel polyampholytic surfactants were studied.

Keywords: RedOx polymerization, telomerization, block copolymers, polyampholytes.

1. Introduction

The synthesis of novel polyelectrolyte based surfactants for biomedical applications is the subject of a special interest of many research groups. As usual such polyelectrolytes of cationic type are suggested to be used as drug or DNA delivery vehicles. Negatively charged DNA can interact with cationic groups of polyelectrolyte resulting in formation of interpolyelectrolyte complexes (polyplexes) surely protected from damages by enzymes. Due to their high transfection efficiency poly-L-lysine (PLL) [1, 2], polyethylenimine (PEI) [3], polylactic-co-glycolic acid (PLGA) [4], poly(dimethylaminoethyl methacrylate) (PDMAEM) [5] and their branched and block copolymers [6-8] are the most widely used as nonviral vectors for targeted DNA delivery. Grafting PEG chains that form the outer hydrophilic shell of the polyplexes is the promising way to improve biocompatibility of such carriers delivering DNA. Besides reducing the cytotoxicity, PEGylation of polymers enhances their solubility, protects polyplexes against nonspecific interaction with blood proteins and recognition by the host immune defense system [9, 10].

The perspective route of the synthesis of novel peroxide-containing polyampholytic surfactants of block

structure *via* radical telomerization of DMAEM using PEG-OH-Ce⁴⁺ as the initiating RedOx system and functional peroxide telogen as the chain length controller was studied in this paper

2. Experimental

2.1. Materials

Poly(ethylene glycol) methyl ether (mPEG), molecular weight of 550, 2-(dimethylamino)ethyl methacrylate (DMAEM, 98%), ammonium cerium (IV) nitrate (98%) were received from Aldrich Chemical Co. and used without further purification. Other reagents and solvents such as nitric acid, hydrochloric acid, dimethyl sulfoxide (DMSO), dimethylformamide (DMF), ionol, toluene, chloroform, hexane, and acetone were obtained from SFERA SIM (Ukraine) and used as received. 1-isopropyl-3(4)-[1-(tert-butyl peroxy)-1-methylethyl] benzene (MP, Fig. 1) was synthesized from *tert*-butyl hydroperoxide and 2-(4-isopropylphenyl)-2-propanol in the acetic acid solution as described earlier [11].

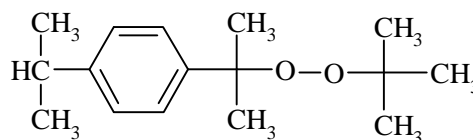


Fig. 1. Structure of MP

2.2. Methods

2.2.1. Polyampholyte synthesis

Di-block-copolymers PEG-oligo(DMAEM)-MP were synthesized *via* polymerization in water under

constant stirring in the flask protected from light at 291 K. Suitable amounts of DMAEM (0.45 mol/l), PEG (0.08 mol/l) and concentrated nitric acid were dissolved in 20 ml of distilled water. After that water insoluble MP was added to the solution. The mixture was purged by argon flow and thermostated under stirring for 1 h. The reaction was started by injecting the cerium(IV) ammonium nitrate (0.076 mol/l) dissolved in 5 ml aqueous acidic solution. Polymerization was carried out for 4 h. The resulting mixture was cooled to room temperature and dried. The precipitated polymer was thoroughly washed with acetone to remove a residual PEG, DMAEM, MP and Ce(IV) salt and dried under vacuum to a constant weight.

The synthesis of tri-block-copolymers PEG-block-oligo(DMAEM)-MP-block-oligo(DMAEM) was carried out in DMF at 353 K. DMAEM (3.125 g), macroinitiator PEG-block-oligo(DMAEM)-MP (0.335 g) was dissolved in 6.7 ml DMF. Monomer conversion was measured using dilatometric techniques. Resulting mixture was cooled to room temperature, dried, dissolved in acetone, multiply purified by precipitation into hexane and dried again in vacuum to the constant weight.

2.2.2. Polymer characterization

The content of DMAEM links and the number of average molecular weight were determined using elementary and functional analyses; the content of MP fragments was determined by the method based on the decomposition of peroxide groups at 483 K and subsequent gas-liquid chromatography determination of the decomposition products [12].

