Novel Amphiphilic Poly(ϵ-caprolactone)-*g*-poly(L-lysine) Degradable Copolymers

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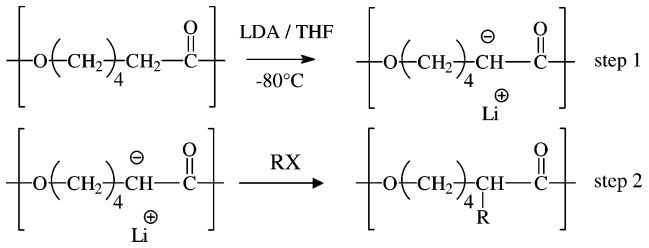
As part of the search of novel degradable polymers, amphiphilic and cationic poly(ϵ-caprolactone)-*g*-poly(L-lysine) (PCL-*g*-PLL) copolymers have been synthesized following a grafting "onto" or a grafting "from" method both applied to a macropolycarbanionic PCL derivative. The first approach led to PCL-*g*-PZLL containing 36% of ϵ-caprolactone and 64% of *N*-ϵ-Z-L-lysine units, by reaction of activated poly(*N*-ϵ-Z-L-lysine) on the macropolycarbanion derived from PCL. The second route was based on the anionic ring opening polymerization of *N*-carboxyanhydride of *N*-ϵ-benzyloxycarbonyl-L-lysine initiated by the macropolycarbanion derived from PCL and led to a similar copolymer containing 45% of of ϵ-caprolactone and 55% of *N*-ϵ-Z-L-lysine units. After deprotection of the lysine units, PCL-*g*-PLL copolymers were obtained. These copolymers are water-soluble and form nanometric micelle-like objects with mean diameters between 60 and 500 nm in distilled water depending on the synthesis route.

Introduction

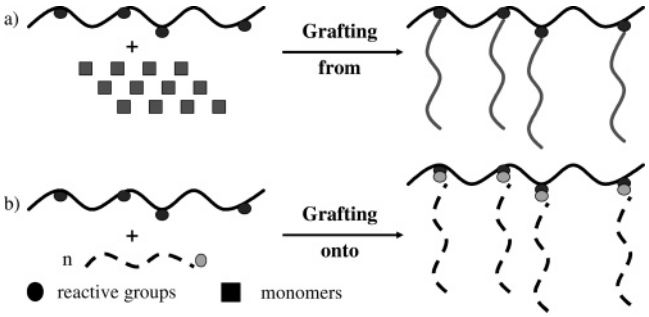
Poly(ϵ-caprolactone) (PCL) is a biocompatible degradable aliphatic polyester of interest for biomedical and pharmaceutical applications which has been proposed to elaborate sustained release drug delivery systems or scaffolds for tissue engineering. However, whereas PCL degrades rapidly under outdoor conditions or *in vitro* in the presence of nonspecific enzymes, its hydrophobicity and the absence of suitable enzymes tend to limit its *in vivo* degradability and consequently limit the use in biomedical and pharmaceutical fields compared to poly(lactic acid). Amphiphilic PCL-based graft copolymers have structures exhibiting original properties as well as a faster degradation of the polyester chain. We recently proposed a simple general method to chemically modify aliphatic polyesters chains, especially PCL.[1] This method is based on the anionic activation of the PCL chain by removal of a proton of the methylene group in α position of the ester carbonyl, using a non nucleophilic base such as lithium diisopropylamide (LDA). In a second step, various electrophilic reagents are added to the macropolycarbanion generated in the first step (Scheme 1). The method was applied to the formation of PCL-based graft copolymers via i) a grafting "from" technique, in which anionic polymerization of methyl methacrylate was initiated by the macropolycarbanion,[2] and (ii) a grafting "onto" technique, in which activated α-methoxy,ω-hydroxy-poly(ethylene oxide) was grafted on the macropolycarbanion[3] (Scheme 2). These grafting techniques with monomers leading to hydrophilic and degradable and/or biocompatible structures give access to novel PCL-based graft copolymers exhibiting an amphiphilic behavior. Among these structures, poly(α-amino acids) (PAA) are interesting candidates to form side chains on the PCL backbone, as they are considered to be biocompatible and biodegradable. Another advantage is the variety of existing α-amino acids that offers a wide range of functionalities and potentially leads to a family of degradable amphiphilic PLC-based copolymers. However, only a few degradable polymers composed of aliphatic polyesters and poly-(α-amino acid) chains have been reported so far. Most of them are block copolymers based on poly(lactic acid) (PLA) hydrophobic segment. PLA-*b*-PAA diblocks copolymers with either neutral, anionic, or cationic PAA segments have been described.[4−8] Some graft copolymers have also been synthesized in which PLA is the hydrophobic side chain. Here again, some neutral and cationic poly(L-lysine) (PLL) copolymers[9−11] are described. Inverse structures, with a hydrophobic PLA backbone and a grafted PAA have been synthesized by Langer et al. who started from a poly(lactic acid-*co*-lysine) copolymer containing 2% of amino-protected lysine[12,13] to obtain nonionic PLA-*g*-poly(alanine) (PLA-*g*-PAla), anionic PLA-*g*-poly(aspartic acid) (PLA-*g*-PAsp), and cationic PLA-*g*-PLL.[14,15] Beside these PLA-based structures, amphiphilic copolymers with PCL as hydro-

Scheme 1. Simplified General Scheme for PCL Anionic Modification[a]



[a] RX= Reagent, R standing for electophilic species.

