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FUNCTIONAL POLYMERS

Amphiphilic Linear-Branched Copolylactides and Disperse Systems on Their Basis

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Abstract—The linear-branched copolylactides containing linear side poly(ethylene oxide) blocks are synthesized and characterized. The critical micelle concentrations and the aggregative stability and the dispersity of oil/water emulsions stabilized by these copolymers are estimated. The polylactide microparticles are obtained by emulsification followed by evaporation of an organic solvent using acetylsalicylic acid as a model drug. The structure of copolylactides strongly affects the properties of the microparticles. On one hand, the presence of large poly(ethylene oxide) blocks in the linear-branched macromolecules leads to the formation of colloidal systems with a higher aggregative stability of emulsions and a lower size of particles, and on the other hand, the microparticles formed from these copolymers possess a lower incorporation efficiency relative to water-soluble low-molecular-mass compounds and the profile of the release of these compounds is nonlinear and contains the region of accelerated release.

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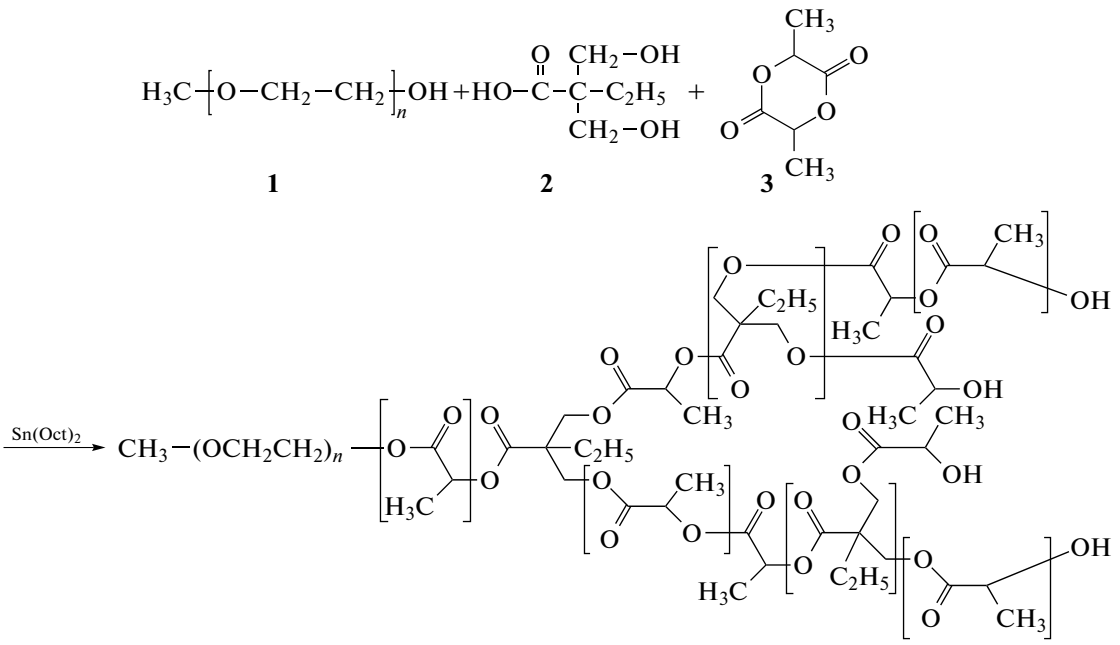
Linear-branched aliphatic polymers are of particular interest owing to their unusual properties; in particular, hyperbranched polymers with substituted terminal functional groups are able to serve as efficient carriers of low-molecular-mass compounds [1–6]. It is known that these copolymers have the core–shell structure, readily form stable nanosized micelles in water at low concentrations, and can even form monomolecular micelles [7–11]. The most promising polymers for biomedical application are biodegradable ones, for example, those obtained from hydroxy acids, such as lactic and glycolic, since their decomposition affords natural metabolism products [12, 13]. Unfortunately, the number of works devoted to branched biodegradable amphiphilic copolymers is extremely limited. The existing data are insufficient to define the dependence of surface-active properties of branched polymers on their structures. This information is necessary for the directed synthesis of polymer surfactants with the desired complex of properties.

