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Polylactide containing nanoparticles — new carriers of active compounds[**)]

Summary — Two amphiphilic block copolymers poly(L-lactide)-*b*-polyglycidol-*b*-poly(ethylene oxide) (PLA-*b*-PGly-*b*-PEOX) were synthesized: PLA-*b*-PGly-*b*-PEOX(I) with $\overline{M}_n$ equal 6260 (PLA), 510 (PGly) and 6600 (PEOX) and PLA-*b*-PGly-*b*-PEOX(II) with $\overline{M}_n$ equal 3600 (PLA), 500 (PGly) and 5500 (PEOX). Polyglycidol segment in PLA-*b*-PGly-*b*-PEOX(II) was modified in reaction with succinic anhydride — the reaction leading to conversion of $-CH_2OH$ groups of polyglycidol monomeric units to $-CH_2OC(O)CH_2CH_2COOH$ moieties with carboxyl groups [modified PLA-*b*-PGly-*b*-PEOX(II) was denoted as PLA-*b*-PGly-*b*-PEOX(IIc)]. Molecules of all three copolymers did self assemble in water into polymeric nanoparticles with number average diameters equal 20.0±0.8 nm [PLA-*b*-PGly-*b*-PEOX(I)], 20.9±0.3 nm [PLA-*b*-PGly-*b*-PEOX(II)] and 33±1 nm [PLA-*b*-PGly-*b*-PEOX(IIc)]. Critical aggregation concentration (*CAC* — a concentration above which nanoparticles are formed) was equal $1.55 \cdot 10^{-2}$, $7.0 \cdot 10^{-2}$ and $2.51 \cdot 10^{-1}$ g/L for PLA-*b*-PGly-*b*-PEOX(I), and PLA-*b*-PGly-*b*-PEOX(IIc), respectively. Nanoparticles were formed also in presence of pyrene and partition of pyrene between solution and nanoparticles was determined. For example, for concentrations of PLA-*b*-PGly-*b*-PEOX(II) and pyrene equal $1.95 \cdot 10^{-3}$ g/L and $4.94 \cdot 10^{-7}$ g/L the fraction of encapsulated pyrene was *ca.* 10 % whereas for the same concentration of pyrene but for higher concentration of PLA-*b*-PGly-*b*-PEOX(II), above $2.50 \cdot 10^{-1}$ g/L, the whole amount of pyrene was incorporated into nanoparticles. Profile of pyrene release from nanoparticles was bi-exponential with the rate constant for "fast" (k_f) component equal $9.5 \cdot 10^{-2}$, $3.8 \cdot 10^{-2}$ and $1.5 \cdot 10^{-1}$ L/h, for PLA-*b*-PGly-*b*-PEOX(I), PLA-*b*-PGly-*b*-PEOX(II) and PLA-*b*-PGly-*b*-PEOX(IIc), respectively, and with the rate constant for "slow" (k_s) component essentially the same for nanoparticles from all terpolymers equal $(8.4 \pm 0.2) \cdot 10^{-3}$ L/h. It has been suggested that "fast" rate describes the release of pyrene from shells of nanoparticles whereas the "slow" one describes the release from their cores.

Key words: amphiphilic block copolymers, nanoparticles, pyrene, encapsulation, kinetics of release.

Some proteins and proteinaceous aggregates (for example, human serum albumin and viral capsids) function as carriers is to transport effectively bioactive compounds in living organisms. Since usage of natural drug carriers is often accompanied with unwanted side effects or with potential risk of contamination with pathogenic species there is growing an interest in synthetic carriers of bioactive compounds. Low molecular weight amphiphilic compounds have been used for preparation of micellar and liposomal carriers [1]. Carriers of this type are used in medicine and in cosmetics; however, in some instances their application is limited due to insufficient mechanical and colloidal stability. Micelles from low molecular weight compounds quickly decompose below critical micelle concentration (*CMC*). Liposomes are quite labile under shear forces.

Polymeric nanoparticles with solid hydrophobic cores and loose hydrophilic stabilizing shells are good

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candidates for more stable carriers. Due to cohesion forces between insoluble polymer chains in the cores the polymer nanoparticles once formed may preserve their integrity more effectively that micelles from the low molecular weight compounds. Obviously, like all other drug carriers introduced into an organism, polymeric nanoparticles after serving their function should be decomposed and (bio)eliminated. One class of polymers with the mentioned above property consists of linear polyesters [for example, poly(ε-caprolactone) and polylactides] [2]. In last years we developed a polymerization method allowing the formation of polyester microspheres by self-assembly of propagating macromolecules into particle seeds and further propagation in seed particles converting them into microspheres [3—7]. However, this method suitable for synthesis of poly(ε-caprolactone) and polylactide particles with a narrow diameter distribution allowed obtaining only rather large particles (of diameter exceeding 0.5 μm). Recently, in our laboratory, there was elaborated a synthesis of poly(L-lactide)-*b*-polyglycidol-*b*-poly(ethylene oxide) (PLA-*b*-PGly-*b*-PEOX) block copolymers with controlled block lengths [8]. These amphiphilic copolymers were suitable for self-assembly in water into nanoparticles.

In this paper we report on studies on incorporation of pyrene, a model hydrophobic low molecular weight compound, into (PLA-*b*-PGly-*b*-PEOX) nanoparticles and into nanoparticles from copolymers in which $-CH_2OH$ groups of polyglycidol monomeric units were converted to $-CH_2OC(O)CH_2CH_2COOH$ moieties bearing carboxyl groups. Presented are also the results of our studies on kinetics of the release of pyrene from nanoparticles.