Surface tension of the solutions of polyampholytes was studied on the device PPNL-1 (Ukraine) using the maximum bubble pressure method (MBPM) [13]. The sizes of the micelle-like structures (MLS) of polymers were measured by dynamic light scattering (DLS) on Zetasizer Nano ZS (Malvern) photon correlation spectrometer using NIBS (Non-Invasive Back-Scatter) technology at 298 K. Reduced viscosities of polymer solutions were measured by the known method [14]. IR-spectra of the polymers were recorded on the Specord-M80 in KBr tablet, vaseline oil or in polymer films obtained from solutions in tetrahydrofuran.

3. Results and Discussion

It is evident from Fig. 2 that polymerization of DMAEM initiated by RedOx system PEG-OH-Ce⁴⁺ with participation of MP as a functional chain transfer agent obeys to well known regularities of low inhibited polymerization [15]. The increase of chain transfer agent polymerization and molecular weight of resulting polymers are responsive appreciably on pH value of the

reaction system. Since DMAEM is less soluble in alkaline water solution in comparison with acidic media its redistribution between water and organic phases is shifted to the organic phase, which is enriched by water insoluble MP. As a result one can see the more noticeable influence of MP concentration in the system on the copolymer yield and MW when polymerization occurred in the alkaline medium. Molecular weight of block copolymers varies from 13.000 to nearly 5.000 g/mol at alkaline pH, while it is almost independent on MP in the neutral medium.

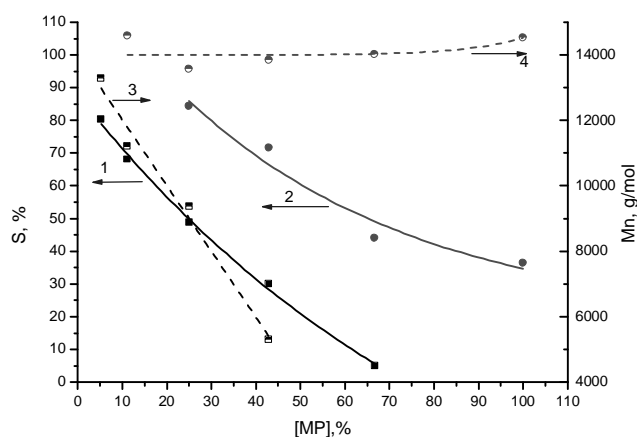


Fig. 2. Dependence of the yield (1, 2) and molecular weight (3, 4) of polymers on the concentration of MP in the initial monomer mixture at different meanings of pH: pH = 10 (1, 3) and pH = 7 (2, 4); [Ce⁴⁺] = 0.076 mol/l; [PEG] = 0.08 mol/l

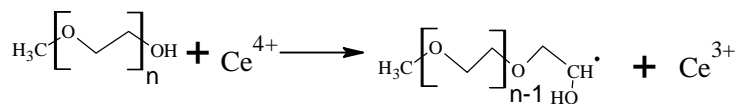
At the same time the increase of the MP concentration in the initial monomer system leads to the enhancement of its relative content in block-copolymer molecules (Table 1). Taking into account the mechanisms of the initiation by the system PEG-OH-Ce⁴⁺ [16-20] and the termination of growing radicals via interaction with MP molecules [21] the assumed scheme of di-block copolymer synthesis containing the reactive peroxide fragment is proposed (Fig. 3).

In accordance with the di-block copolymer scheme consisting of PEG block as the fragment of the initiator, poly(DMAEM) block and end peroxide containing MP fragment are formed. The structure of block-copolymers formed was confirmed by the functional analysis, CN analysis (Table 2) and IR-spectroscopy (Fig. 4).

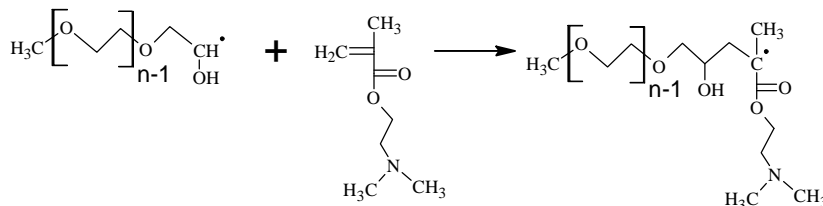
The same absorption bands that correspond to functional groups of the polymer chain are present on the spectra of samples, obtained at different conditions.

Quite narrow and intense band at 1150 cm⁻¹ attributed to vibrations of -C-O-C group proves the presence of PEG fragments in the polymer structure.