This study deals with amphiphilic copolymers of the core–shell structure, the shell of which is formed by poly(ethylene oxide) oligomer blocks and the core is formed by aliphatic copolylactides of the branched structure. The goal of the present work is to establish the dependence of colloidal-chemical properties of the copolymers on their structure.

EXPERIMENTAL

This study was concerned with *L*-lactide (Aldrich, 98%), 2,2-bis(hydroxymethyl)butyric acid (BHM) (Acros Organics, 98%), tin 2-ethylhexanoate ($Sn(Oct)_2$) (Acros, 97%), 1,6-diphenylhexatriene (Aldrich, 98%), acetylsalicylic acid (ASA) (Aldrich, ≥99.0%), and poly(vinyl alcohol) with a molecular mass of $M_w = 8.9 \times 10^4$–9.8×10^4 (PVA) (≥99.0%, Aldrich), which were used as received. Poly(ethylene glycol) monomethyl ethers (MPEG) with $M_w = 550$ and 1900 (Aldrich) and $M_w = 750$ (Acros Organics) were dried by azeotropic distillation with toluene (after drying, the content of water defined by the Fischer coulometric titration was 0.01%). Solvents toluene and chloroform (high-purity grade, Khimmed) were purified by conventional techniques [14]. The copolymers were synthesized according to the following procedure.

A round-bottom flask equipped with a magnetic stirrer, an inert gas inlet, and a calcium chloride tube was charged with 0.001 mol of MPEG (comonomer **1**) and 0.01 mol of *L*-lactide (comonomer **3**):



1 **2** **3**

The reaction mixture was heated to 100°C, and the solution of $Sn(Oct)_2$ (0.0001 mol) in 3 mL of toluene was added under vigorous stirring. The reaction was carried out for 1 h, and BHM (comonomer **2**) (0.05 mol) and *L*-lactide (comonomer **3**) (0.24 mol) were added to the flask. The resulting mixture was stirred for 1 h at 100°C, the temperature was elevated to 150°C, and the reaction was continued in vacuum (200 Pa) under stirring for 18 h. The resulting polymer was dialyzed against water to remove the chloroform solution. The product obtained after dialysis was additionally purified by column chromatography on silica gel (eluent, toluene), the resulting solution was evaporated, and the polymer was dried under vacuum to a constant mass (100 Pa) at 50°C for 72 h.

The ^1H and ^{13}C NMR spectra were registered using 10% copolymer solutions in $CDCl_3$ on a Bruker spectrometer operating at frequencies of 600.22 and 150.94 MHz, respectively (using tetramethylsilane as an internal standard), at the Laboratory of Nuclear Magnetic Resonance, Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences.

The gel permeation chromatography of the copolymers was performed on a Knauer chromatographer calibrated against polystyrene standards (eluent, THF; 1 mL/min, PL-GEL 5u MIXC column 300 × 7.5 mm) at the National Research Centre, Kurchatov Institute.

The critical micelle concentrations (CMC) were determined according to the published procedure [15] by fluorescence spectroscopy using a water-insoluble compound solubilized by micelles; 1,6-diphenylhexatriene was used as a fluorescence probe (excitation and registration wavelengths were 366 and 430 nm, respectively).

The values of hydrophilic-lipophilic balance (HLB) of the copolymers were estimated by the Griffin method [16]. The analytical expression of HLB for the surfactant molecules has the form of 20 (M_h/M), where M_h and M are molecular masses of the hydrophilic moiety and the whole molecule.

The emulsions were obtained by dispersing 2 mL of a 2.5% solution of the copolymers in methylene chloride in 20 mL of water for 30 s using a UZDN-A sonicator; the ultrasound power was 15 W. The concentrations of all of the copolymers in water were much higher than the CMC.