EXPERIMENTAL

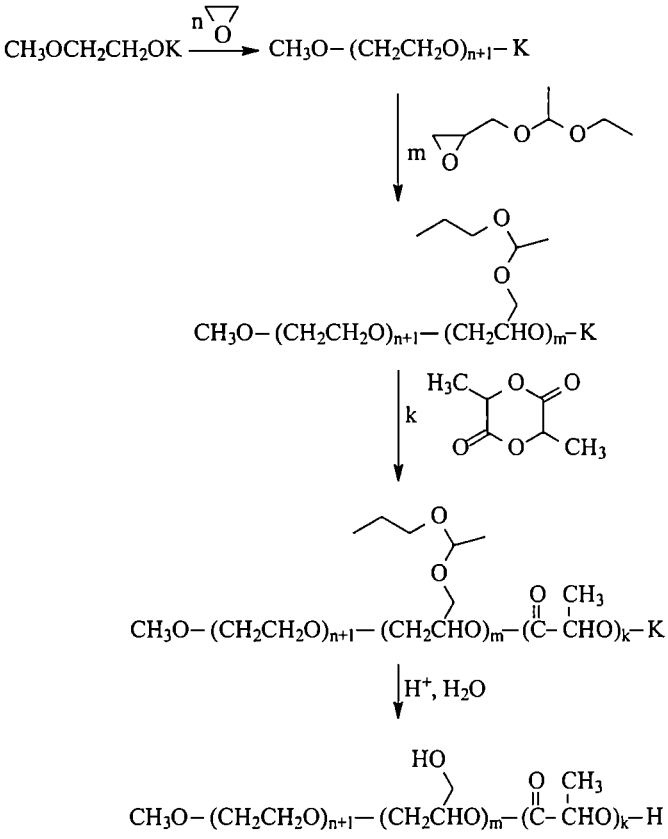
Materials

1,4-Dioxane (POCh, Poland) and tetrahydrofuran (THF) (POCh, Poland) were distilled and then dried in a common way over Na wires. Finally, the solvents were dried over Na-K alloy in an ampoules equipped with Teflon® stopcock and stored *in vacuo*. Needed amounts were distilled from the ampoule just before using.

Pyrene (Aldrich) was used as received.

Synthesis of block copolymers

Poly(L-lactide)-*b*-polyglycidol-*b*-poly(ethylene oxide) (PLA-*b*-PGly-*b*-PEOX) block copolymers (briefly called later terpolymers) were synthesized by three step anionic polymerization of ethylene oxide, 1-ethoxyethylglycidyl ether (EEGly — glycidol in which $-CH_2OH$ groups were blocked with ethoxyethyl groups) and L-lactide with subsequent deblocking of hydroxyl groups. Reactions involved in the synthesis are shown in Scheme A.



Scheme A. Synthesis of PLA-b-PGly-b-PEOX block copolymer

Detailed description of the synthesis is given in [8]. Briefly, first 1-ethoxyethylglycidyl ether was synthesized according to the known method [9]. Thereafter, ethylene oxide (EOX) was polymerized in THF solution. Polymerization initiated with 2-methoxyethanolate was carried out in a reactor protecting polymerizing mixture from contact with air (elimination of termination by water from air). Progress of polymerization was monitored by ^1H NMR. GPC trace for withdrawn polymer sample was recorded. After completion of the synthesis of PEOX block the required amount of blocked glycidol (EEGly) was added to PEOX with alcoholate active centers. Progress of polymerization was monitored again by ^1H NMR and GPC trace was recorded. Synthesis of poly(L-lactide) (PLLA) block started after addition of L-lactic acid (crystallized from 2-propanol, sublimed and stored *in vacuo* before using). Product (PLA-*b*-PEEGly-*b*-PEOX), isolated by precipitation into diethyl ether (at -50 °C), was characterized by ^1H NMR and GPC. GPC traces indicated that after addition of each comonomer molecular weight of copolymer did increase. ^1H NMR spectra of obtained product (Fig. 1) compared with spectra of PLA-*b*-PEEGly-*b*-PEOX reported in the literature [8] proved that synthesized copolymers had the desired structure.

Hydroxyl groups in synthesized terpolymers were deprotected using 10 % solution of oxalic acid (Aldrich)

