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Biodegradable Nano- and Microparticles with Controlled Surface Properties

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Summary: Methods suitable for formation of polyester nano- and microspheres with controlled surface properties are described. The title nano- and microspheres composed of polylactides or poly(ε-caprolactone) were obtained either directly, during dispersion polymerization of corresponding monomers, or by self-assembly of block copolymers containing polyester blocks. Surface properties of the particles were controlled by using surfactants with required functional groups for dispersion polymerization, by modification of interfacial layer of synthesized particles, and by using for self-assembly copolymers with appropriate functional groups. Particles with alkyl, amino, hydroxyl, carboxyl, and 4-(phenyl-azo)phenyl groups in interfacial layer were obtained. Stability of suspensions of particles and particle's integrity did depend on the nature of functional groups in their interfacial layer and on external stimuli such as pH of suspending medium and irradiation with UV light.

Keywords: microspheres; nanospheres; polyesters; surface properties

Introduction

Nano- and microspheres (bio)degradable to biocompatible products that could be removed from an organism via natural pathways are important candidates for drug delivery.[1,2] It is desirable to design particles that also would be able to reach the required tissue, cells and in some instances even precisely determined intercellular compartments, not destroyed by defense mechanisms of an organism. Such particles should have properly tailored surface properties.

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Various strategies could be used for production of nano- and microspheres with required chemical groups in their surface layers. The first one is based on synthesis of copolymers with (bio)degradable blocks and blocks containing monomeric units with needed functionalities and on self-assembly of the copolymers into nanoparticles. The second one consists of synthesis of particles carried on in presence of molecules equipped with appropriate functional groups, molecules that are incorporated predominantly into surface layer of particles during synthesis. The third one is based on chemical modification of polymer macromolecules in interfacial layer of preformed particles. In the paper we discuss application of the mentioned above strategies for production of polyester-containing (polylactide and poly(ε-caprolactone)) nano- and microparticles with hydrophobic, ionic (cationic and anionic) groups in their surfaces or groups changing properties of particles in result of irradiation with UV light.

Experimental

Materials. Monomers: L-lactide (L-Lc), ε-caprolactone (CL), and ethylene oxide (EO) (Aldrich) were purified and stored as it was described earlier.[3,4] 1-Ethoxyethylglycidyl ether (EEGly) was synthesized and purified according to the known procedure.[5] Dodecyl acrylate (DA) was purified from a stabilizer by distillation. n-Octadecyl methacrylate (ODMA) (Polysciences Inc.) was purified by dissolution in hexane and extraction four times with 5% aqueous NaOH. After drying the organic phase over magnesium sulfate, the solution was passed through neutral alumina and solvent was removed under reduced pressure. Dimethylaminoethyl methacrylate (DMAEMA), (Aldrich) was passed through a column filled with neutral alumina, just before use. Solvents: 1,4-Dioxane, THF and heptane (Aldrich) used for syntheses were first purified by distillation and dried over Na wires according to standard procedures and then dried over Na-K alloy. Toluene was distilled from sodium benzophenone ketyl solution and stored over molecular sieves. Initiators: Diethylaluminum ethoxide, tin(II) 2-ethylhexanoate and α,α'-azoisobutyronitrile (Aldrich) were used as received. Other initiators and components of initiating systems, like potassium 2-methoxyethanolate, hydroxyethyl 2-bromoisobutyrate, dinonylbipyridine (DNBIPY) were synthesized from the readily available precursors. Copper(I) chloride

(CuCl)(Acros) was washed with glacial acetic acid in order to remove any soluble oxidized species, filtered, washed with ethanol and dried.
4-(Phenylazo) benzoic acid (Aldrich) and its acid chloride derivative for polymer labeling were used as received.