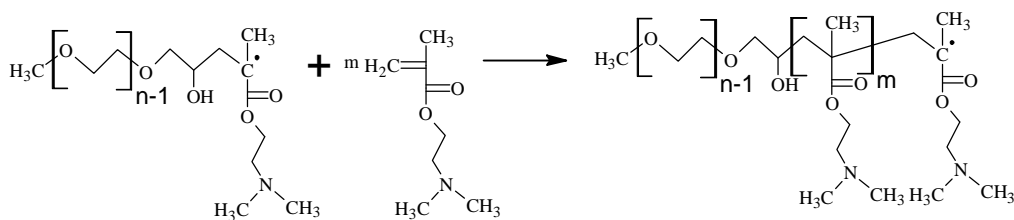
1) formation of free PEG – radical via redox reaction



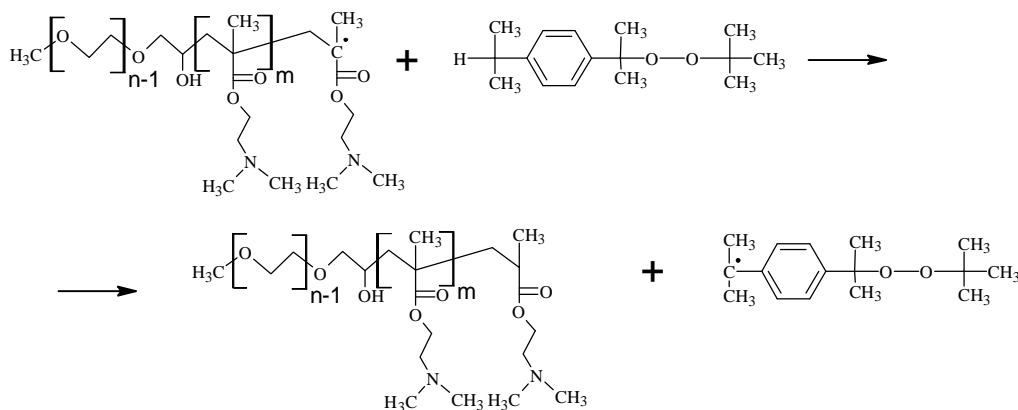
2) interaction of PEG – radical with the monomer



3) propagation of the polymer chain



4) interaction of the growing radical with MP



5) termination of the polymer chain due to the interaction (recombination) of the growing radical and MP radical

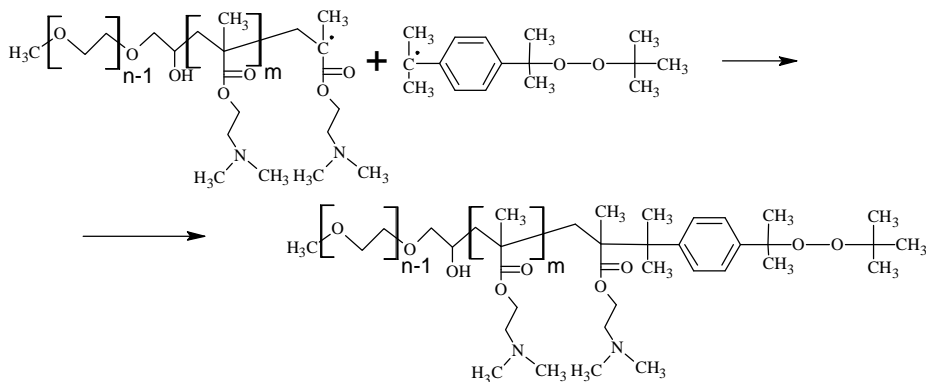


Fig. 3. Scheme of di-block copolymer synthesis

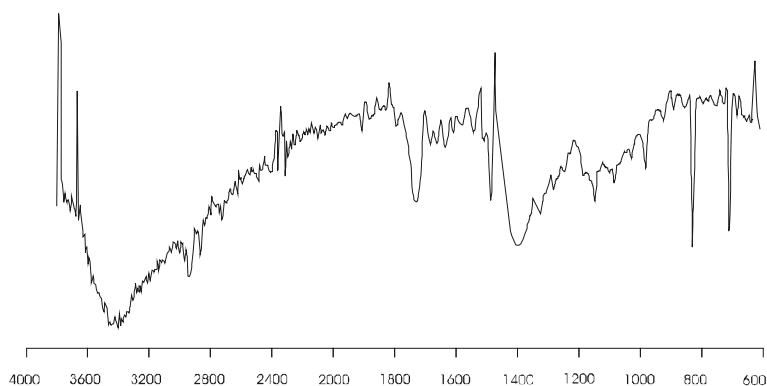


Fig. 4. IR-spectrum of PEG-oligo(DMAEM)-MP

Table 1

Characteristics of the initial monomer mixtures and PEG-oligo(DMAEM)-MP copolymers