The microparticles were produced by dispersing methylene chloride solutions of the copolymers and ASA in a 2% aqueous solution of PVA followed by evaporation of the organic solvent as described in [17, 18].

The average size of emulsion droplets and the diameter of microparticles were determined with a Photocor–FC photon correlation spectrometer (Photocor Instruments Inc., United States) using a Coherent He–Ne laser (United States, Model 31-2082, 632.8 nm, 10 mW) as a light source.

All the microparticles were separated from solutions containing 10 wt % ASA relative to the copolymer amount. The efficiency of ASA incorporation into the microparticles was evaluated according to the following procedure: the microparticles (10 mg) were dissolved in methylene chloride (1 mL), and the content of ASA in solution was determined by fluorimetry (excitation at 290 nm, registration at 360 nm) using the preliminarily recorded calibration curves. The efficiency of ASA incorporation was determined as the

Table 1. Structures and molecular masses of branched copolymers

Polymer	Yield, %	M_w of comonomer **1**	Molar ratio of comonomers **1** : **2** : **3**		Molecular mass, $M_n \times 10^4$			M_w/M_n
			in reaction mixture	in copolymer	theoretical	NMR	GPC	
1	85	550	1 : 25 : 250	1 : 22 : 210	2.18	1.85	1.80	1.9
2	83	750	1 : 25 : 250	1 : 20 : 204	2.20	1.82	1.77	1.8
3	81	1900	1 : 25 : 250	1 : 21 : 201	2.31	1.91	1.86	2.0
4	83	750	1 : 50 : 500	1 : 44 : 410	4.32	3.60	4.20	1.8
5	86	1900	1 : 50 : 500	1 : 45 : 423	4.44	4.44	4.32	2.0

ratio of the actual amount of ASA in microcapsules relative to the theoretical one. For each sample, measurements were performed three times (the arithmetic mean values).

The sizes of particles of the polymer suspensions were estimated by electron scanning microscopy on a LEO-1430 VP unit.

The investigation of the in vitro release of ASA was carried out in a Tris buffer solution. The dispersion of microparticles (10 mg) in Tris buffer solution (10 mL) was placed in a dialysis bag (the molecular mass cutoff MWCO was 3500 Da), which, in turn, was placed in a glass containing 50 mL of a neat Tris buffer solution. The temperature in the system was maintained at a level of 37°C; stirring was conducted with a magnetic stirrer. At 12-h intervals, the amount of ASA in the external buffer solution was determined by fluorimetry using preliminarily recorded calibration curves, and the buffer solution was exchanged for a similar amount of fresh one. The amount of ASA released from the microparticles was determined by fluorescence spectroscopy on a Fluorat-02 M Panorama spectrophotometer (Lyumeks, Russia). For each sample, measurements were performed three times (the arithmetic mean values are presented).

The Tris buffer solution (pH 7.6) of normal molarity had the following composition, mmol/L: 145 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgSO_4$, and 5.4 Tris-HCl.

RESULTS AND DISCUSSION

The diblock copolymers containing linear poly(ethylene oxide) and branched polylactide blocks were synthesized. The branched copolymers were studied by NMR spectroscopy, elemental analysis, and GPC (Table 1).

In particular, an analysis of the NMR spectra revealed signals due to protons of $-CH_2-O-$ and $-O-CH_3$ groups in MPEG (3.50–3.58 and 3.32 ppm, respectively), signals due to protons of methyl and methylene groups in BHM (0.88 and 1.37 ppm, respectively), and signals due to protons of $-CH_3$ (1.45–1.65 ppm) and $-CH<$ (5.0–5.2 ppm) groups in *L*-lactide (Fig. 1).