Analytical techniques. Polymers were characterized by ^{1}H NMR using a Bruker AC 200 spectrometer and GPC (in CH_2Cl_2 or THF). GPC analyses were performed either by using a system combining an LKB 2150 pump (LKB, Sweden), TSK-Gel G4000XHL and G2000XHL columns (TOSOHAAS, Japan), a Dawn F MALS detector (Wyatt), and an Optilab 903 Interferometric Refractometer (Wyatt) (dichloromethane eluent, flow rate of 0.8 mL /min) or a system with Waters 515 pump, PSS columns (Styrogel 10^5, 10^3, 10^2 Å) and Waters 410 differential refractometer (THF eluent flow rate of 1 mL/min). In the latter case linear poly(methyl methacrylate) standards were used for calibration. Absorption spectra were registered using a Hewlett Packard 8452A diode array spectrometer. SEM pictures were recorded using a JEOL 5500LV scanning electron microscope. Size of nanoparticles was determined by quasielastic light scattering method using a ZetaSizer 3000HSa (Malvern). Concentration of nanoparticles was related to the number of counts per second during determination of particle size with ZetaSizer 3000HSa.

Results and Discussion

Block copolymers stabilizing poly(L-lactide) and poly(ε-caprolactone) microspheres in suspension during synthesis and used as building blocks suitable for self-assembly into nanoparticles.

Dispersion (pseudo)anionic polymerizations of L-Lc and CL, leading to polyester microspheres, that were developed in our laboratory,[3,6-8] must be carried on in aprotic solvents. For these polymerizations the custom designed surfactants were needed. Suitable surfactants contain block(s) or grafts (e.g. polyCL) promoting adsorption of surface active molecules at surface of growing polyester particles and blocks soluble in reaction medium.

Synthesis of poly(dodecyl acrylate)-*g*-poly(ε-caprolactone) (poly(DA)-*g*-poly(CL)) graft copolymers

Syntheses of poly(DA)-*g*-poly(CL) copolymers were described in details in our earlier papers.[3,6] Here we quote a recipe for a typical two-step synthesis. The first step consists on functionalization of hydroxyl-terminated poly(CL) with methacryloyl function. For example, poly(CL) (4.7 g, M_n = 3090) and methacryloyl chloride (0.785 g) reacted in 50 ml of toluene in presence of triethylamine (0.4 g). After 48 h triethylaminehydrochloride was filtered off and poly(CL) methacrylate was precipitated into cold methanol. Poly(CL) methacrylate (2.5 g, M_n = 3090), dodecyl acrylate (10 g), AIBN (0.135 g) and toluene (15 ml) were placed into an ampoule. Polymerization was carried on at 70 °C for 72 h. Poly(DA)-*g*-poly(CL) with M_n = 49000 and with ca 4.1 poly(CL) grafts per one copolymer macromolecule was obtained. In similar manner there were synthesized poly(DA)-*g*-poly(CL) copolymers with M_n from 20000 to 55000 and with M_n of poly(CL) grafts from 2000 to 10000.

Synthesis of poly(ε-caprolactone)-*b*-poly(octadecyl methacrylate)-*b*-dimethyl-aminoetyl methacrylate (poly(CL)-*b*-poly(ODMA)-*b*-poly(DMAEMA)) and poly(ε-caprolactone)-*b*-poly(octadecyl methacrylate-*co*-dimethyloaminoethyl methacrylate) (poly(CL)-*b*-poly(ODMA-co-DMAEMA)) copolymers

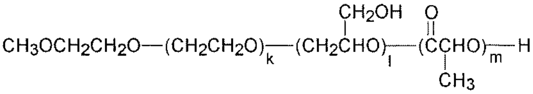
Detailed report on syntheses of copolymers of ε-caprolactone, dimethyloaminoethyl methacrylate and octadecyl methacrylate are given in a paper fully devoted to this subject.[9] Here there is given only a short description of synthesis of copolymers that were used as surfactants in dispersion polymerization leading to poly(L-lactide) (poly(L-Lc)) microspheres.