Sample	[MP]* in monomer mixture, mol %	pH	m (DMAEM)	M_w , g/mol	Yield, %	[MP] in polymer, mol %
PDM-1	5.26	10	80.08	13300	80.36	0.24
PDM-2	11.11	10	66.71	11200	68.12	0.30
PDM-3	25	10	54.99	9400	48.82	0.36
PDM-4	42.86	10	28.88	5300	30.12	-
PDM-7	25	10	64.84	10900	60.52	0.17
PDM-8	42.86	10	66.67	11200	36.95	0.31
PDM-12	11.11	7	85.55	11300	98.52	0.36
PDM-13	11.11	7	88.37	14600	90.01	0.17
PDM-17	25	7	81.88	13600	84.31	0.21
PDM-16	42.86	7	83.60	13900	71.56	0.14
PDM-15	66.67	7	84.77	14000	44.14	0.17

* against content of DMAEM

Narrow intense band at 1720 cm^{-1} corresponds to vibrations of C=O group of DMAEM units. Stretching and deformation vibrations of amino groups absorb at $2760\text{--}2720\text{ cm}^{-1}$ and $1640\text{--}1636\text{ cm}^{-1}$, respectively. The presence of several narrow or single intense bands at $2950\text{--}2880\text{ cm}^{-1}$ corresponds to vibrations of CH_2 groups of the carbon skeleton of polymers.

In all cases, there are narrow and low-intensity absorption bands at $1250\text{--}1225$ and $940\text{--}920\text{ cm}^{-1}$ due to the presence of the *tert*-butyl fragment of the terminal MP group. That fact that polymer structure has terminal MP groups is also confirmed by the presence of bands at $1600\text{--}1580$, $860\text{--}824$ and $724\text{--}680\text{ cm}^{-1}$, corresponding to the vibrations of the aromatic ring and C-H groups of disubstituted benzene derivatives. Presence of broad intense bands at $3450\text{--}3390$ and $1405\text{--}1360\text{ cm}^{-1}$ can be attributed to stretching and deformation vibrations of O-H groups due to the formation of hydrogen bonds by PEG fragments.

The formation of two main products (acetone and *tert*-butyl alcohol) of the decomposition of block copolymers testifies to the availability of end peroxide-

containing fragments in their molecules and witnesses the ability to form free radicals at heating.

The polyampholytes are water-soluble surface-active substances at wide range of pH. They decreased the surface tension of water solutions and form hydrophobic micelle like structures (MLS) at reaching the definite critical concentration (CMC) (Fig. 5).

DLS studies of polymer water solutions at CMC confirm formation of MLS (Fig. 6). One can see that the sizes of MLS increase with the increase of polymer chain length. Reduced viscosity of PEG-oligo(DMAEM)-MP synthesized in water-alkaline medium (pH = 10) corresponds to the dependence of the viscosity on the concentration peculiar to uncharged polymer solutions. The value of reduced viscosity diminishes with the decrease of the polymer concentration in DMF solution. On the contrary, reduced viscosity of PEG-oligo(DMAEM)-MP synthesized in water, wherein HCl was added to provide the neutral pH value of the medium enhances sharply at the increase of its concentration in DMF (Fig. 7).

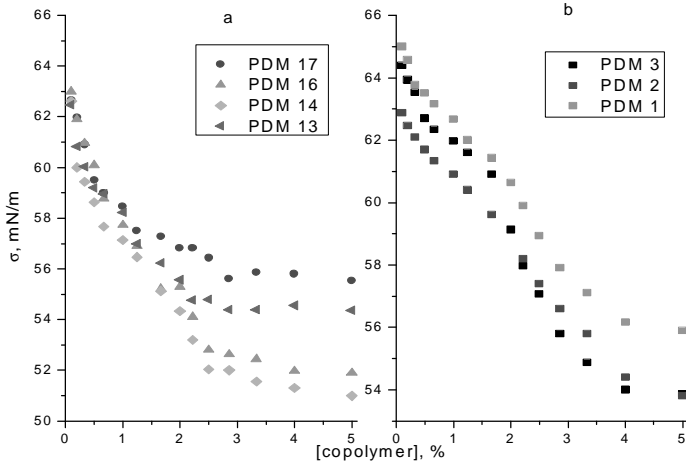


Fig. 5. Surface tension isotherms of polymers water solutions, obtained from neutral (a) and alkaline (b) medium of initial mixtures