Scheme 2. Main Strategies for the Synthesis of Graft Copolymers: (a) Grafting "from", (b) Grafting "onto"



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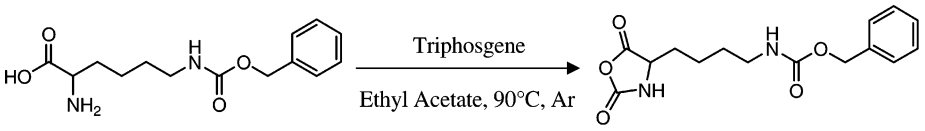
Scheme 3. Synthesis of *N*-Carboxyanhydride of *N*-ϵ-benzyloxycarbonyl-l,lysine



phobic chain have also been described, but even if some of them are biocompatible, the hydrophilic segments, namely poly(ethylene oxide), which is associated under the form of di-[16−18] or triblocks,[19−21] star[22,23] or graft[3] copolymers, poly(ethyloxazoline),[24,25] poly(acrylic) derivatives,[26−31] poly(vinylic) derivatives[32] or poly(ethylene imine),[33] are not degradable. Degradable PCL-*b*-PAA diblocks and PAA-*b*-PCL-*b*-PAA triblocks with glycine, alanine, phenylalanine, and γ-benzyl-L-glutamate as α-amino acids have been synthesized by Kricheldorf et al.[34] but these copolymers were not amphiphilic. Indeed, only a very limited number of amphiphilic and potentially fully degradable copolymers based on PCL have been reported such as chitosan-*g*-PCL,[35,36] poly((*R*,*S*)-β-malic acid)-*g*-PCL,[37] and poly(asparagine)-*g*-PCL (PAsn-*g*-PCL),[38] which is, as far as we know, the only amphiphilic structure exhibiting both PCL and poly(α-amino acid) segments.

These examples underscore the lack of amphiphilic structures based on poly(ϵ-caprolactone) and poly(α-amino acids) and especially the absence of amphiphilic and degradable graft copolymers exhibiting a hydrophobic backbone associated with hydrophilic side chains. As a consequence, it appeared to be of interest to propose new architectures of this type, especially with PCL as a main hydrophobic chain grafted with cationic side chains, to obtain novel degradable amphiphilic structures. In this work we focus on the synthesis of new water soluble PCL-*g*-PLL copolymers which represent the first examples of amphiphilic polyester/ poly(α-amino acids) graft copolymers with a PCL backbone. Properties of these copolymers in aqueous solution are also investigated.

Experimental Section

Materials. Poly(ϵ-caprolactone) ($\overline{M_n}$ = 42500 g/mol; $\overline{M_w}$ = 80000 g/mol), lithium diisopropylamide (2 M LDA in THF/n-heptane/ethylbenzene) and diethylamine (Et_2NH, ≥98%) were obtained from Aldrich (St. Quentin Fallavier, France). *N*-ϵ-Benzyloxycarbonyl-L,lysine (*N*-ϵ-Z-Lys,>98%), bromoacetyl chloride (>95%), and ammonium chloride (NH_4Cl) were obtained from Fluka (St. Quentin Fallavier, France). Triphosgene, hydrobromic acid in glacial acetic acid (33 wt %), trifluoroacetic acid (TFA, >99%), and anhydrous $Na_2S_2O_3$ were obtained from Acros Organics (Noisy-le-Grand, France). Ethyl acetate was obtained from Carlo-Erba (Val de Reuil, France), $MgSO_4$ was obtained from Prolabo (Paris, France), and dichloromethane, methanol, dioxane, and heptane were obtained from Riedel de Haën (St. Quentin Fallavier, France). All these chemicals were used as received. THF was obtained from Acros Organics (Noisy-le-Grand, France) and distilled on benzophenone/sodium until the formation of a deep blue color.

***N*-ϵ-Benzyloxycarbonyl-L-lysine *N*-carboxyanhydride (*N*-ϵ-Z-Lys NCA): Synthesis and Characterization.** The following synthesis was adapted from the literature.[39,40] Typically *N*-ϵ-Z-lysine (10 g, 36 mmol) and triphosgene (3.7 g, 12.4 mmol) were dissolved in ethyl acetate (310 mL) and placed in a two-necked round-bottomed flask equipped with a water condenser linked to two flasks, one empty and one containing 1 N NaOH aqueous solution. The atmosphere was kept inert by bubbling argon in the stirred reaction medium whose temperature was kept at 90 °C for about 4 h (Scheme 3). The reaction was stopped when the milky suspension turned to a clear solution. The temperature was decreased to room temperature and ethyl acetate was partly evaporated under argon atmosphere. Heptane was added up to a ratio ethyl acetate/heptane = 1/2 (v:v) to precipitate the NCA as slow as possible. The mixture was maintained at 4 °C overnight under argon. The white precipitate was filtered off under argon, washed two times with heptane, dried under vacuum, and kept at 4 °C under argon. The product was characterized by FT-IR spectrometry and ^{1}H NMR in $CDCl_3$ or deuterated trifluoroacetic acid (TFA-*d*). In $CDCl_3$ the resonance peaks of the *N*-ϵ-Z-Lys NCA were the following: 1.42−1.49−1.81 and 1.92 ppm: CH_2 of the lateral chain, 3.18 ppm: CH_2 in α position of carbamate, 4.24 ppm: C*H* of the ring, 4.92 ppm: N*H* of the carbamate, 5.08 ppm: CH_2 of the benzyl, 6.91 ppm: N*H* of the ring and 7.32 ppm: benzylic protons (Figure 1). The overall reaction yield was 90%.

Synthesis of Poly(*N*-ϵ-Z-L-lysine) (PZLL). The method used was adapted from the literature[41−43]. Typically, *N*-ϵ-Z-Lys NCA (5 g, 17 mmol) was dissolved in dioxane (100 mL) in a two-necked round-bottomed flask. The solution was stirred 5 min, and diethylamine (18 μL, 0.17 mmol) was added. The reaction was achieved at room temperature and revealed by the formation of carbone dioxide. After 60 h, the reaction medium was poured into cold methanol (1000 mL). A white precipitate was obtained, filtered off, and dried under vacuum. FT-IR spectrometry (specific polypeptide absorption band at 1650 cm^{-1}, disappearance of the specific absorption bands of the NCA at 1850; 1820 and 1775 cm^{-1}) and ^{1}H NMR in TFA-*d* (aromatic protons of the protecting group at 7.13 and 7.09 ppm, benzyl OCH_2 at 5.18 and 4.96 ppm, main chain C*H* at 4.36 ppm, side chain HNCH_2 at 3.00 ppm, and lateral chain CH_2 between 1.62 and 1.35 ppm) showed that there was no residual NCA, and poly(*N*-ϵ-Z-L-lysine) was obtained in a 80% yield. Differential scanning calorimetric thermograms of the polymer showed the typical poly(L-lysine) glass transition temperature T_g at 28 °C. Molecular weights, measured by SEC in $CHCl_3$ are $\overline{M_n}$ = 60000 and $\overline{M_w}$ = 104000 (PD = 1.73).