Poly(CL)-*b*-poly(ODMA-*co*-DMAEMA) was synthesized as follows. Poly(CL) block was synthesized in polymerization of CL initiated with hydroxyethyl 2-bromoisobutyrate/ tin(II) hexanoate system. CL (10.3 g), hydroxyethyl 2-bromoisobutyrate (0.38 g), tin(II) hexanoate (0.01 g) and 10 ml of dry toluene were placed in an ampoule under nitrogen. Polymerization was carried on at 90 °C for 22 h. Poly(CL) with M_n = 8300 (GPC, based on polystyrene standards) and M_w/M_n = 1.22 has been used as macroinitiator for subsequent ATRP polymerization of ODMA and DMAEMA.[10-12] Poly(CL) (1.1 g), DMAEMA (0.39 g), ODMA (1.80 g), $CuCl_2$ (1 mg), CuCl (13 mg), DNBIPY (108 mg) and diphenyl ether (50 ml) were heated to 70 °C for 17 h. Obtained copolymer (Mn = 21900, Mw/Mn = 1.15 (GPC, based on polystyrene standards) did contain 35, 14 and 51 mol % of poly(CL), poly(DMAEMA) and poly(ODMA) monomeric units, respectively (fractions of copolymers based on ^{1}H NMR).

Poly(CL)-*b*-poly(ODMA)-*b*-poly(DMAEMA) was synthesized similarly as poly(CL)-*b*-poly(ODMA-*co*-DMAEMA), but comonomers were added to 2-bromoisobutyrate bearing poly(CL) consecutively. Copolymer with Mn = 23100, Mw/Mn = 1.15 and molar fractions of poly(CL), poly(ODMA) and poly(DMAEMA) units equal 43.8, 31.6 and 24.6% was obtained.

Synthesis of poly(L-lactide)-b-poly(glycidol)-b-poly(ethylene oxide) (poly(L-Lc)-b-poly(Gly)-b-poly(EO)) copolymers

Poly(L-Lc)-*b*-poly(Gly)-*b*-poly(EO) copolymers with a general structure



$$\mathrm{CH_3OCH_2CH_2O{-}(CH_2CH_2O)_k{-}(CH_2\overset{\overset{CH_2OH}{|}}{C}HO)_l{-}(\overset{\overset{O}{\|}}{C}\underset{\underset{CH_3}{|}}{C}HO)_m{-}H}$$

Scheme 1. Poly(L-Lc)-b-poly(Gly)-b-poly(EO) block copolymer.

were synthesized by anionic sequential polymerization of EO, EEGly (glycidol with hydroxyl groups blocked with 1-ethoxyethyl) and L-Lc, followed by deblocking the hydroxyl groups in poly(EEGly) blocks at acidic conditions, according to the procedure known from the literature.[4] Copolymers with various length of blocks have been synthesized. An example of a recipe is given below. First poly(EO) was synthesized by initiating polymerization of EO (6.4 g) with potassium 2-methoxyethanolate (0.4 g) in dry THF solution (43 ml). Polymerization was carried on at 37 °C for 72 h. Then, EEGly (1.8 g) was added to the mixture and polymerization was carried on at 37 °C for another 72 h. Thereafter, a solution of 10.5 g of L-Lc in 23 ml of dry THF was added to a living copolymer and polymerization was continued for 12 h at room temperature. Yield of terpolymer 87 %. Hydroxyl groups in poly(EEGly) blocks were deprotected with oxalic acid (10 wt/v % in acetone:water 1:1 v/v/ mixture). Deprotection was carried on for 90 min at 37 °C. Copolymers with Mn from 6000 to 24000 with Mn of poly(L-Lc) in the range from 2400 to 12000, Mn of poly(EO) from 1700 to 11000 and Mn of poly(Gly) 770 ± 30 were obtained by varying comonomers and initiator ratios. Mn of blocks was determined from ^{1}H NMR spectra by end-group (**CH_3**-OCH_2CH_2O-, 3.38, s) analysis.

Poly(L-Lc)-*b*-poly(Gly)-*b*-poly(EO) derivatives with carboxyl groups

The title copolymers were synthesized in reaction of poly(L-Lc)-*b*-poly(Gly)-*b*-poly(EO) with succinic anhydride.