From the ratio of integral intensities of the signals due to protons in groups characteristic of comonomers **1**, **2**, and **3**, the relative content of the units of these comonomers in the copolymers was determined. Because each macromolecule contained only one poly(ethylene oxide) moiety with known molecular mass, a comparison of the amount of protons in this fragment and the integral intensity of the signals due to protons characteristic of poly(ethylene oxide) and other comonomers made it possible to experimentally determine the ratios of comonomer units in the polymers and to calculate the molecular masses of the corresponding polymers (Table 1). It is obvious that the ratio of comonomer fragments in the copolymers differs from the ratio of comonomers in the reaction mixture. This finding and a low yield of the product (81%) may be explained by the facts that the chosen organotin catalyst is not optimal for two different reactions, namely, the ring-opening polymerization of *L*-lactide and the polycondensation of BHM, and that the catalyst is poisoned with water released during polycondensation.

A comparison of the molecular masses calculated from the NMR spectra and determined using GPC shows that these values are close.

In order to evaluate the surface activity of the amphiphilic copolymers, the critical micelle concentrations were determined (Table 2).

It is apparent that, in terms of mass, the polymers may be arranged in two series: $(3.53–3.37) \times 10^4$ and $(7.5–8.45) \times 10^4$. An increase in the mass of hydrophobic polylactide blocks in the copolymer macromolecules leads to a reduction in the values of CMC; on the contrary, in the case of PEO blocks, the values of CMC increase. Since the molecular masses of polylactide blocks for all of the copolymers were different, the values of hydrophilic-lipophilic balance of the copolymers calculated by the Griffin method were used for the correct comparison of these samples. This