Synthesis of Poly(ϵ-caprolactone)-*g*-poly(*N*-ϵ-Z-L-lysine) by the Grafting "onto" Technique. First, PZLL was activated by reaction of the amino-chain end with bromoacetyl chloride. Typically, PZLL (2 g, 8.6×10^{-5} mol) was dissolved in dioxane (100 mL) overnight under stirring at room temperature. Bromoacetyl chloride (0.5 mL, 6×10^{-3} mol) was added, and the reaction was carried out for 3 h under stirring at room temperature. Dioxane and unreacted bromoacetyl chloride were removed by evaporation under vacuum to give a sticky wax, which was then directly dissolved in anhydrous THF (100 mL). In parallel, PCL (1 g, 8.8 mmol) was dissolved in anhydrous THF (100 mL) in a reactor equipped with mechanical stirring, and kept at −78 °C under argon atmosphere. A solution of LDA (4.40 cm^3, 8.8 mmol) was injected with a syringe through a septum to form a macropolycarbanion (PCL^-), and the mixture was kept at −78 °C for 30 min. The ω-bromoacetyl-PZLL THF solution was then injected with a syringe through a septum in the reaction medium. The reaction was carried

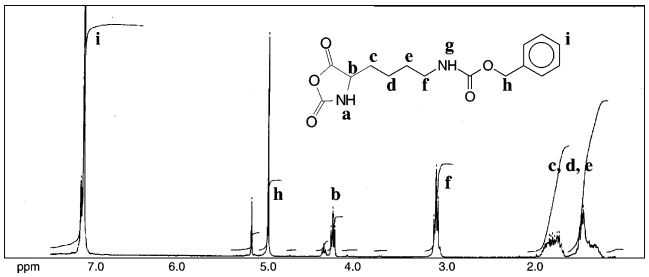
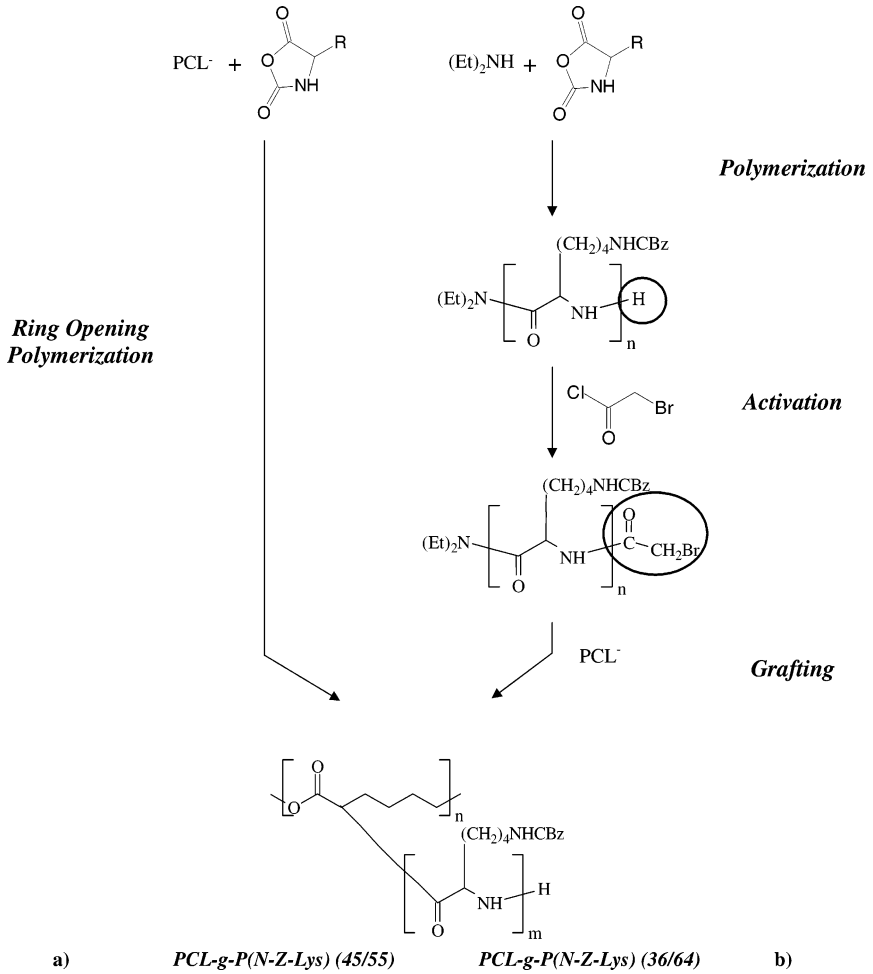


Figure 1. ^{1}H NMR of *N*-carboxyanhydride of *N*-ϵ-benzyloxycarbonyl-L-lysine in TFA-*d*.

Scheme 4. Strategies for the Synthesis of Poly(ϵ-caprolactone)-*g*-poly(*N*-ϵ-Z-L-lysine) (PCL*g*LPZLL) Copolymers by (a) Grafting "from" Technique or by (b) Grafting "onto" (here R = $(CH_2)_4NHCOOCH_2C_6H_4$)



out 1 h between −50 °C and −40 °C under stirring. The mixture was then hydrolyzed by 200 mL of a 2 M aqueous solution of NH_4Cl. The pH was adjusted to c.a. 7 by addition of 37% HCl. The copolymer was then extracted twice with 100 mL of dichloromethane. The combined organic phases were dried using anhydrous $MgSO_4$ and filtered, and the solvent was partly evaporated under reduced pressure. The concentrated copolymer solution was precipitated in an excess of cold methyl alcohol, filtered, and finally washed with cold methyl alcohol. The copolymer was dried under vacuum (10^{-3} mbar) for several hours (yield = 61%).