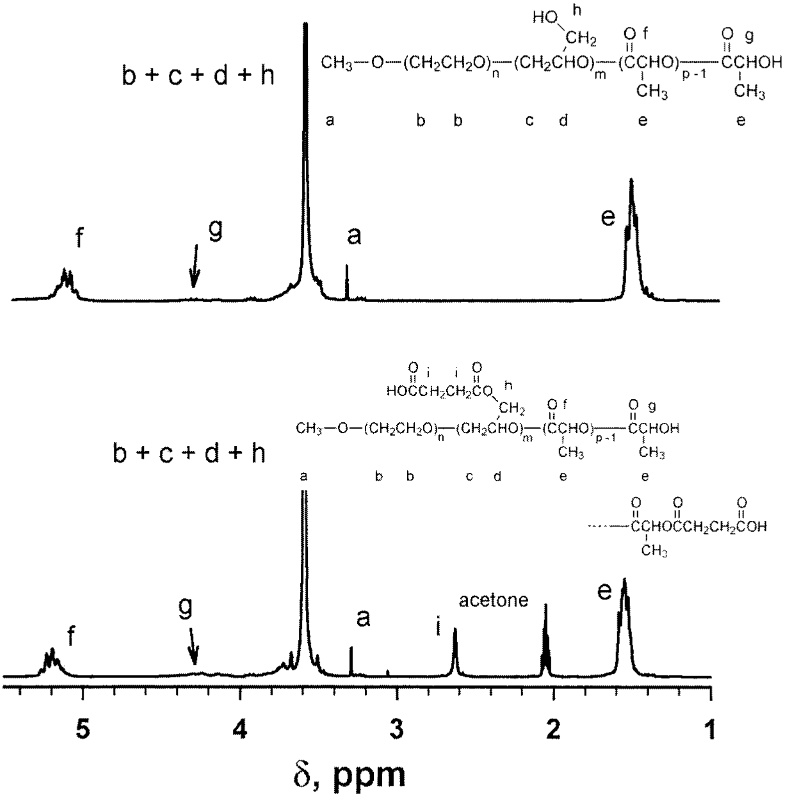


Figure 1. ^{1}H NMR spectra of copolymer with carboxyl groups and parent poly(L-Lc)-*b*-poly(Gly)-*b*-poly(EO). DP$_n$ of poly(L-Lc), poly(Gly) and poly(EO) blocks equals 51.3, 4.05 and 62.5, respectively.

Reaction was carried on in acetone:pyridine (5:1, v/v) solution. Succinic anhydride was used in 5-fold excess with respect to hydroxyl groups. ^{1}H NMR spectrum revealed that 100% of hydroxyl groups in poly(Gly) block did react with the anhydride. Obviously, one could expect that also terminal $-C(CH_3)OH$ groups will react with succinic anhydride. However, since this signal was very weak, we could not conclude whether functionalization of end groups was quantitative.

Poly(L-Lc)-*b*-poly(Gly)-*b*-poly(EO) derivatives with 4-(phenyl-azo)phenyl labels

Derivatives of poly(L-Lc)-*b*-poly(Gly)-*b*-poly(EO) labeled with 4-(phenyl-azo)phenyl moieties were obtained by reacting poly(L-Lc)-*b*-poly(Gly)-*b*-poly(EO) with 4-(phenylazo) benzoic acid or with its acid chloride derivative.