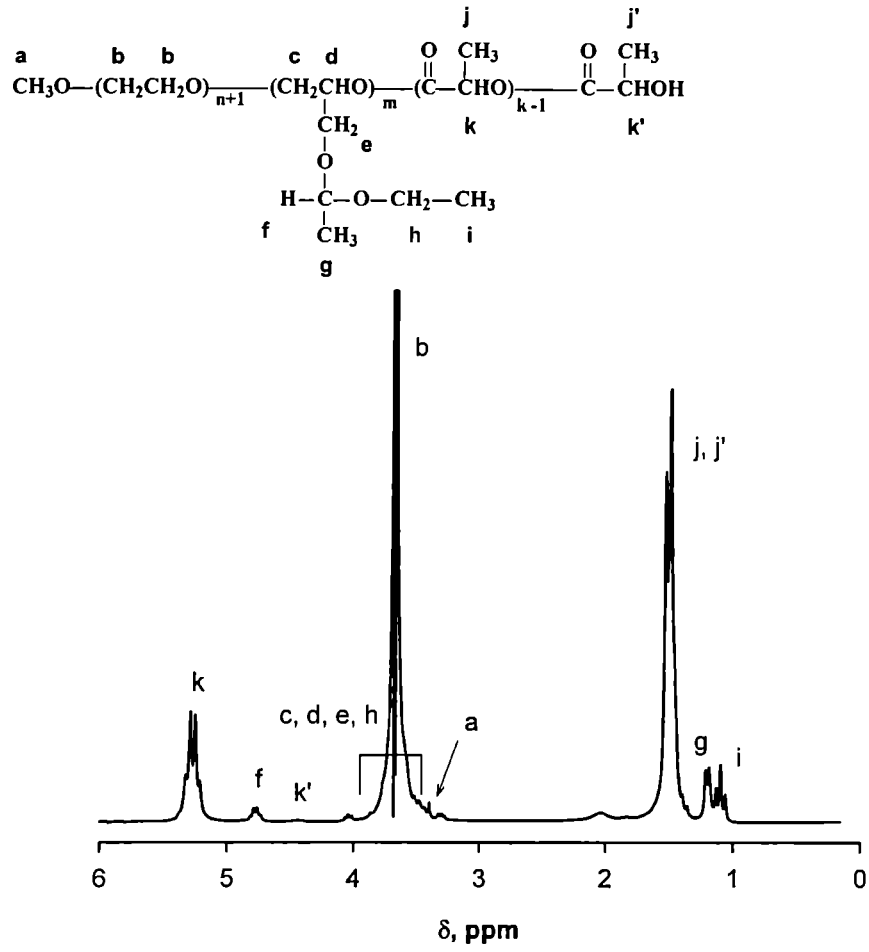


Fig. 1. ^1H NMR spectrum of PLA-b-PEEGly-b-PEOX

dissolved in acetone:water (50:50 v/v) mixture. Deprotection was carried out at 37 °C for 90 min. Product was lyophilized, dissolved in THF and precipitated in cold diethyl ether. The last purification step was repeated once more and finally the pure PLA-*b*-PGly-*b*-PEOX was dried for two days at high vacuum. Two samples of terpolymer with different block lengths were synthesized according to the recipes shown in Table 1.

T a b l e 1. Reagents used for synthesis of PLA-*b*-PGly-*b*-PEOX terpolymers

	PLA-*b*-PGly-*b*-PEOX(I)	PLA-*b*-PGly-*b*-PEOX(II)
THF, mL	62	39
$CH_3OCH_2CH_2OK$, g	0.31	0.21
EOX, g	11.5	10.3
EEGly, g	2.39	1.16
L,L-LA, g	13.6	9.2

Modification of PLA-*b*-PGly-*b*-PEOX

Properties of nanoparticles formed by self assembly of PLA-*b*-PGly-*b*-PEOX block copolymers may be changed by modification of PGly block leading to replacement of $-CH_2OH$ groups with groups with different size, polarity, ability for ionization, *etc.* In this work we decided to check how the replacement of $-CH_2OH$ groups by $-CH_2COOH$ in PLA-*b*-PGly-*b*-PEOX terpolymer affects the properties of nanoparticles. Modification was done by reaction of known sample of PLA-*b*-PGly-*b*--PEOX(II) with succinic anhydride. Reaction was carried out at room temperature, in dry 1,4-dioxane under argon for 5 hours. Succinic anhydride was used in excess (300 %) with respect to hydroxyl groups in PLA-*b*-PGly--*b*-PEOX(II). Product was precipitated into cold diethyl ether and dried under high vacuum for three days. Purified polymer was denoted as PLA-*b*-PGly-*b*-PEOX(IIc). ^1H NMR spectra of PLA-*b*-PGly-*b*-PEOX(II) before and after reaction with succinic anhydride are shown in Fig. 2.

Formation of nanoparticles

Stock solutions of PLA-*b*-PGly-*b*-PEOX(I), PLA-*b*--PGly-*b*-PEOX(II) and PLA-*b*-PGly-*b*-PEOX(IIc) were prepared in three times distilled water. Polymer concentration in stock solution was 2.5 g/L. Solutions of nanoparticles were prepared by diluting (with triple distilled

water) samples of the stock solutions to required polymer concentrations. These solutions were equilibrated at 4 °C for 18 hours. Presence of nanoparticles in solutions was detected by monitoring intensity of light scattered by the sample (number of counts per second registered by detector of Malvern apparatus). Quasielastic light scattering was used also for determination of diameters and diameter distributions of nanoparticles.

Encapsulation of pyrene into PLA-*b*-PGly-*b*-PEOX nanoparticles

Encapsulation of pyrene into PLA-*b*-PGly-*b*-PEOX(I), PLA-*b*-PGly-*b*-PEOX(II) and PLA-*b*-PGly-*b*-PEOX(IIc) was performed in the following way. At first stock solutions of pyrene and terpolymers in 1,4-dioxane were prepared. Than, the required amounts of these solutions were mixed together. To the obtained solution water was added gradually until concentration of 1,4-dioxane decreased to 10 % (v/v). Concentrations of pyrene and terpolymer in each sample were calculated from their concentrations in stock solutions and from the degree of dilution. The samples were conditioned for 12 hours and then deaerated by bubbling argon. Thereafter their emission spectra were registered.

Kinetics of release of pyrene from PLA-*b*-PGly-*b*-PEOX nanocomposities

Kinetics of the release of pyrene from nanoparticles was monitored by measuring changes of the concentration of pyrene, encapsulated into nanoparticles placed into the dialysis bag (Serva, regenerated cellulose, molecular weight cut-off 1000; concentration of pyrene averaged over the whole volume of liquid containing nanoparticles). Content of the dialysis bag (5 mL) was dialyzed against water (container with 800 ml of three times distilled water). Water in the container was exchanged every 12 hours. During dialysis the released pyrene was transferred across the membrane from the liquid containing nanoparticles to the external container ("sink") with water.