Synthesis of Poly(ϵ-caprolactone)-*g*-poly(*N*-ϵ-Z-L-lysine) by the Grafting "from" Technique. Typically, PCL (1 g, 8.8 mmol) was dissolved in anhydrous THF (50 mL) in a reactor provided with a mechanical stirrer and kept at −78 °C under an argon atmosphere. A solution of LDA (2.20 cm^3, 4.4 mmol) was injected with a syringe through a septum, and the mixture was kept at −78 °C for 30 min. *N*-ϵ-Z-Lys NCA (3.5 g, 11.4×10^{-3} mol) was dissolved in a minimum of THF and added to the reaction medium containing the macropolycarbanion PCL^-. The reaction was carried out 2 h under stirring at a temperature ranging from −70 °C to −40 °C. The reaction medium was then treated according to the procedure reported for the grafting "onto" technique. The specific yields in ϵ-caprolactone (ϵ-CL) units and *N*-ϵ-Z-lysine units were 55% and 52%, respectively.

Deprotection of the Poly(*N*-ϵ-Z-L-lysine) Chains. Typically, PCL-*g*-PZLL (0.5 g, 1.5 mmol of protecting group Z) was dissolved in TFA (10 mL) under stirring in a two-necked round-bottomed flask placed in a water/ice bath and equipped with a transfer funnel and an evacuation tube. HBr in glacial acetic acid (33 wt %, 2.4 mL, 12 mmol of HBr) was introduced dropwise in the reaction vessel. The reaction was kept under stirring 16 h at room temperature, and then reaction medium was poured in a large excess of cold diethyl ether. The yellowish precipitate was filtered, washed two times with cold diethyl ether, and dried under vacuum. Poly(ϵ-caprolactone)-*g*-poly(L-lysine) (PCL-*g*-PLL) was obtained with a yield c.a. > 95%.

Characterization. Molecular weights were measured by size exclusion chromatography (SEC) using a Waters equipment fitted with a 60 cm long 5 μm mixed C PLgel column as the stationary phase. THF at 1 cm^3/min flow rate was the mobile phase, and a Waters 410 refractometric detector was used. Typically, polymer (10 mg) was dissolved in THF (2 mL). The resulting solution was filtered on a 0.45 μm Millipore filter and injected in a 20 μL sample loop. Molecular weights $\overline{M_n}$ and $\overline{M_w}$ were expressed with respect to polystyrene standards. ^1H and ^{13}C NMR spectra were recorded at room temperature on a Brucker spectrometer operating at 300 MHz. Deuterated chloroform or dimethyl sulfoxide was used as solvent, and chemical shifts were expressed in ppm from the signal of tetramethylsilane (TMS). FT-IR spectra were recorded on a Perkin-Elmer 1760 FTIR spectrometer, polymers being cast on NaCl plates from solvent solution. The mean diameter of the micelle-like objects was determined by dynamic light scattering at 90° using a Spectra-Physics Stabilite 2017 LASER (514 nm, 200 mW, 30 A) and a Brookhaven 9863 collimator. Samples were filtered using a 0.45 μm filter, kept at 25 °C in a decalin bath, and data were treated by the default CONTIN method of the supplied software. Thermal transitions were measured under nitrogen flow by differential scanning calorimetry using a DSC6 Perkin-Elmer apparatus. Scanning conditions are the following: heating from 20 to 150 °C at 10 °C/min, cooling to −70 °C at 10 °C/min, and heating to 150 °C at 10 °C/min. Thermal transitions are measured on the second heating scan.