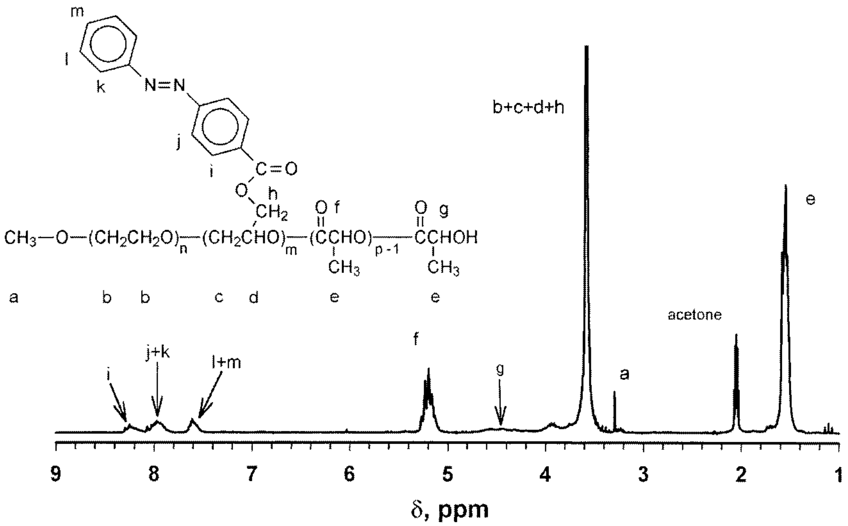


Figure 2. ^{1}H NMR spectrum of poly(L-Lc)-*b*-poly(Gly)-*b*-poly(EO) copolymer with poly(Gly) block labeled with 4-(phenyl-azo)phenyl labels. DP$_n$ of poly(L-Lc), poly(Gly) and poly(EO) blocks equals 28.4, 5.4 and 70.4 respectively.

For example, poly(L-Lc)-*b*-poly(Gly)-*b*-poly(EO) (0.5 g, with Mn = 18600 and in average 6 –OH groups per macromolecule), 4-(phenylazo) benzoic acid (0.102 g), dimethylaminopyridine (6 mg) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (96 mg) were dissolved in 16 ml of CH_2Cl_2. Reaction was carried at room temperature, in dark, for 12 h. Functionalized polymer was purified by precipitation into ethyl ether and dialysis from dioxane solution against water. UV and ^{1}H NMR indicated that in average each macromolecule was substituted with 4.1 4-(phenyl-azo)phenyl groups (some end groups could be functionalized as well however, week signal of these groups did not allow for quantitative determination of end group substitution).

Nanoparticles by self assembly of poly(CL)-*b*-poly(ODMA-*co*-DMAEMA), poly(CL)-*b*-poly(ODMA)-*b*-poly(DMAEMA), poly(L-Lc)-*b*-poly(Gly)-*b*-poly(EO), and their derivatives with carboxyl and 4-(phenylazo)phenyl groups.

Nanoparticles form the title copolymers were obtained by equilibration of copolymers in media that were bad solvents for polyester blocks (e.g. water or heptane:1,4-dioxane (4:1 v/v) mixture) and good solvents for other blocks (e.g. water for poly(EO) and poly(Gly) and heptane:1,4-dioxane for poly(ODMA) and poly(DMAEMA)) or by dialysis of copolymer solutions against these solvents. Polyesters formed cores of particles and other segments formed stabilizing shells.

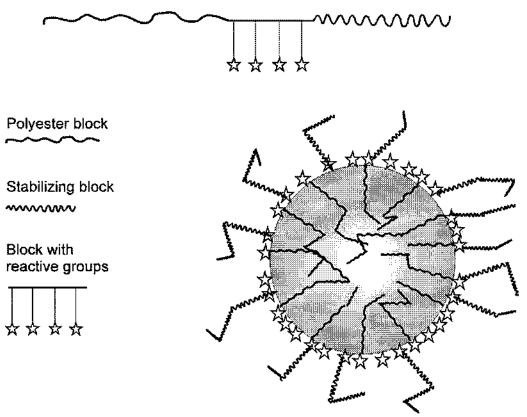


Figure 3. Scheme illustrating self-assembly of block copolymers into nanoparticles with reactive groups at interface.

Critical micelle concentration concentrations (CMC) and diameters of nanopartciles obtained from selected copolymers in the way described above are given in Table 1.

Table 1. Critical micelle concentration concentrations (CMC) and diameters of nanoparticles (D_n) obtained from various copolymers.

Copolymer			
Type of copolymer Proportion of monomeric units (in moles) Suspending medium	M_n	CMC g/l	D_n nm
Poly(CL)-*b*-poly(ODMA-*co*-DMAEMA) 3.3:1.0:2.6; heptane :1,4-dioxane 4:1	21 900	0.10	96 ± 5
Poly(CL)-*b*-poly(ODMA)-*b*-poly(DMAEMA) 1.8:1.3:1.0; heptane :1,4-dioxane 4:1	23 100	< 0.1	1700 ± 360
Poly(Lc)-*b*-poly(Gly)-*b*-poly(EO) 6.3:1.0:30.6 water	9 400	0.07	21 ± 3
Poly(Lc)-*b*-poly(Gly)-*b*-poly(EO) with carboxyl groups 6.3:1.0:30.6 water	10 300	0.25	35 ± 4
Poly(L-Lc)-*b*-poly(Gly)-*b*-poly(EO) with 4-(phenylazo)phenyl labels 7.8:1.0:10 water	6 000	0.07	41 ± 7