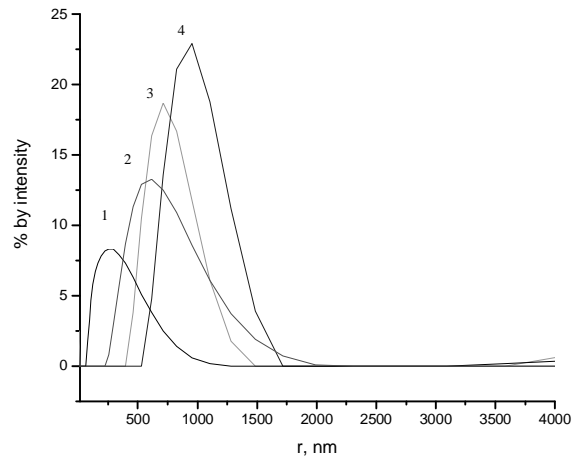


Fig. 6. DLS images of micelle-like structures of di-block copolymers: 8500 g/mol (1); 10900 g/mol (2); 13600 g/mol (3) and 14500 g/mol (4)

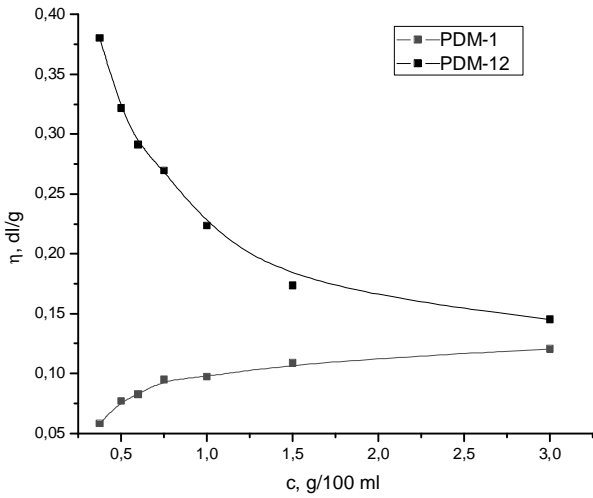


Fig. 7. Reduced viscosity η of different polymers in DMF

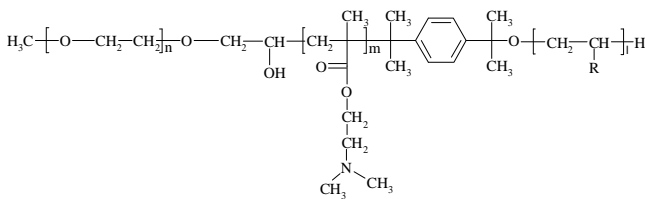


Fig. 8. Chemical structure of tri-block copolymers, where R means any functional group

The distinction of reduced viscosities dependences of close structured block-copolymers, however, synthesized in water systems with different values of pH, can be explained, in our opinion, by their polyelectrolyte nature. As a result of the interaction of HCl molecules with ternary amino groups of DMAEMA links of the oligo(DMAEMA) block of the copolymer positively charged quaternary ammonium fragments along the chain

are formed. This causes unwrapping the chain as a result of charged side groups repulsion and the increase of the polyelectrolyte solution viscosity, so called “polyelectrolyte effect”.

The specific feature of the functional polyampholytes is the availability of the end peroxide moiety capable to generate free radicals. One of them is the macroradical consisting of PEG and oligo(DMAEMA) blocks providing initiation of the polymerization leading to the formation of triblock-copolymers of desired nature (Fig. 8).

It is obvious from the kinetic curves (Fig. 9) that the increase of the content of initiating end MP fragments is accompanied by the enhancement the polymerization rate and yield of the final tri-block-copolymer. Large difference of molecular weight of purposeful tri-block copolymer and homopolymer, which is also formed, provides their easy separation.