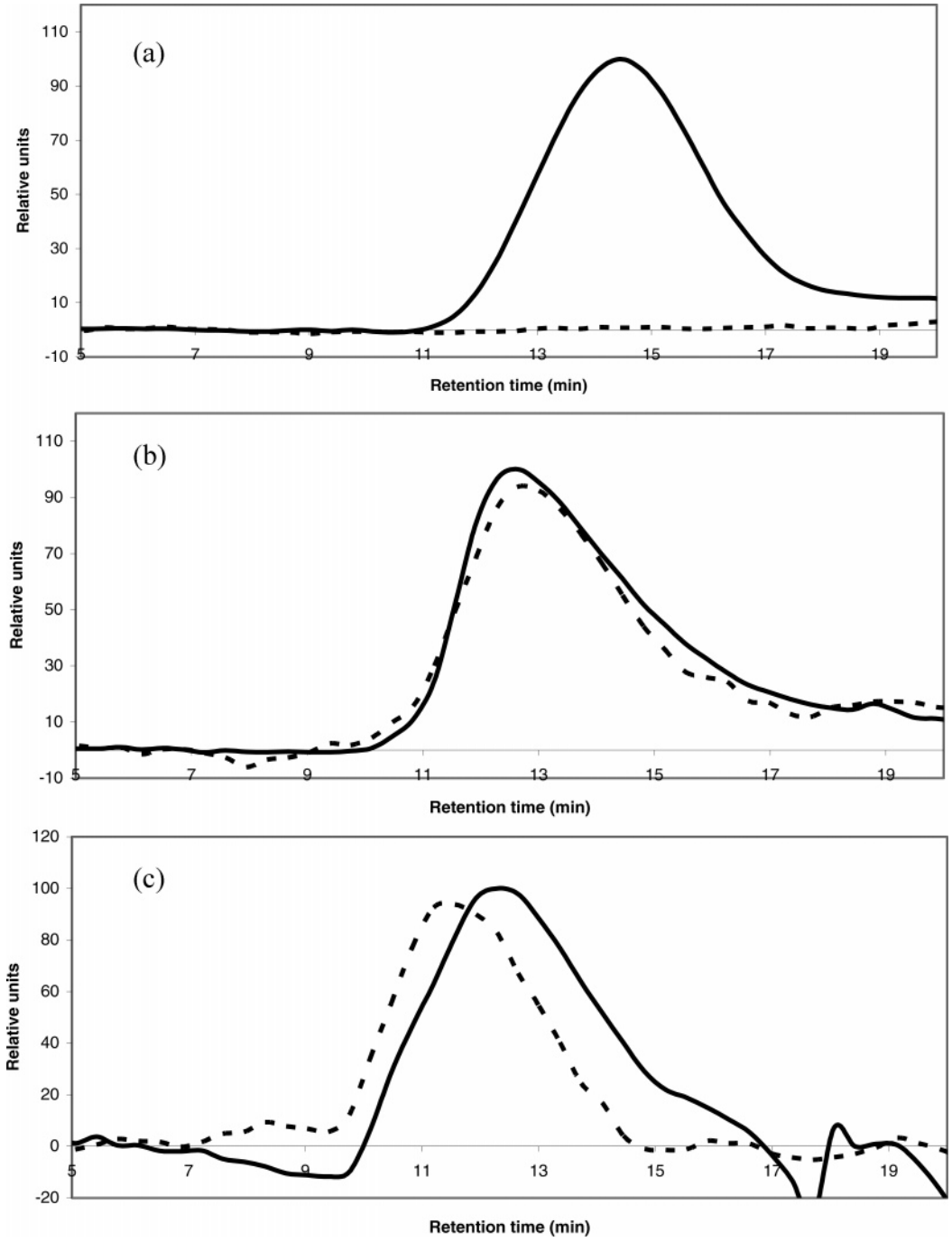


Figure 2. SEC Chromatograms in THF of (a) poly(ϵ-caprolactone), (b) poly(*N*-ϵ-Z-l-lysine), and (c) poly(ϵ-caprolactone)-*g*-poly(*N*-ϵ-Z-L-lysine) copolymer synthesized by the grafting "onto" method and detected by refractometric index detector (full line) and fluorometric detector (dotted line).

Results and Discussion

As outlined in the introduction, only a few examples of amphiphilic degradable graft copolymers have been so far described. This is especially the case for cationic structures, which despite their potential for many valuable applications are mainly based on nondegradable poly(methacrylic) or poly(ethyleneimine) derivatives. For this reason it appeared to be of interest to combine the well-known "grafting onto" and "grafting from" techniques with our anionic chemical modification technique of PCL. First this combination leads to novel amphiphilic PCL-based graft copolymers which present an original architecture compared to the existing structures. Second "grafting from" copolymerizations mainly rely on the initiation by pre-existent functional hydroxyl or amine groups,[15,35] while "grafting onto" copolymerizations rely on coupling reactions between activated pendant functional groups of the hydrophilic backbone and the chain-end moieties of the hydrophobic side chains.[11,33,36,38] Finally it is noteworthy that the same is found with the macromonomer strategy which is generally based on radical polymerization.[27,29] As a consequence, the synthesis of PCL-*g*-PZLL copolymers starting from anionic PCL intermediate appeared to be original.

Synthesis of Poly(ϵ-caprolactone)-*g*-poly(*N*-ϵ-Z-L-lysine) by the Grafting "onto" Technique. PZLL was synthesized by anionic ROP of *N*-ϵ-Z-Lys NCA, initiated by diethylamine. Reaction scheme of this strategy is shown in Scheme 4b. The mechanism can be considered as living, which allowed the

Table 1. Molecular Weights an Polydispersity (PD) of Poly(ϵ-caprolactone) (PCL), Poly(*N*-ϵ-Z-L-lysine) (PZLL), and Poly(ϵ-caprolactone)-*g*-poly(*N*-ϵ-Z-L-lysine) (PCL-*g*-PZLL) Synthesized by the Grafting "onto" Method According to SEC Analysis in $CHCl_3$

polymer	M_n (g/mol)	M_w (g/mol)	PD
PCL	40000	58000	1.45
PZLL	63000	104000	1.65
PCL-*g*-PZLL	137000	197000	1.44

modulation of the degree of polymerization ($\overline{DP}$) by adjusting I/M ratio, I and M standing for the concentrations in initiator and monomer respectively. Expected $\overline{DP}$ was 100 according to experimental conditions. SEC analysis of PZLL in $CHCl_3$ gave $\overline{M_n} = 60000$ g/mol which corresponds to $\overline{DP} = 215$. However, the molecular weight of PZLL being expressed with respects to polystyrene standards it is not possible to confirm the controlled nature of the polymerization.