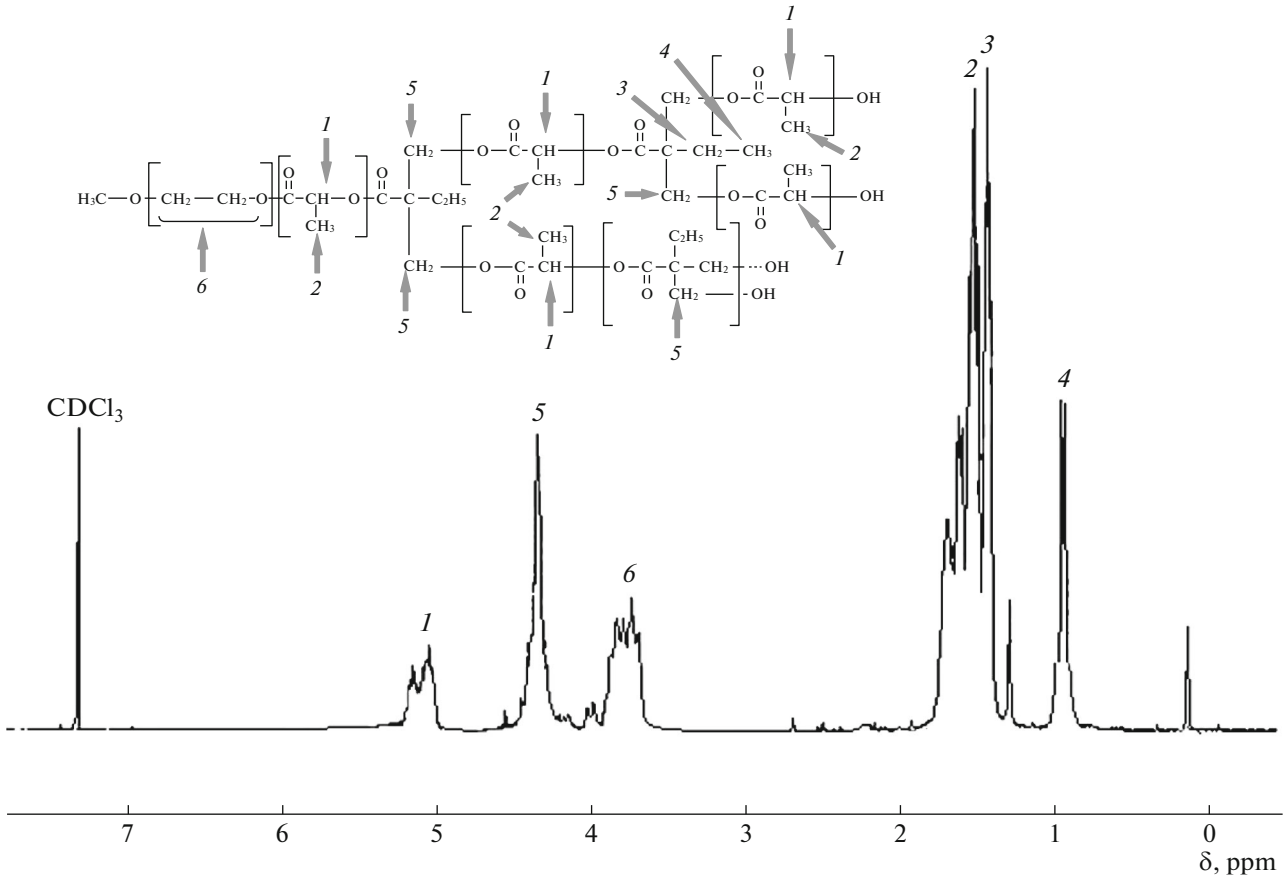


Fig. 1. Typical ^{1}H NMR spectra of the copolymers and assignment of the signals due to proton groups.

parameter characterizes the ratio of masses of hydrophilic and hydrophobic blocks in a copolymer. For all of the copolymers, these values were within 0.4–2.2, implying their high hydrophobicity. Indeed, the values of CMC obtained for the copolymers under investigation were relatively low and ranged from 5.1×10^{-9} to 8.2×10^{-7} M. At the same time, there was an almost linear dependence of the critical micelle concentrations on the hydrophilic-lipophilic balance; that is, an increase in the HLB of the copolymer afforded growth in CMC. An analogous dependence on HLB was revealed for the size of emulsion droplets obtained using the copolymers as surfactants. Thus, the lower the value of HLB of the corresponding copolymer, the higher the size of emulsion droplets at the beginning of the experiment. At the same time, the linear dependence of the size of emulsion droplets on HLB was valid only in initial measurements. For the polymers with a large fraction of hydrophilic PEO blocks (consequently, with a high HLB), increase in the size of emulsion droplets in 30 min characterizing the aggregative stability of emulsions was much lower than that

Table 2. Properties of the block copolymers and characteristics of the emulsions on their basis

Polymer	Block molecular mass, M_w		HLB	CMC, wt %	Size of emulsion droplets, nm	
	PEO	PL			initial, D_0	in 30 min, D_{30}
1	550	33700	0.6	0.0029	30	64
2	750	31200	0.9	0.011	29	55
3	1900	35300	2.2	0.032	25	47
4	750	75000	0.4	0.0026	31	81
5	1900	84500	1.0	0.012	28	53

Table 3. Characteristics of the microparticles obtained from copolymers 1–5

Polymer	Average diameter of microparticles, μm	Efficiency of ASA incorporation into microparticles, %	ASA released in 5 days, %
1	17.6	77	26.7
2	14.2	76	34.0
3	11.7	69	48.4
4	19.5	78	22.3
5	15.7	75	38.2

for the polymers with a low content of PEO blocks. This is likely connected with the peculiarities of association of branched copolymers at the interfacial boundary.

Using copolymers **1**–**5** as a polymer matrix, the polymer microparticles with immobilized ASA were obtained. The micrographs of the microparticles were obtained, their sizes were determined, the efficiency of ASA incorporation was evaluated, and the kinetics of ASA release from the microparticles was studied. The resulting data are presented in Table 3.

The particles of all of the suspensions are spherical in shape (Fig. 2). At the same time, the diameter and size distribution of the microparticles based on copolymers **1**–**5** are different. For example, according to the dynamic light scattering data, the particles with the smallest diameter are obtained in the presence of copolymer **3**, while the particles with the largest diameter are synthesized in the presence of copolymers **1** and **4**. Furthermore, the size distribution of particles was the narrowest for copolymer **3**. In addition, the dependence of the microparticle size and their size distribution on the fraction of poly(ethylene oxide) blocks in the copolymer was observed: as the content of PEO blocks increased, the sizes of the microparticles decreased and the size distribution became narrower.