Methods

^{1}H NMR spectra were recorded for polymer solutions in $CDCl_3$ using a Bruker AC 200 spectrometer operating at 200 MHz.

GPC traces were recorded using a system including LKB 2150 pump (LKB, Sweden) TSK-Gel G6000HR and G3000HR columns (TOSOHAAS, Japan), a Dawn F MALS detector (Wyatt, USA) and an Optilab 903 Interferometric Refractometer (Wyatt, USA).

Suspensions of nanoparticles were investigated by quasielastic light scattering method using ZetaSizer 3000HSa apparatus (Malvern, UK) with 10 mW He-N laser (emission at λ = 633 nm) and Malvern 7132 correlator. The signal of light scattered at 90° was analyzed using the CONTIN method. Results of five measurements for each sample were averaged.

Absorption spectra were registered using Hewlett-Packard 8452A diode array spectrometer.

For registration of stationary emission spectra Perkin-Elmer LS 50 spectrometer has been used.

RESULTS AND DISCUSSION

Synthesis of PLA-*b*-PGly-*b*-PEOX

Integration of appropriate signals in ^{1}H NMR spectra registered after each step of polymer synthesis allowed determination of $\overline{M}_n$ of each block (see Fig. 1). In the case of the first block (PEOX) molecular weight was calculated from integration of the signal of -CH_3 end-groups (*s*, 3.38) and signal of the main chain -CH_2CH_2- groups (*m*, from 3.50 to 3.90). Molecular weight of PEEGly in PEOX-*b*-PEEGly-*b*-PLA was calculated from integral of the signal -CH_3 end-groups (*s*, 3.38) and integrals of the signals of -OCH($\underline{CH_3}$)O- (*d*, 1.27) and $\underline{CH_3}CH_2O$- (*t*, 1.18) of PEEGly. Molecular weight of PLA blocks was determined from the integrals of the signal of -CH_3 end-groups (*s*, 3.38) and integrals of the signals of -OCH($\underline{CH_3}$)C(O)- groups of PLA (signal of main chain *d*, 1.56; signal of end-group, *d*, 1.51). After deprotection the signals of -OCH($\underline{CH_3}$)O- and $\underline{CH_3}CH_2O$- groups of PEEGly were absent indicating a complete removal of blockig groups. Since GPC traces before and after deprotection were identical it was possible to conclude that this process did not lead to a noticeable hydrolysis of main chains of macromolecules. Values of $\overline{M}_n$ characterizing PLA-*b*-PGly-*b*-PEOX(I) and PLA-*b*-PGly-*b*-PEOX(II) are given in Table 2.

Table 2. Molecular weight ($\overline{M}_n$) of PLA-*b*-PGly-*b*-PEOX terpolymers)**

Block	PLA-*b*-PGly-*b*-PEOX(I)	PLA-*b*-PGly-*b*-PEOX(II)
PLA	6260	3600
PGly	510	500
PEOX	6600	5500
Terpolymer	13 370	9600

*) Determined from ^{1}H NMR spectra.

Modification of PLA-*b*-PGly-*b*-PEOX

Integration of signals of -CH_2OC(O)$\underline{CH_2CH_2}$COOH groups and of -CH_3 end-groups allowed evaluation of the average number of carboxylate groups per one copolymer chain. Comparison of these data with number of -CH_2CH(CH_2OH)- and -C(O)CH(CH_3)OH groups in PLA-*b*-PGly-*b*-PEOX(II) (equal in average 7.75 -CH_2OH groups per one polymer chain) revealed that functionalization was 100 % efficient (Fig. 2).