According to the polymerization mechanism, PZLL exhibited two amine groups at chain ends, a tertiary amine issued from the initiator and a primary amine due to the polypeptidic chain. This primary amine is reacted with a bromoacetyl halide to yield highly reactive α-bromo-ketone species. This strategy has been described for the grafting of α-methoxy,ω-hydroxy-poly(ethylene oxide) on PCL.[3] In the present work, bromoacetyl chloride was used to activate primary amine on the chain-end. Only 0.1 equiv of PZLL chain for each ϵ-CL unit was used to avoid a precipitation that occurs when classical conditions (≈1 equiv) were applied.[1,3] The grafting of PZLL chain was shown by SEC using a dual detection based on a refractometer and a fluorometer ($\lambda_{ex} = 255$ nm, $\lambda_{em} = 265$ nm). These wavelengths are characteristics of the excitation and emission of the benzyloxycarbonyl protecting group. SEC chromatograms of PCL, PZLL, and grafted PCL-*g*-PZLL copolymer are shown in Figure 2. PCL was detected only by refractometry (Figure 2a) while homo-PZLL was detected by both detectors (Figure 2b). The copolymer is also detected by fluorometry (Figure 2c), proving the grafting of the PZLL chains on the PCL backbone. Figure 2c shows that molecular weight of the copolymer was higher than that of the homopolymers precursors. The shift of the SEC peaks observed between the two chromatograms is explained by the fact that only grafted copolymers are detected by fluorometry while refractometry takes also into account the nongrafted PCL chains with lower molecular weights. The molecular weight of the PCL-*g*-PZLL measured in $CHCl_3$ was $\overline{M_n}$ =137000 with a polydispersity PD = 1.44. In order to roughly evaluate the number of grafted chains on the PCL backbone, the final molecular weight of copolymer was compared to those of homopolymers (Table 1). If it is supposed that all macromolecular species have similar conformations in chloroform, two PZLL chains are grafted per PCL chain. Taking into account this approximation, molar composition is 44% of ϵ-CL and 56% of *N*-ϵ-Z-lysine units approximatively. A typical ^{1}H NMR spectrum of PCL-*g*-PZLL in TFA-*d* is shown in Figure 3. Composition was evaluated by the ratio of signals at 4.36 ppm corresponding to the C*H* protons of the PZLL chain (Figure 3, peak f), and at 4.00 ppm corresponding to the methylene CH_2O of PCL (Figure 3, peak c). A composition of 36% of ϵ-CL and 64% of *N*-ϵ-Z-lysine was found, in agreement with the hypothesis of two PZLL chains per PCL backbone. Considering this composition, specific yields in PCL and PZLL were 35% and 74%, respectively.

Synthesis of Poly(ϵ-caprolactone)-*g*-poly(*N*-ϵ-Z-L-lysine) by the Grafting "from" Technique. This strategy relies on the use of the polycarbanionic intermediate PCL$^-$ as a macroinitiator to polymerize *N*-ϵ-Z-L-Lys NCA as shown in Scheme 4a. The molar ratio LDA/ϵ-CL is 0.5, LDA and ϵ-CL standing for the concentrations in lithium diisopropylamide and ϵ-caprolactone units, respectively. These conditions were selected to carry out the formation of the carbanion and the ROP of NCA monomers in a one-pot reaction and to minimize the homopolymerization of NCA monomers that occurred simultaneously.

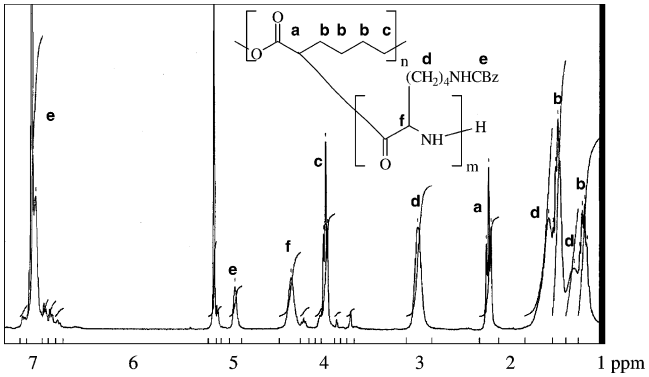
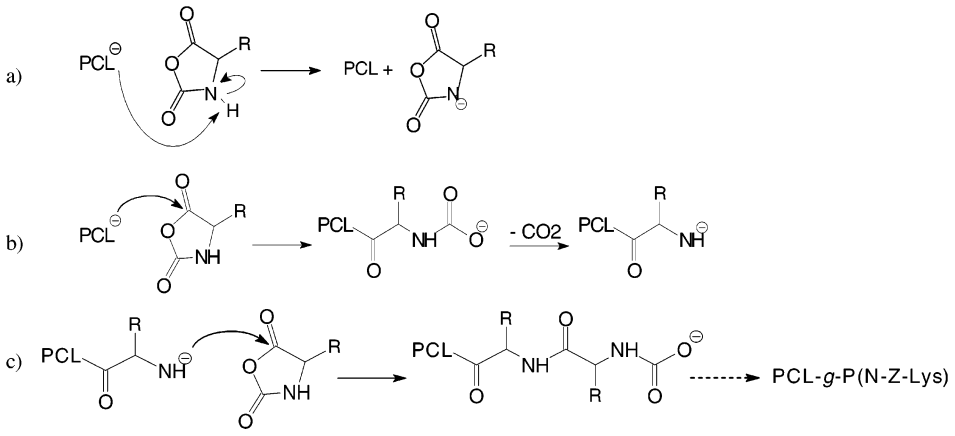


Figure 3. ^{1}H NMR of poly(ϵ-caprolactone)-*g*-poly(*N*-ϵ-Z-L-lysine) copolymer synthesized by the grafting "from" technique.

The initiation of the ROP of NCA ring by the macropolycarbanion was expected to proceed by a nucleophilic attack of the carbon 5 of the NCA ring by the carbanionic sites of the activated PCL$^-$ (Scheme 5b and 5c) leading to a graft copolymer according to the grafting "from" technique. However, the transfer of amidic protons can occur in parallel and deactivate some of the carbanionic sites to regenerate PCL-type repeating units (Scheme 5a). Therefore, a competition between the two mechanisms was expected. FT-IR spectrum of the recovered solid exhibited absorption bands of polypeptides at 1650 cm^{-1}, N−H at 3290 cm^{-1}, and ester of PCL at 1730 cm^{-1}. ^{1}H NMR spectrum in TFA-*d* is shown in Figure 3. Signals typical of both PCL and PZLL moieties were detected, and a 45/55% of ϵ-CL/*N*-ϵ-Z-lysine molar composition was calculated. Specific yields in PCL and NCA were 55% and 52%, respectively. SEC analysis in THF gave $\overline{M_n} = 7200$ g/mol and PD = 1.40. The monomodal aspect of the chromatogram suggested the absence of homo-PZLL. DSC thermograms showed a glass transition at 28 °C, close to glass transition of PZLL homopolymer, and a melting point at 50 °C, characteristic of substituted PCL derivatives.[1,2,44] These data show the effective macroinitiation of NCA monomer, even in the presence of amidic labile hydrogen on the ring. Side reaction was probably limited to the formation of oligomers that did not precipitate during the solvent−non-solvent purification, thus explaining the low yield in terms of NCA incorporated in copolymer chains.