At the same time, an increase in the fraction of PEO blocks leads to a decrease in the efficiency of ASA immobilization. This is likely due to the formation of hydrogen bonds between ASA and PEO blocks of the copolymers [19]. On one hand, these complexes favor the immobilization of ASA in the organic phase of emulsions; on the other hand, they facilitate the transfer of ASA from the interfacial boundary followed by its release into the external aqueous phase. As a result, a part of ASA in the microparticles is dispersed in the bulk of the polymer matrix, and a part of ASA is bound with the amphiphilic copolymers in the vicinity of the microparticle surface.

The release of ASA from the microparticles was studied in a buffer solution modeling the physiological medium of the body. It is obvious that, for different copolymers, release occurs in a different manner (Fig. 3). Thus, copolymers **2**, **3**, and **5** feature a rapid release of ASA for five days after the onset of extraction (Table 3), and the greatest increase in the concentration of ASA is observed during the first three days. It is obvious that the rate of the initial ASA release correlates with the content of PEO blocks in the amphiphilic copolymer. Usually, the stage of this rapid initial release is explained by the release of immobilized compounds that occurs either in the vicinity or on the surface of microparticles [20]. This idea coincides with our concepts about the existence of a part of ASA bound to the PEO blocks of the copolymer. Furthermore, the accelerated release of ASA can be facilitated by the swelling of PEO blocks of the copolymers in water. As a result, the ASA–PEO complexes decompose. In five days, this phase is exchanged for the decelerated release phase during which ASA is released with a gradually decreasing rate. It is believed [21] that this character of release is connected with the combined action of diffusion and erosion of the polymer matrix.

At the same time, the microparticles formed from copolymers **1** and **4** containing the lowest amounts of PEO blocks are practically devoid of phases of the active ASA release. Presumably, this may be explained by a much lower degree of swelling of these microparticles in water which does not provide the rapid decomposition of ASA–PEO complexes and the accelerated erosion of the polymer matrix.

Hence, on one hand, the presence of large poly(ethylene oxide) blocks in linear-branched macromolecules is responsible for the formation of colloidal systems with a high aggregative stability of emulsions and lower particle sizes; on the other hand, the microparticles formed from these copolymers possess a lower efficiency of incorporation relative to water-soluble low-molecular-mass compounds and are characterized by a nonlinear profile of the release of these compounds containing the region of the accelerated initial release.

The studied linear-branched copolymers may be used as surfactants in designing new transport systems for biomedical applications.