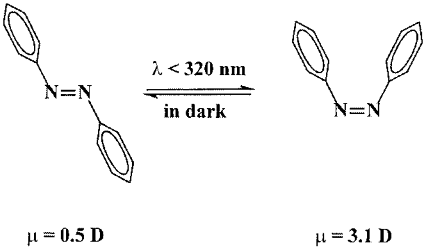
CMC is a copolymer concentration above which polymeric micelles (polymer nanoparticles) starts to form. When comparing surface active copolymers we will considered these with lower CMC to form more stable nanoparticles since they do not decompose (dissolve) at lower copolymer concentration.

It is worth noting that for poly(CL)-b-poly(ODMA)-b-poly(DMAEMA) CMC was much lower and diameters of formed particles were much larger than for that for poly(CL)-b-poly(ODMA-*co*-DMAEMA) with similar molecular weight of copolymer macromolecules but with higher fraction of poly (DMAEMA) and poly(ODMA) monomeric units (molar fraction of poly (DMAEMA) and poly(ODMA) monomeric units in poly(CL)-b-poly(ODMA)-b-poly(DMAEMA) was equal 56.5 % and in poly(CL)-b- poly(ODMA-*co*-DMAEMA) was equal 66%).

Comparison of CMC and D_n for a sample of poly(L-Lc)-*b*-poly(Gly)-*b*-poly(EO) and its derivative obtained by substitution of –OH groups in poly(Gly) block with hydrophilic carboxyl groups revealed that after substitution nanoparticles are less stable (higher CMC) and have larger diameters. For example, for poly(L-Lc)-*b*-poly(Gly)-*b*-poly(EO) (with M_n = 9400 and DP_n of poly(L-Lc), poly(Gly) and poly(EO) equal 25.7, 4.1 and 125.6, respectively) CMC = 0.07 g/L and D_n = 21 ± 3 nm, whereas and its derivative with carboxyl groups CMC = 0.25 g/L and D_n = 35 ± 4 nm.

There are reports on using Ca^{+2} cations as chelating agents crosslinking polymers with anionic groups.[13] We noticed that in the case of nanoparticles from poly(L-Lc)-*b*-poly(Gly)-*b*-poly(EO) (with poly(Gly) segments bearing $-COO^-$ groups) an addition of of Ca^{+2} cations (in concentration $[Ca^{+2}] = 2[-COO^-]$) leads to complete incorporation of cations into nanoparticles. In effect, the nanoparticles are converted from the negatively charged to the neutral ones. After attachment of Ca^{+2} cations the diameters of nanoparticles did not change significantly. For example, for copolymer (M_n = 7400 and DP_n of poly(L-Lc), poly(Gly) and poly(EO) equal 26.3, 5.6 and 63.6, respectively; poly(Gly) segments bearing $-COO^-$ groups) without and with incorporated Ca^{+2} cations the diameters of nanoparticles were equal 44 ± 3 and 47 ± 3 nm, respectively.

It is well known that irradiation of 4-(phenylazo)phenyl groups with light at $\lambda < 325$ nm results in *trans-cis* transition accompanied with change in the polarity of this group and that polymers with labels in *cis* position are more easily soluble in water.[14,15]



Scheme 2. *Cis-trans* isomerization of azobenzene induced by UV irradiation and dipole moments of isomers.

Therefore, one may expect that after irradiation with UV light poly(L-Lc)-*b*-poly(Gly)-*b*-poly(EO) copolymers labeled with 4-(phenyl-azo)phenyl labels will be better soluble in water. Better solubility of irradiated copolymers should manifest itself by higher CMC (i.e., by lower stability of nanoparticles) and therefore their CMC will be higher (this means that stability of nanoparticles formed by self-assembly of irradiated copolymers will be lower).