Deprotection of Poly(ϵ-caprolactone)-*g*-poly(*N*-ϵ-Z-L-lysine). Amine groups of PCL-*g*-PZLL copolymers were regenerated by hydrolysis of Z groups in TFA using a 33 wt % hydrobromic acid solution in glacial acetic acid according to literature.[45] Reaction time and number of equivalents of HBr were adapted to obtain a total deprotection despite the steric hindrance due to the copolymeric structure. ^{1}H NMR spectra of PCL-*g*-PLL in TFA-*d* showed the absence of aromatic protons. Deprotection ratio was in the range 95−100%. Almost no change was observed in the composition of copolymers after acidic treatment. In copolymer prepared by the grafting "onto" method, deprotection led to a PCL-*g*-PLL copolymer containing 40% (initially 36%) of ϵ-CL units; in copolymer prepared by the grafting "from" method, deprotection also led to PCL-*g*-

Scheme 5. Polymerization Mechanisms for NCA with PCL⁻ as Macroinitiator: (a) Amine Hydrogen Extraction, (b) Nucleophilic Attack on the Carbon 5, and (c) Propagation



PLL copolymer containing 40%, (initially 45%) of ϵ-CL units. The same acidic treatment was carried out on PCL, showing no chain breaking, as measured by SEC. The variation in composition detected in ^{1}H NMR spectra of copolymers was probably due to the formation of microdomains in TFA-*d* used for the ^{1}H NMR analysis, in which part of the chains are not detected. This behavior was assessed by ^{1}H NMR analysis of a PCL-*g*-PLL copolymer in D_2O, which exhibited only peaks of PLL protons, whereas 40% of ϵ-CL units are detected in TFA-*d*. In D_2O, hydrophobic PCL chains are assumed to be masked in the inner core of micelle-like objects whose formation is discussed later. Thus, even if degradation cannot be excluded, composition changes observed by ^{1}H NMR are likely due to conformation changes of the copolymers in TFA-*d* after deprotection. Therefore, it was concluded that removal of Z group is quantitative and did not lead to PCL backbone degradation.

Study of Poly(ϵ-caprolactone)-*g*-poly(L-lysine) Aqueous Solutions. Poly(L-lysine) is a water-soluble polybase at neutral pH due to the protonation of the primary amine of the lateral chain. The high pK_a (10.2) gives a basic character to the polymer in a broad scale of pH, in particular at a physiological pH. PCL-*g*-PLL copolymers contain 60% of L-lysine units, a proportion high enough to promote water-solubility despite the highly hydrophobic character of the PCL backbone. Solutions of copolymers were prepared in distilled water and in isoosmolar phosphate buffer (PBS, pH = 7.4) at concentrations varying from 0.05 to 10 mg/cm^3 and filtered. PBS solutions were prepared in order to mimic physiological conditions, as it is well-known that the presence of salts screens electrostatic intrachain and interchain interactions in polyelectrolytes. Diameters were evaluated by dynamic light scattering in water or PBS as shown in Figure 4. First it is noteworthy that PCL-*g*-PLL copolymers are soluble in distilled water and PBS, whatever the synthesis route: (i) the grafting "from" technique (copolymer referred as PCL-*g*-PLL "from") or (ii) the grafting "onto" technique (copolymer referred as PCL-*g*-PLL "onto"). Second it is remarkable that these graft amphiphilic copolymers spontaneously form micelle-like objects in solution. A general trend is the decrease of the mean diameter when the concentration decreases. In PCL-*g*-PLL "from", diameter varies from 165 to 60 nm when concentration decreases from 5 to 0.05 mg/cm^3 (Figure 4a, full lines), showing that these objects form plurimolecular aggregates or micelles. The effect of the shielding of charges in PBS is also shown (Figure 4a, dot line). At a 10 mg/cm^3 concentration a strong contraction of the structure was observed, mean diameter decreasing from 165 to c.a. 10 nm. In the case of PCL-*g*-PLL "onto", the same trends were observed, but objects are much bigger. Mean diameter in distilled water is 3 to 8 folds higher than in the case of PCL-*g*-PLL "from", with values ranging from 400 to 500 nm (Figure 4b, full lines). In PBS a drastic contraction was also observed, mean diameter being around 10 nm at a 10 mg/cm^3 concentration.

Differences between mean diameters of the two copolymers at the same concentrations are explained in terms of polymer structures. In the case of the grafting "from" strategy, SEC and ^{1}H NMR analyses showed that an average of two long PZLL chains ($\overline{M_n}$ = 60000 g/mol) are grafted on each PCL backbone (Scheme 6b), leading to a structure similar to a triblock structure. The extension of these PLL chains in distilled water as well as the aggregation of PCL segments is responsible of the formation of large aggregates (Ø ≈ 500 nm). Large aggregates have been described in similar but inverse structures of poly(*N*,*N*-dimethylamino-2-ethyl methacrylate)-*g*-poly(ϵ-caprolactone)[29]