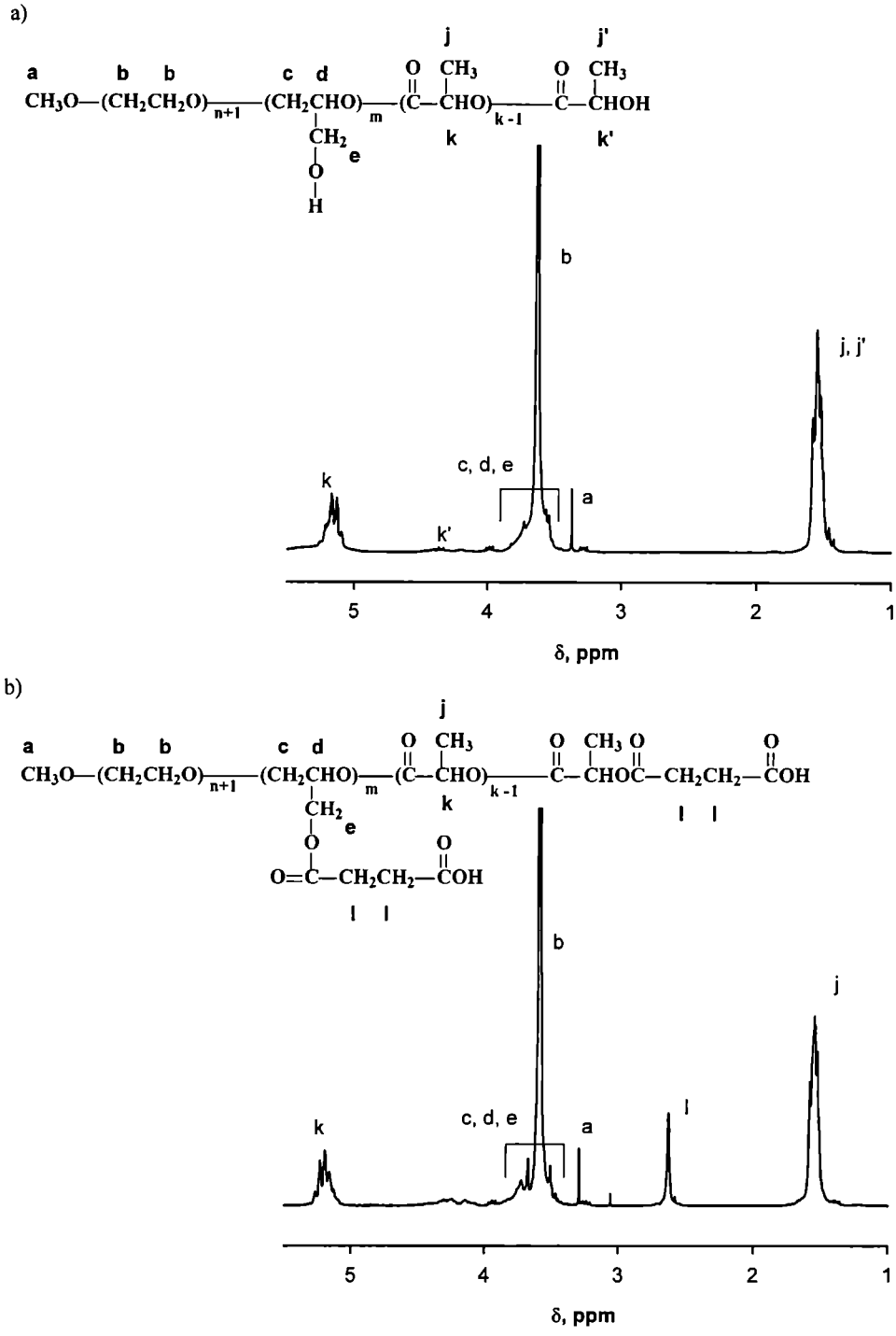


Fig. 2. ^1H NMR spectra of PLA-b-PGly-b-PEOX before (a) and after (b) replacement of -CH_2OH groups with -$CH_2OC(O)CH_2CH_2COOH$ groups

Formation of nanoparticles

Figure 3 shows dependence of the rate of counts on concentration of PLA-*b*-PGly-*b*-PEOX(II). From this dependence one could conclude that intensity of light scattering (manifested by rate of counts) rapidly increases above a certain particular concentration of copolymer (critical aggregation concentration — *CAC*). This critical concentration has similar meaning as critical micelle concentration (*CMC*) observed for amphiphilic low molecular weight compounds.

For all three copolymers *CAC* was determined from intersections of asymptotes to the low and high concentration parts of plots similar to those shown in Fig. 3. Results of these determinations are presented in Table 3.

Figure 4 shows diameter distributions of nanoparticles determined by QELS for monomer concentrations exceeding *CAC* by *ca.* 100 %. Number average diameters

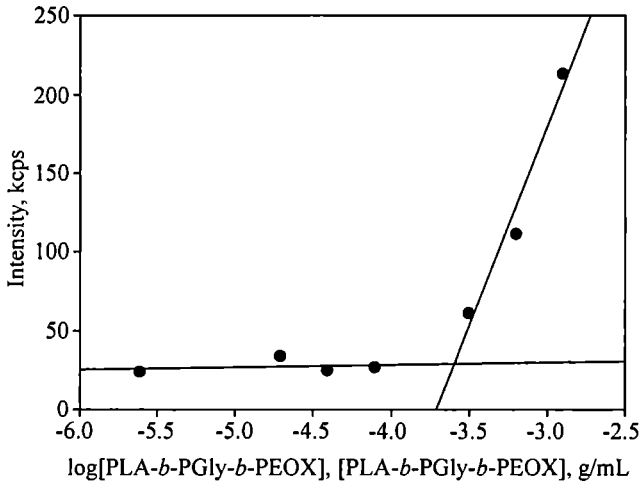


Fig. 3. Dependence of the rate of counts on concentration of PLA-b-PGly-b-PEOX(II)