Values of CMC for poly(L-Lc)-*b*-poly(Gly)-*b*-poly(EO) copolymers (with M_n = 18130 and DP_n of poly(L-Lc), poly(Gly) and poly(EO) equal 68.4, 4.4 and 179.3, respectively) and for its derivative labeled with 4-(phenyl-azo)phenyl labels (before and after irradiation with UV light within the wavelength window 300 nm < λ < 420 nm) are given in Table 2

Table 2. CMC for poly(L-Lc)-*b*-poly(Gly)-*b*-poly(EO) and its derivative with 4-(phenyl-azo)phenyl labels.

Poly(L-Lc)-*b*-poly(Gly)-*b*-poly(EO) Poly(L-Lc) DP_n = 68.4; poly(Gly) DP_n = 4.4; poly(EO) DP_n = 179.3		CMC G/L
Before labeling		8.0 x 10^{-3}
After labeling with azobenzene labels	Before irradiation	6.4 x 10^{-3}
	After irradiation (300 nm < λ < 420 nm) for 20 min	2.2 x 10^{-2}

Indeed, whereas labeling of investigated copolymer with 4-(phenyl-azo)phenyl labels did not lead to significant change of CMC of copolymer the irradiation with UV light resulted in higher value of CMC (lower stability of formed nanoparticles).

Microspheres with amino groups in the surface layer obtained directly by polymerization.

Polymerization of lactides in hydrocarbon rich media yields a shapeless polymer precipitate. The same reaction carried on in presence of poly(CL)-*b*-poly(ODMA-*co*-DMAEMA) or poly(CL)-*b*-poly(ODMA-*co*-DMAEMA) copolymers gave polymer in form of microspheres. Recipe for synthesis of microspheres is given below. Monomer (L-Lc, 2.25 g) and initiator (tin(II) 2-ethylhexanoate; 0.35 g) were placed in a reactor containing heptane:1,4-dioxane mixture (4:1 v/v; 50 ml) with dissolved surface active copolymer (poly(CL)-*b*-poly(ODMA-*b*-DMAEMA) or poly(CL)-*b*-poly(ODMA-*co*-DMAEMA), 90 mg). Air was removed from the solution by bubbling argon through it. Thereafter, the solution was heated to boiling. After ca 15 min, the reaction mixture turned milky. Polymerization was carried on in argon atmosphere for 2 h and then was stopped by pouring the polymerizing mixture to cold heptane containing acetic acid (five-fold excess with respect to initiator). Traces of unreacted monomer that precipitated rapidly in form of crystals were removed from the mixture by filtration. Thereafter, the medium suspending poly(L-Lc) microspheres was exchanged to heptane (by sedimentation of microspheres in gravitational force (without centrifugation) and replacement of supernatant with fresh portions of heptane). Yield of purified microspheres (determined gravimetrically) was equal 65%. The reference poly(L-Lc) microspheres were synthesized in a similar way in polymerization with poly(dodecyl acrylate)-*g*-poly(ε-caprolactone) (poly(DA)-*g*-poly(CL)) graft copolymer was used as a surfactant. Microspheres with D_n from 2.1 to 2.7 μm (depending on the chemical structure of copolymer) and $D_w/D_n < 1.3$ were obtained.

Figure 4 shows SEM microphotograph of poly(L-Lc) microspheres obtained in synthesis with poly(CL)-*b*-poly(ODMA)-*b*-(DMAEMA) surfactant.

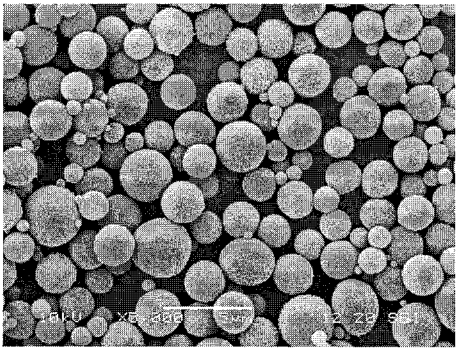


Figure 4. SEM microsphotograph of poly(L-Lc) microspheres obtained in dispersion polymerization of L-Lc carried on in presence of poly(CL)-*b*-poly(ODMA)-*b*-(DMAEMA) surface active copolymer (M_n = 23100, M_w/M_n = 1.15; molar fractions of poly(CL), poly(ODMA) and poly(DMAEMA) units equal 43.8, 31.6 and 24.6%). Number average diameter of microspherers D_n = 2.24 μm, diameter polydispersity factor D_w/D_n = 1.25.