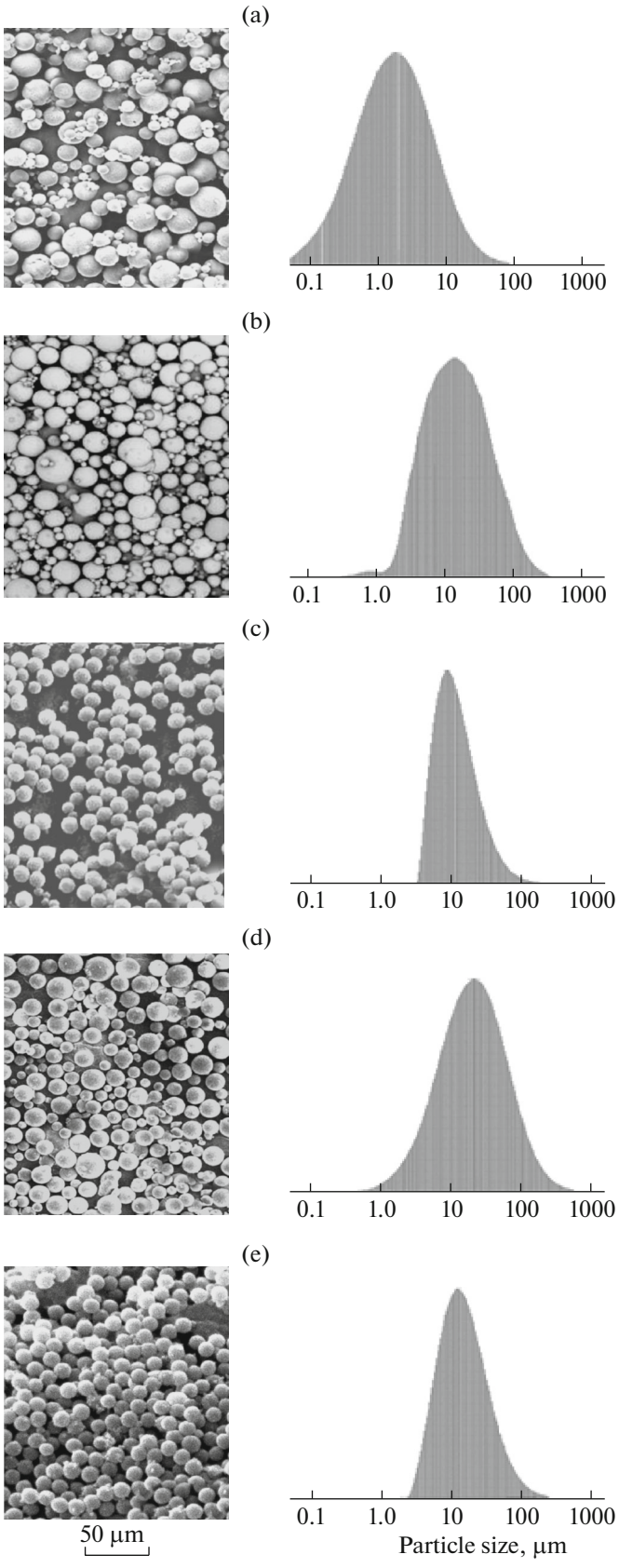


Fig. 2. Micrographs of the microparticles based on copolymers **1**–**5** and size distribution histograms obtained by dynamic light scattering. The notation in the micrographs and histograms (a–e) corresponds to the numeration of the polymers **1**–**5** in Tables.

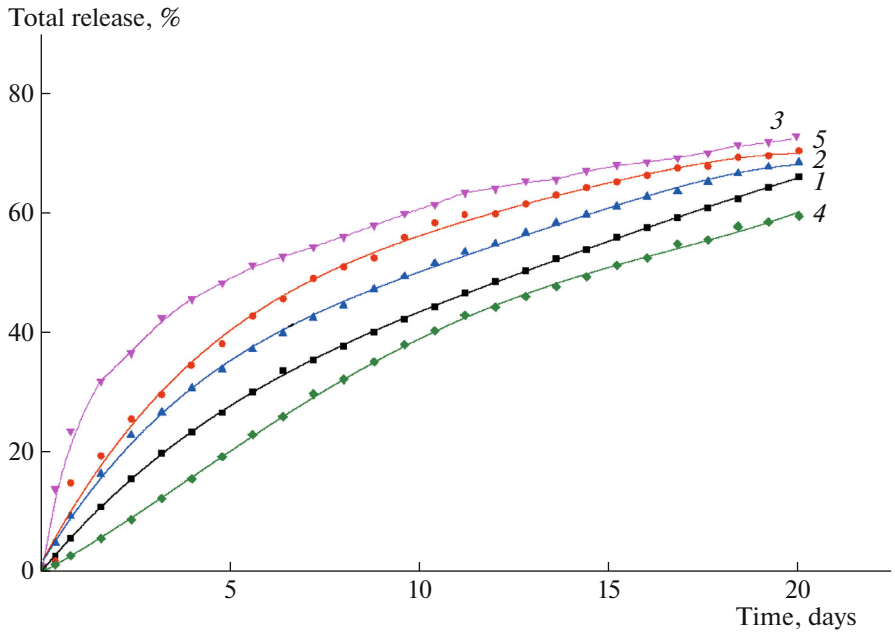


Fig. 3. (Color online) Kinetic curves of ASA release from the microparticles based on copolymers **1**–**5**. The numeration of the curves corresponds to the numeration of the polymers in the tables.

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