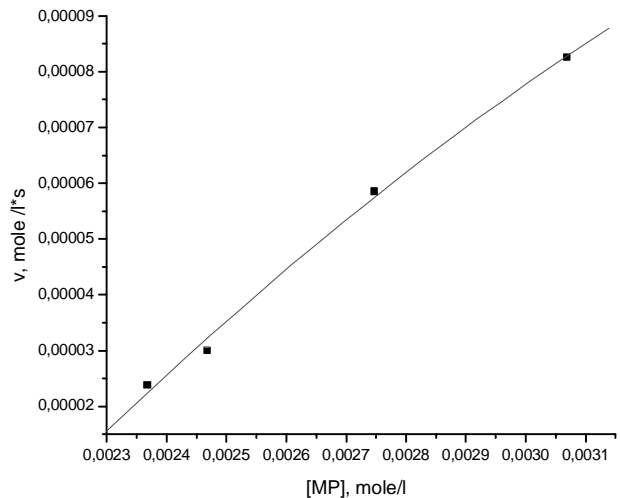


Fig. 9. Rate of DMAEM polymerization, grafted to PEG-oligo(DMAEM)-MP copolymers

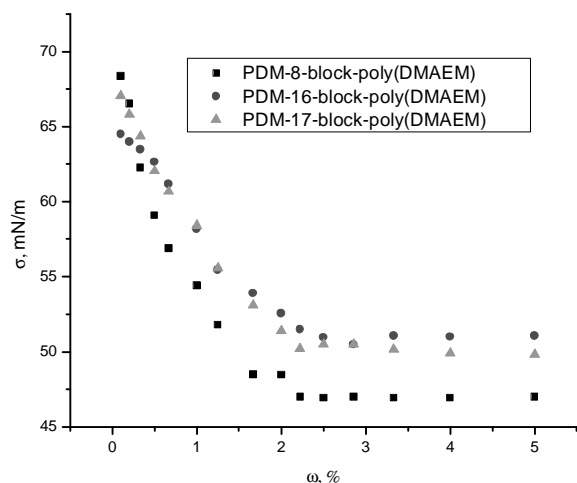


Fig. 10. Surface tension isotherms of tri-block copolymers water solutions

Depending on the nature and chain length of the third polymeric block tri-block copolymers are soluble in water and/or hydrocarbon solvents of various polarities. These parameters also define their colloidal-chemical characteristics. For example, tri-block PEG-block-oligo(DMAEM)-MP-block-oligo(DMAEM) copolymers formed in the result of DMAEMA polymerization initiated by PEG-oligo(DMAEM)-MP are soluble not only in water, DMF and DMSO as initial di-block copolymers, but also in other polar organic media. Moreover, they possess the higher surface activity (Fig. 10) and form MLS of significantly larger sizes (Fig. 11) in comparison with initial di-block copolymers.

4. Conclusions

Finally, there can be concluded that combination of specific initiating RedOx system and peroxide-containing the chain transfer agent at polymerization of functional monomers is a convenient way for functional di- and tri-block copolymers tailored design of the controlled molecular weight, functionality, reactivity and colloidal-chemical characteristics.

Acknowledgments

The studies have been performed owing to financial support of President Ukrainian Grant for young scientists F49/145-2013.

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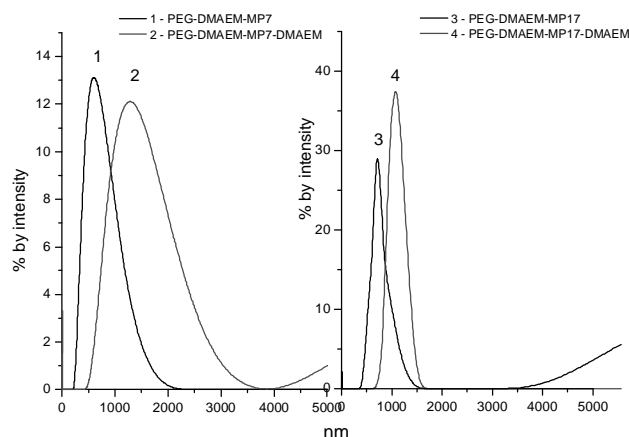


Fig. 11. DLS images of micelle-like structures of different initial di-block and tri-block copolymers

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НОВІ ПЕРОКСИДВМІСНІ ПЕГЕЛЬОВАНІ ПОЛІАМФОЛІТНІ БЛОК-КОПОЛІМЕРИ

Анотація. Методом радикальної полімеризації ініційованої Ce^{4+} -PEG редокс-системою з використанням функціонального пероксидвмісного агента передачі ланцюга, були синтезовані блок-кополімери диметиламіноетилметакрилату і поліетиленгліколю (PEG) з контрольованою довжиною полімерного ланцюга і вмістом кінцевих пероксидних груп. Досліджено реологічні та колоїдно-хімічні властивості отриманих нових поверхнево-активних поліамфолітів.

Ключові слова: редокс-ініціатор, теломеризація, блок-кополімер, поліамфоліт.