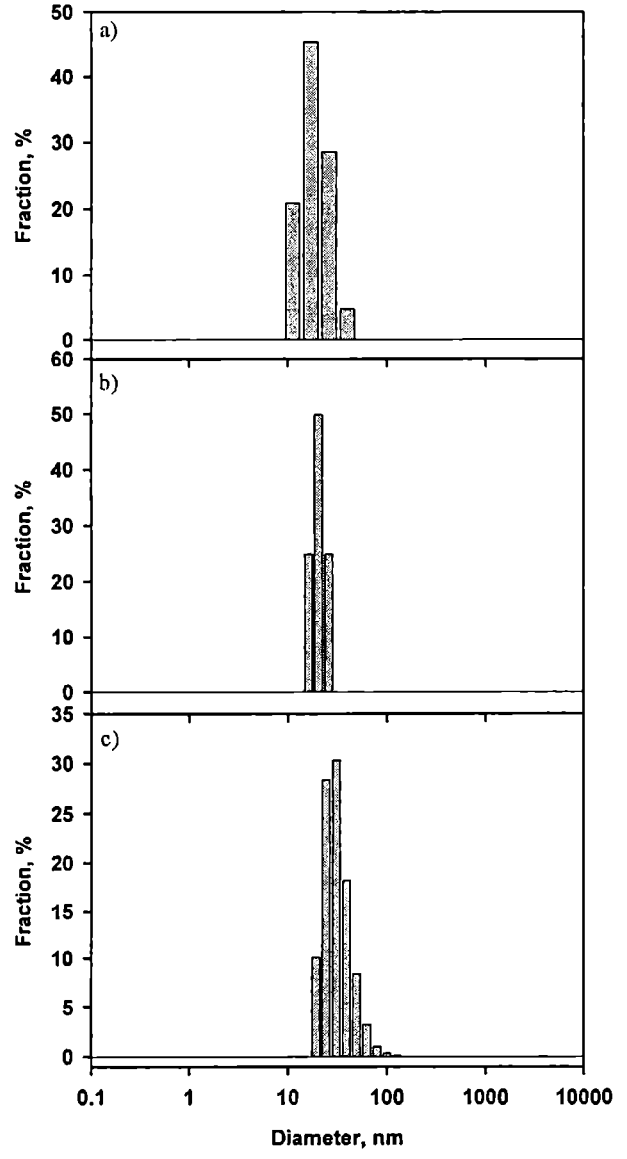


Fig. 4. Diameter distributions of nanoparticles from PLA-b-PGly-b-PEOX(I) — (a), PLA-b-PGly-b-PEOX(II) — (b) and PLA-b-PGly-b-PEOX(IIc) — (c) copolymers

of nanoparticles determined from the described above dependencies are also shown in Table 3.

Table 3. Critical aggregation concentration (*CAC*) and diameters of nanoparticles ($\overline{D}_n$) formed by self-assembly of PLA-*b*-PGly-*b*-PEOX terpolymers

Terpolymer	CAC, g/L	$\overline{D}_n$, nm
PLA-*b*-PGly-*b*-PEOX(I)	$1.55 \cdot 10^{-2}$	20.0±0.8
PLA-*b*-PGly-*b*-PEOX(II)	$7.0 \cdot 10^{-2}$	20.9±0.3
PLA-*b*-PGly-*b*-PEOX(IIc)	$2.51 \cdot 10^{-1}$	33±1

Data in Figs. 3 and 4 and in Table 3 indicate that for PLA-*b*-PGly-*b*-PEOX(I) copolymer with larger hydrophobic block *CAC* is significantly lower than for PLA-*b*-PGly-*b*-PEOX(II) with similar molecular weight of PEOX block but with lower molecular weight of PLA block. It is worth noting, however, that $\overline{D}_n$ for nanoparticles obtained from these copolymers is very close (see Table 3). Apparently, diameters of nanoparticles depend to the greater extent on the length of shell forming hydrophilic PEOX block than on the length of the hydrophobic PLA packed in the core.

Very interesting is the result of introduction of carboxyl groups into PGly blocks. Evidently, the hydrophilic carboxyl groups not only effectively destabilize nanoparticles [*CMC* for PLA-*b*-PGly-*b*-PEOX(IIc) equal $2.51 \cdot 10^{-1}$ g/L is much larger than for the parent PLA-*b*-PGly-*b*-PEOX(II) copolymer ($7.0 \cdot 10^{-2}$ g/L)] but larger is also diameter of these nanoparticles [$\overline{D}_n$ = 33±1 and 20.9±0.3 nm for PLA-*b*-PGly-*b*-PEOX(IIc) and PLA-*b*-PGly-*b*-PEOX(II), respectively].

Encapsulation of pyrene into PLA-*b*-PGly-*b*-PEOX nanoparticles

Pyrene is a convenient model compound for studies of encapsulation into hydrophobic nanoparticles suspended in water media. Pyrene is only slightly soluble in water (at concentrations *ca.* 10^{-7} mol/L). Thus, encapsulation of a compound present in water at such low concentration can be used as a measure of effectiveness of the matrix. Since pyrene is a strongly fluorescent compound its presence can be easily monitored by fluorescence spectroscopy. Moreover, pyrene is particularly attractive as a model because its emission spectra depend on polarity of local environment and thus are different for pyrene in water and in nonpolar (usually hydrophobic) media [10—14]. For pyrene emission the ratio of the intensity of (0,0) band (I_1) to the intensity of (0,2) band (I_3) significantly decreases when pyrene is transferred from a polar to nonpolar environment.

An example of the emission spectrum of pyrene/PLA-*b*-PGly-*b*-PEOX(I) system is shown in Fig. 5.

In a set of experiments with constant concentration of pyrene ([Pyrene] = $4.9 \cdot 10^{-7}$ mol/L) and varied concen-

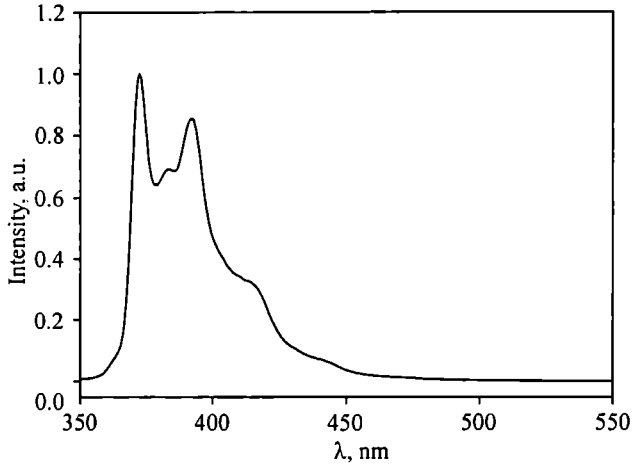


Fig. 5. Emission spectrum of pyrene/PLA-b-PGly-b-PEOX(I) system in 1,4-dioxane:water solution (10 % of 1,4-dioxane v/v); [PLA-b-PGly-b-PEOX(I)] = 1.15 · 10^{-1} g/L, [Pyrene] = 4.94 · 10^{-7} mol/l