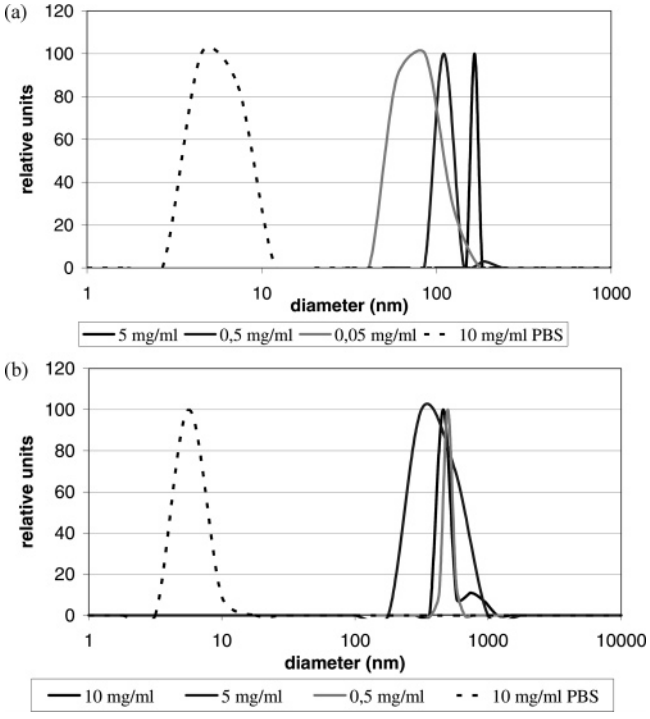
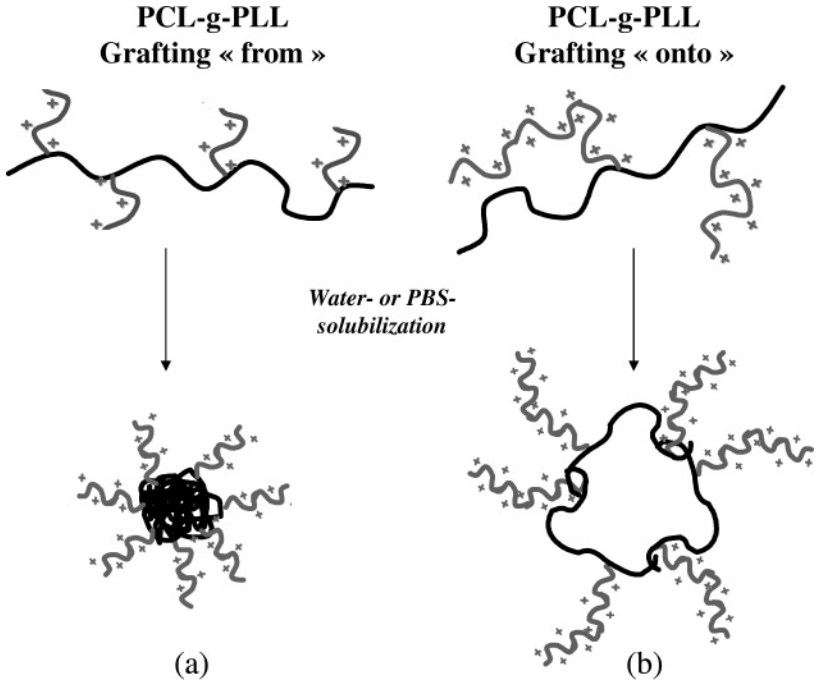


Figure 4. Dynamic light scattering studies of solutions of poly(ϵ-caprolactone)-*g*-poly(L-lysine) obtained by (a) the grafting "from" technique and (b) the grafting "onto" technique in distilled water (full lines) and PBS (dotted lines).

Scheme 6. Expected Differences of Architectures for the Poly(ϵ-caprolactone)-*g*-poly(*N*-ϵ-Z-L-lysine) (PCL-*g*-PLL) Copolymers and the Micelle-like Object Formed in Aqueous Medium as a Function of the Synthesis Strategy: (a) Grafting "from" and (b) Grafting "onto"



copolymers that contain only one PCL side chain. In acidic solutions, these diblock-type structures form at a concentration of 1 mg/cm^3 large aggregates having a mean diameter around 340 nm. In PBS salty medium, because of charge screening, PLL chains are contracted, leading to a contraction of the whole structure, and consequently to the formation of small sized objects (Ø ≈ 10 nm). In the case of PCL-*g*-PLL "onto" copolymer, the situation is quite different. The macropolycarbanionic intermediate PCL$^-$ could initiate many short PZLL chains. After deprotection, the copolymer was expected to present in aqueous solution a homogeneous repartition of the charged PLL side chains along the PCL backbone (Scheme 6a). The structure with a high degree of substitution is close to the structure of polyelectrolytes in which hydrophobic interactions are counterbalanced by electrostatic repulsions, leading to small particles containing only a few macromolecules in water (Ø ≈ 100 nm) as well as in PBS (Ø ≈ 10 nm). In PLL-*g*-PLGA and PAsn-*g*-PCL copolymers the mean diameter of the micelles formed in aqueous solution is decreasing (from 150 to 70 nm in the case of PLL-*g*-PLGA) when the degree of substitution increases.[11,38] These results were attributed to the stronger hydrophobic interaction resulting from the higher packing density of the hydrophobic side chains. In PCL-*g*-PLL copolymers the same tendency is observed although these copolymers exhibit hydrophobic core and hydrophilic side chains.

Conclusion

Novel amphiphilic and water-soluble degradable cationic copolymers have been synthesized. Compared to most of the amphiphilic graft copolymers, which present a hydrophilic backbone and hydrophobic side chains, the originality of these copolymers relies on their inverse structure. The polymeric backbone is PCL, while hydrophilic side chains are formed with cationic poly(L-lysine), which represents as far as we know the first example of inverse amphiphilic degradable graft copolymer. These PCL-*g*-PLL copolymers were obtained by grafting "onto" and grafting "from" strategies based on the reaction between the two activated homopolymeric precursors and on the anionic ring opening polymerization of *N*-ϵ-Z-Lys NCA, respectively. The primary amine groups of the lysine units were recovered by an acidic treatment with c.a. no change in the overall composition and no polyester chain breaking. Furthermore, PCL-*g*-PLL copolymers are water-soluble at physiological pH and spontaneously formed micelle-like objects of nanometric size, whose diameters were ranging from 60 to 500 nm in distilled water depending on the synthetic pathway and the degree of substitution. Smaller objects were obtained for higher degree of substitution. All particles had the same diameter (≈ 10 nm) in PBS. It is noteworthy that the two routes generate similar copolymers with variable compositions and different architectures. Finally, although the hydrolytic degradation of these new PCL-*g*-PLL copolymers was not yet evaluated, it is expected that they are degradable due to the well-known degradability of both homopolymers used.

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