Samples of microspheres synthesized in presence of poly(CL)-*b*-poly(ODMA)-*b*-poly(DMAEMA), poly(CL)-*b*-poly(ODMA-*co*-DMAEMA) and poly(DA)-*g*-poly(CL) surface active copolymers were transfer from heptane to water. Transfer was performed in the following way. First, the suspension of microspheres in heptane (0.5 mL of suspension with 3 wt% solid content) was added to 5 mL of ethanol. When microspheres settled down under gravitational forces the supernatant was removed and a new portion of ethanol was added. This process was repeated three times more. Thereafter, in the same way, ethanol was replaced with water.

Visual observation indicated that the microspheres obtained in polymerizations carried on in presence of poly(DA)-*g*-poly(CL) and poly(CL)-*b*-poly(ODMA-*c*-DMAEMA) strongly aggregated after being transferred to water. On the contrary, observation by optical microscopy revealed that the microspheres synthesized in presence of poly(CL)-*b*-poly(ODMA)-*b*-poly(DMAEMA) did not aggregate in water at pH = 4.0 (pH adjusted by addition of a needed amount of acetic acid). The particles stabilized with poly(CL)-*b*-poly(ODMA)-*b*-poly(DMAEMA) settled down after ca 30 minutes but could be re-suspended again by gentle shaking. Clearly, the poly(DMAEMA) blocks in which amino groups are protonated at low pH provide sufficient ionic and steric stabilization.

Microspheres with carboxyl and hydroxyl groups in the surface layer obtained by polymerization with following hydrolysis of polymer in particle surface layer.

Our studies on direct synthesis of poly(L-Lc) and poly(CL) microspheres with controlled diameters ranging from ca 0.6 to 8 μm have been recently summarized in a review paper.[16] The microspheres were obtained during polymerization carried on in the presence of poly(DA)-*g*-poly(CL) copolymers used as surfactants. The mentioned above hydrophobic surfactants provided excellent steric stabilization of microspheres in hydrocarbons (e.g., hexane and heptane), however, for obvious reasons, were inappropriate for stabilization of microspheres in water. Stabilization of poly(L-Lc) and poly(CL) microspheres in water was achieved by their partial hydrolysis in ethanol solutions of KOH and Triton-405, sodium dodecyl sulfate or ammonium sulfobetaine as surfactants.[17] Hydrolysis yielded particles with interfacial layer rich in –COOH and –OH groups. Maximal surface concentrations of –COOH groups in partially hydrolyzed microspheres ranged from $2.6 \cdot 10^{-7}$ to $7.4 \cdot 10^{-7}$ mol/m^2. Carboxyl groups in interfacial layer of poly(L-Lc) were used for covalent binding of compounds containing amino groups. In this way, fluorescent microspheres labeled with 6-aminoquinoline and Lucifer Yellow CH were obtained.[17]

Conclusions

Biodegradable nanoparticles (with diameters usually from 20 to 100 nm) from poly(L-Lc) and poly(CL), containing functional groups in the surface layer could be obtained by self-assembly of copolymers with polyester blocks and blocks bearing the required functional groups. The stability of nanoparticles was dependent on chemical structure of these groups. Microspheres (particles with diameters above 0.5 μm) with functional groups in the surface layer could be obtained either directly, by dispersion polymerization of cyclic esters carried on in the presence of surfactants containing polyester blocks and blocks with the required chemical groups or by post-treatment (partial hydrolysis) of the earlier synthesized polyester micrsopheres without functional groups. Hydrolysis introduced carboxyl and hydroxyl groups into surface layer of these particles.

Acknowledgement

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