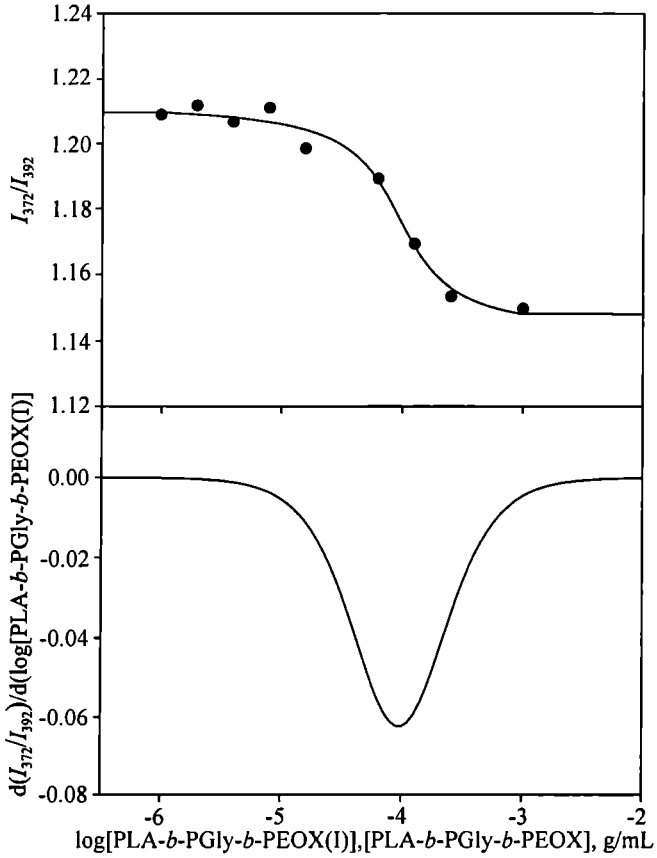


Fig. 6. Ratio of emission intensities (I_{372}/I_{392}) in pyrene spectra as a function of concentration of PLA-b-PGly-b-PEOX(I); [Pyrene] = 4.94 · 10^{-7} mol/L

tration of terpolymer we noticed a gradual change of the ratio I_{372}/I_{392} (where I_{372} and I_{392} are I_1 and I_3 bands in emission spectrum of pyrene). The relevant plot is shown in Fig. 6. In Figure 6 there is given also a plot of $d(I_{372}/I_{392})d(\log[PLA\text{-}b\text{-}PGly\text{-}b\text{-}PEOX(I)]$ the minimum on which indicated polymer concentration at which efficiency of encapsulation changes most rapidly.

The plot in Fig. 6 indicates that for PLA-*b*-PGly-*b*-PEOX(I) concentration below 10^{-6} g/mL all pyrene is in solution (its environment does not change upon further dilution) whereas for polymer concentration above 10^{-3} g/mL all pyrene is encapsulated into nanoparticles (increased polymer concentration does not change pyrene

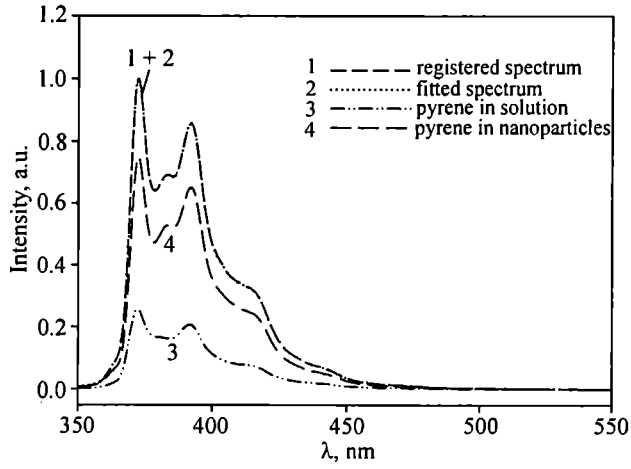


Fig. 7. Emission spectrum of pyrene/PLA-b-PGly-b-PEOX(I) system ([PLA-b-PGly-b-PEOX(I)] = 1.15 · 10^{-1} g/L, [Pyrene] = 4.94 · 10^{-7} mol/L) in 1,4-dioxane:water solution (10 % of 1,4-dioxane v/v), contributing spectra of pyrene in solution and encapsulated into nanoparticles and a fitted one (sum of contributing spectra)

environment into the more hydrophobic one). Thus, the emission spectra of pyrene registered at these concentrations could be assigned to pyrene in solution and in nanoparticles, respectively.

Table 4. Fraction of pyrene in solution (1,4-dioxane:water solution, 10 % of 1,4-dioxane v/v) and in nanoparticles; total concentration of pyrene 4.9 · 10^{-7} mol/L

[PLA-*b*-PGly-*b*-PEOX(I)], g/L	Fraction of pyrene in solution, %	Fraction of pyrene in nanoparticles, %
2.44 · 10^{-4}	89.5	10.5
9.76 · 10^{-4}	85.5	14.5
1.95 · 10^{-3}	90.0	10.0
3.90 · 10^{-3}	86.4	13.6
7.80 · 10^{-3}	62.8	37.2
1.56 · 10^{-2}	56.4	43.6
3.12 · 10^{-2}	25.6	74.4
1.25 · 10^{-1}	13.4	86.6
2.50 · 10^{-1}	0	100
5.00 · 10^{-1}	0	100
1.00	0	100
2.00	0	100
4.00	0	100

Spectra of pyrene in 1,4-dioxane:water solution (10 % of 1,4-dioxane v/v) and in nanoparticles were used as reference for deconvolution of spectra registered at intermediate polymer concentrations (10^{-6} g/mL < [PLA-*b*-PGly-*b*-PEOX(I)] < 10^{-3} g/mL) and for determination of fractions of encapsulated pyrene in each sample. An example of registered spectrum, contributing spectra of pyrene in solution and in nanoparticles, and a fitting calculated as a sum of the last two is shown in Fig. 7. From Figure 7 one could see that the fitting is almost perfect.

Data in Table 4 indicate that for concentrations of PLA-*b*-PGly-*b*-PEOX(I) *ca.* 10 times above *CAC* (*CAC* = $1.55 \cdot 10^{-2}$ g/L) the whole amount of pyrene is in nanoparticles.

Kinetics of release of pyrene from PLA-*b*-PGly-*b*-PEOX nanoparticles

Points in the plot in Fig. 8 could not be fitted with monoexponential decay function, however, the biexponential decay gave an excellent fitting.

$$[Py] = [Py]_0\{A\exp(-k_f t) + B\exp(-k_s t)\} \quad (1)$$

where: *[Py] and $[Py]_0$ — actual and initial concentration of pyrene encapsulated in nanoparticles, A and B — fractions of pyrene released in "fast" and "slow" processes, respectively, k_f and k_s — "fast" and "slow" release rate constants, t — time.*

It is worthy noting that similar (*i.e.* biexponential) release was observed also for nanoparticles from PLA-*b*-PGly-*b*-PEOX(I) and for nanoparticles from copolymer with carboxyl groups in polyglycidol block [PLA-*b*-PGly-*b*-PEOX(IIc)]. The "fast" rate constant was tentatively assigned to the release from shell of nanoparticles that are rich in swollen with water polyether (PGly and PEOX) segments whereas the "slow" was assigned to the release from dense cores made almost exclusively from PLA (Table 5). Data in Table 5 suggest that the "slow" release rate constants are the same for all investigated nanoparticles. Apparently, the release from the densely packed core shows the same rate regardless of the shell structure. The "fast" release rate constants are higher for nanoparticles made of copolymers with longer PEOX chains and introduction of carboxyl groups into PGly segments makes the "fast" release rate constant even higher. The release of 90 % of encapsulated pyrene requires *ca.* two weeks.

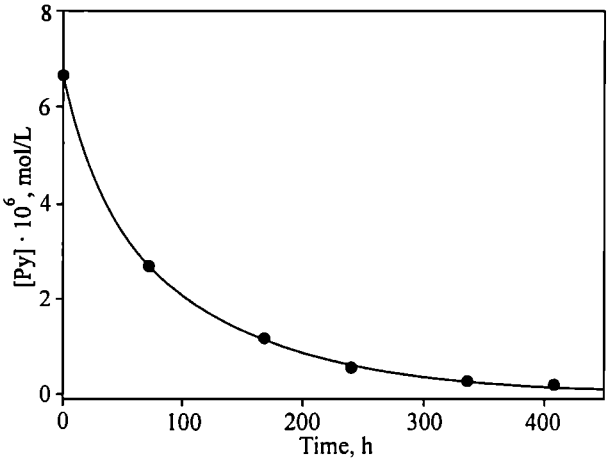


Fig. 8. Kinetic plot of pyrene release from PLA-b-PGly-b-PEOX(II) nanoparticles; $[Py]_0 = 6.63 \cdot 10^{-6}$ mol/L (concentration averaged over the whole volume of liquid containing nanoparticles)

Table 5. Rate constants for "fast" (k_f) and "slow" (k_s) release of pyrene from nanoparticles and fractions of pyrene released by "fast" (*A*) and "slow" (*B*) processes

Nanoparticles	$[Py]_0$ mol/L	*A*	k_f, L/h	*B*	k_s, L/h
PLA-*b*-PGly-*b*-PEOX(I)	$9.60 \cdot 10^{-6}$	0.310	$9.53 \cdot 10^{-2}$	0.690	$8.20 \cdot 10^{-3}$
PLA-*b*-PGly-*b*-PEOX(II)	$6.63 \cdot 10^{-6}$	0.269	$3.76 \cdot 10^{-2}$	0.731	$8.66 \cdot 10^{-3}$
PLA-*b*-PGly-*b*-PEOX(IIc)	$6.54 \cdot 10^{-6}$	0.327	$1.48 \cdot 10^{-1}$	0.673	$8.46 \cdot 10^{-3}$

CONCLUSIONS

Poly(L-lactide)-*b*-polyglycidol-*b*-poly(ethylene oxide) copolymer molecules and copolymer molecules with -CH_2OH groups of polyglycidol monomeric units replaced with -$CH_2OC(O)CH_2CH_2COOH$ groups self assemble in water into nanoparticles. Diameters of nanoparticles made of copolymers with carboxyl groups are significantly larger than diameters of nanoparticles of parent copolymers with -CH_2OH groups. Pyrene present in solution is incorporated into nanoparticles during their formation. For pyrene concentration in 1,4-dioxane:water mixture (10 % of 1,4-dioxane, v/v) [Pyrene] = $4.9 \cdot 10^{-7}$ mol/L the whole amount of pyrene is incorporated into nanoparticles when concentration of terpolymer exceeds *ca.* 10 times the relevant critical aggregation concentration. The release kinetics could be fitted with biexponential functions. The "slow" process, assigned to release from core of nanoparticles, was essentially the same for all investigated nanoparticles. The "fast" one, from shells, was higher for nanoparticles made of copolymers with longer poly(ethylene oxide) blocks and for polyglycidol blocks in which -CH_2OH groups were replaced with -$CH_2OC(O)CH_2CH_2COOH$ groups.

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